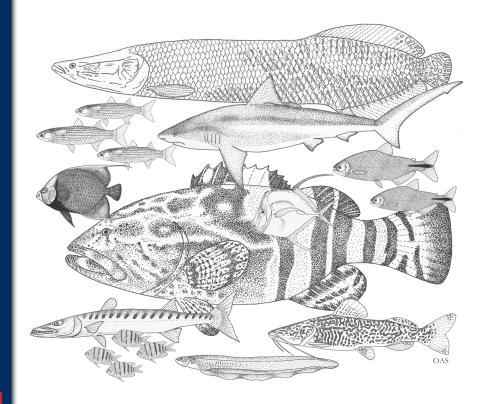


II INTERNATIONAL SYMPOSIUM ON PHYLOGENY AND CLASSIFICATION OF NEOTROPICAL FISHES

# Abstract Book



Londrina, PR - Brazil

October 23<sup>rd</sup> - 27<sup>th</sup>, 2017

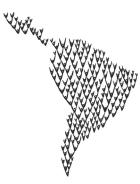
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II INTERNATIONAL SYMPOSIUM ON PHYLOGENY AND CLASSIFICATION OF NEOTROPICAL FISHES October 23 to 27, 2017 at Londrina, Brazil

### Abstracts

Londrina 1st Edition 2017



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II INTERNATIONAL SYMPOSIUM ON PHYLOGENY AND CLASSIFICATION OF NEOTROPICAL FISHES October 23 to 27, 2017 at Londrina, Brazil

Ichthyology in the Neotropics has recently undergone a great and important transformation. Until 40 years ago, most studies of the megadiverse fish fauna of South and Central America were conducted by foreign researchers, who based their research mainly on sparse collections in North American and European museums. These scientists had limited opportunities to study and collect species in their natural habitats. Since then, cooperative projects among researchers working on Neotropical fishes from many different countries have blossomed, and the number of native South and Central American ichthyologists and students has increased tremendously. That increased capacity, and the many opportunities to collect and analyze data afforded by the closer aeographic proximity of researchers to the fishes and their environment has allowed an entirely new view of fish diversity. Ichthyological knowledge in South and Central America has reached an unprecedented level, and the number of studies and publications on the phylogeny and classification of Neotropical fishes has grown exponentially. In turn, this new knowledge about phylogeny and classification catalyzes studies in other areas of biology, and allows researchers to address urgent concerns about the imminent loss of biodiversity caused by the alteration of natural environments.

Twenty years ago, researchers from the Museu de Ciencias da PUCRS, Universidade Federal do Rio Grande do Sul, and United States National Museum organized an international symposium on the Phylogeny and Classification of Neotropical Fishes with the support of the Brazilian Society of Ichthyology. This milestone scientific meeting was the first to gather the great majority of Neotropical fish systematists at a paramount event. The proceedings from the event were published in 1997 as a book on the biogeography and evolution of Neotropical freshwater fishes. Since then, the Brazilian Society of Ichthyology (Sociedade Brasileira de Ictiologia) has strengthened the communication among Neotropical ichthyologists from Brazil and many other countries. The biannual meetings (EBI) have grown from small gathering of about two hundred people to large scientific events attracting more than 1,000 participants. Concurrently, SBI created a well-regarded international scientific journal entitled Neotropical Ichthyology.

Since the publication of the landmark volume in 1997, methods of phylogenetic data have advanced radically, mainly by the growth and refinement of new methods of analyzing DNA sequences. Twenty years ago, most studies focused on osteological characters and analyses of DNA sequences were rare. Today we see not only a wide proliferation of molecular methods, but also growth in the use of other kinds of anatomical data such as myology, neuroanatomy and 3D morphometrics, and combined analyses that integrate morphological and molecular phylogenetics to answer key questions about evolution and diversification.

This second International Symposium on Phylogeny and Classification of Neotropical Fishes occurs in the context of this ichthyological renaissance. The event will reassemble the world's community of researchers working on the evolution of Neotropical fishes twenty years after the first meeting to evaluate and celebrate the advances achieved in the last two decades through the emergence and establishment of new methodologies. Participants will address key questions about the evolution of Neotropical fishes, propose new hypotheses and debate existing ones. A special issue of the society's journal, Neotropical Ichthyology, will disseminate the most event's most interesting and groundbreaking. Like watershed 1997 book Phylogeny and Classification of Neotropical Fishes, this special issue of Neotropical Ichthyology will represent a new landmark for studies on Neotropical fishes.

The event will also pay homage to the late American researcher Richard Vari of the Smithsonian Institution, who was one of the most productive and well-loved researchers in the history of Neotropical fish systematics. Aside from his enormous body of published work, Dr. Vari left a great legacy to Neotropical ichthyology by mentoring, guiding and collaborating with generations of students and postdoctoral researchers from many South American countries. Through his kind and humble gifts of time, wisdom and financial support, he inspired and encouraged the many researchers who now follow in his footsteps.

José L. O. Birindelli President of the II International Symposium on Phylogeny and Classification of Neotropical Fishes

# Vari's Legacy



# An introduction to Richard Vari's legacy, with examples from a decade of collaboration on the systematics and diversification of Anostomoidea

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During his long and storied professional career, Richard P. Vari established himself as one of the world's foremost systematic ichthyologists, mentored dozens of junior scientists, devoted countless hours to professional service, developed a globe spanning network of professional collaborations, and maintained a deep and consistent commitment to his loving family and friends. As one of his longest collaborators, I will introduce this symposium honoring his legacy by summarizing his many impacts and illustrating how his wise mentorship and exceptional knowledge shaped me as a scientist and guided our decade of collaborative study on the systematics, evolution and diversification of Neotropical headstanding fishes and their close relatives.

Keywords: Phylogenetics; taxonomy; professional development; osteology; Characiformes Financial support: NSF Grant DEB-1257898 and a generous travel award from the Smithsonian Institution

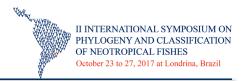


### Rich Vari: Early years and the American Museum of Natural History

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Rich Vari and I were both privileged to be graduate students of Donn E. Rosen in the Department of Ichthyology at the American Museum of Natural History, New York, in the mid-1970s. Donn was a gregarious, erudite man who was an influential and encouraging mentor. Many of ways that Rich Vari worked throughout his career were developed during his time as a graduate student of Donn in the AMNH. Ultimately Rich developed his own style and was an influential mentor himself to many students of systematic ichthyology.



### Systematics of Trachelyichthys: exploring biodiversity

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The taxonomy of the Auchenipteridae has greatly improved due to revisionary studies of many genera over the last two decades. However, some groups still await more encompassing studies seeking to clarify generic limits and to describe unnamed species. This is the case for Trachelyichthys, currently represented by two valid species, T. decaradiatus from Guyana (Rupununi River basin), and T. exilis from Peru (Nanay River, upper Amazon basin), both known from only a few specimens. The rarity of available material of both species has hampered the recognition of new species from the Amazon and Orinoco basins. We conducted a revisionary study of the aenus, revealing four additional species of Trachelvichthys from the upper Nearo River and Orinoco basins; the middle Negro River basin; the Madeira River basin; and the main channel of the Amazon basin. The four new species are distinguished from each other by the presence or absence of black spots on the body, the presence of a black stripe along the lateral line region; the number of pterygiophores free supporting the branched dorsal-fin rays; the number of branched dorsal-fin rays; the number of pelvic-fin rays; the shape of the posterior nuchal plate; the length of the transformator process of the tripus; the shape of the posterior process of the epioccipital; the length of the posterior process of the coracoid; the shape of the anterior portion of the pectoral airdle; and other morphometric proportions of the body and head. A family-level phylogeny based on combined morphological and molecular analysis recovered Trachelyichthys as a monophyletic unit, sister taxa of Trachelyopterichthys, supported by both morphological and molecular synapomorphies. Trachelyichthys clade is formed by the new species from Madeira River as the sister to all congeners, and the new species from the upper Nearo River basin and Orinoco basin as subsequent taxa, sister to the remaining species; and a clade formed by two independent groups, Trachelyichthys decaradiatus + Trachelyichthys new species from middle Negro River, and Trachelyichthys exilis + Trachelyichthys new species from the main channel of the Amazon basin (represented by populations from Tapajós, Xingú, and Amazon basins). The genetic data of col for two species, Trachelyichthys exilis and Trachelyichthys new species from the Amazon basin, which are sister taxa, resulted in 94.5% of genetic similarity, a relatively large genetic distance compared to other species in the family. The beautiful new species from middle Negro River basin will be named in honor to Richard Vari as recognition of his great devotion, fascination and contributions to the study of the Auchenipteridae and other Neotropical fishes.

Key words: Taxonomy; Driftwood; Catfish; Total Evidence Phylogeny; Auchenipteridae Financial support: CNPq, CAPES, and Smithsonian short-term visitor Grant to BBC



### Advances in the systematics and taxonomy of Chilodontidae, Curimatidae and Prochilodontidae

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The superfamily Anostomoidea includes approximately 280 species and encompasses Chilodontidae, Curimatidae and Prochilodontidae, three partially or exclusively detrivorous families, plus Anostomidae, a species-rich family of omnivores, insectivores and herbivores widely distributed across the Neotropics. Over the last four decades Richard Vari alone or with colleagues published a series of studies hypothesizing most intergeneric and intraspecific relationships in the superfamily and further contributed to knowledge of species-level diversity through taxonomy. More recently, several projects have treated diversity and evolution within Anostomoidea. Multilocus phylogenies, for example, have elucidated some longstanding polytomies within Chilodontidae and Prochilodontidae and, most notably, have placed the three detritivorous families in a clade sister to the omnivorous Anostomidae. Here, we present novel results from a multilocus analysis resolving intergeneric and interspecific relationships within the Curimatidae. We also show results from the analyses of more than 1000 nuclear loci of ultraconserved elements (UCEs), a novel source of molecular phylogenetic data that have been successfully applied in the field of molecular systematics and evolutionary biology. UCE results corroborate previous multilocus hypotheses of intrageneric relationships within Chilodontidae and Prochilodontidae and provide better resolution within some problematic genera. The phylogeny also corroborates intergeneric resolution in Curimatidae and provides stronger support at species-level relationships in the most derived and problematic clades that contain Curimatella, Cyphocharax and Steindachnerina. These results provide support for putative nomenclatural changes that are of necessity in some clades, especially within Curimatidae. We also present an overview of recent taxonomic results that continue revealing undescribed and/or cryptic taxa, particularly within Curimata, Curimatopsis, Cyphocharax, Potamorhina, Prochilodus, and Steindachnerina.

Key words: Characiformes; Neotropical; Ostariophysi Financial support: NSF DEB-1257898 and FAPESP 2011/08374-1, 2014/26508-3



#### The legacy of Richard P. Vari: scientific excellency, reliability, openmindedness and generosity

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An overview is presented of the career of Richard P. Vari and his role in the history of ichthyology. Vari's contributions covered a number of different fish groups from several continents and established standards which pervade the field in the long term. Such influence owes as much to his published body of work as to his personality. As one of the most productive fish taxonomists in modern times, R. Vari played a fundamental role in keeping fish systematics a truly universal scientific endeavor. Vari's international interaction was one of his notable professional traits, surpassing national and cultural barriers. That achievement results from a combination of four factors inherent to his personality: scientific excellency, reliability, open-mindedness and generosity. Literally hundreds of young and established ichthyologists worldwide benefitted from his interaction, which changed for the better the outlook of the field. His association with Brazilian scientists was particularly active, especially with students just beginning their careers. Richard Vari is probably the most important foreign benefactor in the history of Brazilian zoology. Such influence was instrumental in preventing the Brazilian community of ichthyologists from falling into provincialism during a critical phase in its development. As a corollary, it largely contributed to the thriving state of the field in the country. His tireless editing of manuscripts, both for content and form, warranted a long-lasting tradition of objectivity, formal cleanliness and conciseness in writing which lives on in the work of many academic descendants.

Key words: scientific legacy, systematics, biography, Smithsonian Institution, ichthyology, taxonomy Financial support: FAPESP 2015/26804-4, CNPq 308962/2013-3



#### Morphology-based phylogeny of the glass knifefishes Eigenmanniinae (Teleostei: Gymnotiformes: Sternopygidae)

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The electric glass knifefishes of Eigenmanniinae comprise five genera and 39 valid species. They are widespread in the Neotropical region, ranging from the Río Tuíra in Panama to La Plata basin in Argentina. There is no consensus on the phylogenetic relationships among genera or on the monophyly of the genus Eigenmannia. Here we advance the knowledge of the group by proposing a hypothesis of phylogenetic relationships to 36 ingroup species of Eigenmanniinae based on the parsimony analyses of 144 characters including external morphology, osteology, myology and neuroanatomy. The results recuperated the monophyly of Eigenmanniinae and recognize the following genera: Archolaemus, Distocyclus, Eigenmannia, "Eigenmannia" goajira, Japigny and Rhabdolichops. Two most parsimonious trees were recovered, with their strict consensus demonstrating the following relationships: (Japigny (Rhabdolichops ((Distocyclus conirostris ("Eigenmannia" goajira Archolaemus)) Eigenmannia))). Relationships within Rhabdolichops are similar to those formerly proposed. On the other hand, relationships within Archolaemus are somewhat different from previously proposed, and imply a series of innovations associated with bone elongation in the lower jaw, neurocranium and suspensorium toward a more elongated snout. For the first time the monophyly of the problematic genus Eigenmannia was recovered. Eigenmannia macrops is the sister group to all remaining congeners. The clade composed of E. humboldtii, E. limbata and E. nigra is sister to the remaining species. Eigenmannia meeki is sister to the Eigenmannia trilineata species aroup. Within the latter, E. matintapereira is the sister of the remaining species and two main clades were recovered. The first clade includes species having subterminal mouths: (E. besouro (E. waiwai (E. virescens (E. vicentespelaea (Eigenmannia sp. "Ventuari" and D. guchereauae))))). The second clade including species with terminal mouths: (E. trilineata (E. desantanai (E. microstoma (E. guairaca (E. sayona (E. antoinioi (E. pavulagem E. muirapinima))))))). The hypothesis of monophyly of Distocyclus including D. conirostris and D. guchereauae is rejected because D. guchereauae is nested within Eigenmannia. As consequence of the new hypothesis of relationships, nomenclatural changes in Eigenmanniinae are required. "Eigenmannia" goajira, previously assigned as incertae sedis in Eigenmanniinae, should be assigned to a new genus; and the nominal species D. guchereauae should be allocated to Eigenmannia.

Key words: Systematics; Taxonomy; Freshwater; South America, Neotropics. Financial support: CNPq (GMD 300066/2016-3, WBW 300940/2015-7 and 405144/2013-0), FAPESP (LAWP 2013/09926-3 and 2015/24709-4; VPA 2014/11397-1; Diversity and Evolution of Gymnotiformes Project 2016/19075-9)



### The legacy of Rich Vari in ostariophysan systematics: genome-wide analyses resolve the morphological tree of S. Fink and W. Fink

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Ostariophysan fishes constitute the dominant group in freshwater habitats around the world. They diversified into more than 10,000 species, having gone through one of the major evolutionary radiations among jawed vertebrates. For the past three decades the most widely accepted hypothesis of relationships among otophysan orders has been based on an intuitive groundplan cladistic analysis of morphology by S. Fink and W. Fink. Subsequent molecular studies have produced conflicting phylogenetic results that differ from the canonical morphological tree, disagreeing about the interrelationships of all major groups (Cypriniformes, Characiformes, Siluriformes and Gymnotiformes), and some even failing to resolve the monophyly of the order Characiformes (including a recent UCE-based phylogenomic analysis). To address this challenging phylogenetic question, we collected genome-wide sequence data from 1,051 exons using target capture and Illumina sequencing for 225 ostariophysan species representing all major lineages. Initial results using a wide range of analytical approaches (concatenation or species trees), applied on multiple data subsets (e.g., strong signal, conserved genes, etc.) and alternative data types (DNA or protein sequences) revealed conflicting phylogenetic signals, finding support for ten alternative evolutionary histories among otophysan lineages. Therefore, these best practice methodologies applied to minimize systematic error in phylogenomics seem to exacerbate the problem rather than to settle the dispute. Neither concatenation nor species tree methods, nor DNA-based or protein-based analyses converge on a single topology. To gain additional insight, we developed a novel analytical approach (Gene Genealogy Interrogation or GGI) based on topology tests to gauge the strength of genealogical signal contained in each gene alignment in favor of alternative hypotheses. In contrast to the results based on standard methodologies, our GGI analyses reveal that the vast majority of gene genealogies are congruent with the canonical morphological tree. These results are consistently obtained regardless of data type used (DNA or protein). Coalescent analyses using the gene tree distributions obtained with GGI as input also support the null morphological tree. In summary, our analyses using genome-wide data in combination with the new methodological approach reconcile a long history of molecular and morphological conflict, supporting the monophyly of Characiformes and a single evolutionary origin of electroreceptive sensory organs within Otophysi, conditions shared only by catfishes and knifefishes.



#### Afro-Amazonian ichthyological comparisons fostered by Dr Richard Peter Vari

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My colleague and close friend the late Richard ('Rich') Vari conducted ground-breaking systematic research on tropical fishes of South America and Africa; and benevolently supported the studies of many others, including my own. Using his curatorial position in the Smithsonian Institution (USNM), Rich enabled me to collect fish specimens and complete fieldwork (1979-1994) in remote West-Central African drainages of Nigeria, Niger, Cameroon and Gabon. From prior experience, I anticipated finding only a few species in a survey of Korup Rainforest National Park, Cameroon (1988). Surprisingly, 140 were discovered in 61 genera, 24 families and 11 orders. Several were new to science, including a remarkable species of Polypterus a paleoniscoid, since described as P. teugelsi. From this, Rich encouraged my explorations of the Cross River drainage of Korup, found to contain more species than known for the far larger Nile and Zambesi rivers, respectively. Further discussions with Rich prompted an outline (1996) ichthyogeographical re-evaluation of the entire Guinea-Congo region of West-Central Africa (>1,400 fish species?). I hypothesised pre-Miocene trans-continental and inter-continental relationships, including with the Amazon River Basin (>2,400 fish species); and contrasted this with traditional Neogene (11 – 0.005 ma) scenarios of taxa dispersing from centres of origin. Prior to continental separation (ca 136 ma) a massive Afro-Brazilian (freshwater?) lake existed; which may correlate with the phyletic origin of some taxa. The last geological contact between coastal Cameroonian hydrography and an emerging Brazilian Amazon may have occurred in the Upper Cretaceous ca 60 ma, accepting a rebounding tectonic conjunction. The presentday shared occurrence of closely comparable aquatic ecosystems and habitats (some marine or estuarine) must somehow reflect prehistory. There are postulated evolutionary parallelisms in freshwater fish morphology, physiology and ecology - as seen in rheophilic taxa and those with mildly electrogenic organs. Eight or more higher-level recent taxa are shared: rajiforms, synbranchiforms, cyprinodontiforms, dipnoans, osteoglossiforms, characiforms, siluriforms and cichlids. Some taxa might be discounted as biogeographically uninformative, e.g. in the case of 'peripheral' euryhaline groups or where monophyly is not established. Complicating matters, numerous new species and other taxa (both recent and fossil) are recognised every year in both continents through original descriptions and resurrections from synonymy. Certainly, to determine unique Afro-Amazonian systematic and phylogeographical relationships there is a need for in-depth cladistic analyses of candidate taxa at successive levels of generality; and to correlate results with ancient patterns of hydrographical (or eco-physiological?) fragmentation or coalescence. These data will be invaluable for other biological research and vital to the assessment and conservation of fish diversity threatened by identical factors on both sides of the Atlantic. Such substantial and ongoing scientific opportunities constitute only a small part of the precious ichthyological legacy bequeathed to us by Richard Vari. We all thank him for this!

Key words: Ichthyogeography; Freshwater; Estuarine; Guinea-Congo; Neotropics Financial support: USNM Research Fellowship, travel grants and equipment 1979-1994



### Catalog of the species of Characiformes (Teleostei: Ostariophysi)

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A list of all valid names and available synonyms of fishes of the Order Characiformes was organized as part of the project "South American Characiformes Inventory" (SACI). It represents the result of a joint effort of 43 specialists, mostly from Brazil, a few from Belgium, and Richard Vari from the United States. The starting point for preparing this catalog was the online version of the Catalog of Fishes by Eschmeyer, Fricke, van der Lagn, from which a list of nominal species and genera and associated taxonomic information was extracted. Specialists were in charge of checking, correcting and providing additional information on species, genus and/or family group names. In order to accomplish the task of putting together a vast amount of information provided by such large number of experts, various computer programs were developed by one of us (EGB), at different stages throughout the process, that allowed us to check, compare, split or combine information. In addition, a website was created to help the editors to validate and organize the generated data. The list is comprehensive through July 2017 and includes 2151 valid species distributed among 293 genera and 17 families. Almost 90% of the diversity of recent species occurs in the Neotropical region (1889 species), 240 species are found in the African continent, and 22 species are represented by fossils. In addition to providing an up-to-date listing of taxa together, we aimed to organize as much as possible, all relevant information that would help either to clarify or explain taxonomic uncertainties or conflicting views at the species-group, genus group and family group levels. Geographic distributions of valid species are expressed in terms of their occurrence in the freshwater ecoregions of the world delineated by Abell et al. In our view this should facilitate comparisons of distribution patterns of species by future workers. Although initially aimed at producing a printed version of the cataloa, those computer macros, programs and website could be also used as a template to produce checklists of other taxa. In addition, they could be further developed and made more widely available in order to allow for continuous update of taxonomic information, not only for characiforms but for the entire Neotropical freshwater ichthyofauna.

Key words: Checklist, Freshwater fishes, Neotropical, Geographic distribution Financial support: FAPESP (process 2011/50282-7)



### A complete morphological supermatrix for the superfamily Anostomoidea (Teleostei: Characiformes). What can it tell us about history?

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The freshwater superfamily Anostomoidea is endemic to the Neotropical realm, and is very diverse with nearly 300 species unequally assigned to 27 genera in four families. Decades of study at the familial and generic levels have resulted in numerous monographs, and, more germane to the present study, a vast number of morphological character-state descriptions for various focal ingroup. From these publications 463 anatomical characters were culled, compiled, with character-states revised or added to, if necessary, in order to account for all observations in the species examined. Each character was then scored for 222 terminals via direct specimen examination. The resultant completed supermatrix was analyzed using parsimony and Bayesian inferences in order to propose a hypothesis of phylogenetic relationships based solely on morphological characters. We find that each family is strongly supported as monophyletic with the following pattern of relationships: ((Anostomidae, Chilodontidae), (Prochilodontidae, Curimatidae)). Within the Chilodontidae both Caenotropus and Chilodus are recovered as monophyletic. In Anostomidae those genera where more than one species is recognized and investigated herein Leporellus, Hypomasticus, Rhytiodus and Schizodon are recovered as monophyletic, while Leporinus, Anostomoides, Laemolyta, Pseudanos, Anostomus and Petulanos are not. Reasons for this pattern in Anostomoides, Laemolyta, Pseudanos, Anostomus and Petulanos are investigated. Within the Prochilodontidae Ichthyoelephas, Prochilodus, and Semaprochilodus are each monophyletic with Ichthyoelephas recovered as sister to Semaprochilodus. In the Curimatidae the genera Curimatopsis, Potamorhina, Curimata, Psectrogaster, and Steindachnerina are recovered as monophyletic while Cyphocharax, Curimatella, and Pseudocurimata are not. Characters uniting each family and genus are enumerated and compared with their previous treatments in the literature.

Key Words: Phylogeny, Systematics, Characters, Synthesis Financial Support: NSF Grant DEB-1257898 and a generous travel award from the Smithsonian Institution



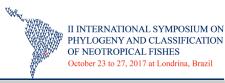
#### The tempo and mode of lineage and morphological diversification in the freshwater fish order Characiformes

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The freshwater fish lineage, Characiformes, is one of the most speciose and ecomorphological diverse radiations of freshwater fishes exhibiting a multitude of divergent body shapes, but the macroevolutionary processes responsible for the biodiversity remain largely unexplored. Here, I reconstruct the pattern of diversification in this major radiation using a new time-calibrated molecular phylogeny, trophic ecology database, geometric and linear morphometric dataset, and phylogenetic comparative methods. I found that body shape disparity is significantly higher early in cladogenesis, quickly reducing as the lineages fill. Shifts in trophic ecology drive some aspects of body shape diversification, and in particular the evolution of piscivory drives strong convergence towards elongate bodies and large heads. However, a model of early body shape diversification fits substantially better than a model based on different adaptive peaks for each trophic ecology, suggesting that other ecological variables may drive much of the body shape diversification. I found that characiform body shape is represented by three independent modules: cranial, precaudal, and caudal modules. The evolutionary dynamics differ substantially between modules; most of the precaudal variation evolved very early in cladogenesis, while in contrast, caudal shape shows a relatively recent explosion of diversity. Rates of morphological evolution vary significantly across clades, with the precaudal region of the body evolving at least five times faster than either the head or caudal region for all clades. However, there are no significant differences in the evolutionary rate across trophic groups, indicating that other ecological variables may drive the shifts in rate between clades. Lineage diversification is faster early in cladogenesis, with the rate of speciation slowing down as lineages fill, following the pattern of disparity observed in body shape. The evolution of Characiformes exhibits many signatures of an adaptive radiation, but without a clear ecological correlative, the exact mechanisms and processes responsible for diversification remain unknown.

Key words: Evolution, Morphology, Neotropical, Comparative phylogenetics Financial support: NSF grant DEB – 1257898, OSU Provost Fellowship, Smithsonian Graduate Fellowship and ASIH Raney Award



# Species delimitation analyses in *Potamorhina* (Characiformes: Curimatidae) based on molecular data

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Curimatidae is the fourth largest family in Characiformes containing about 112 species widely distributed from Costa Rica to Argentina. These species are allocated in eight genera, including Potamorhina Cope, 1878, which has five species: P. altamazonica from Amazon and Orinoco basins, P. laticeps from Lago Maracaibo, P. latior and P. pristigaster from Amazon basin and P. squamoralevis from Paraná-Paraguay basin. They perform long-distance migrations and represent an important food source for local population and for regional fisheries. The taxonomic revision along with interspecific relationships within Potamorhina through morphological data provided diagnoses for each species and details of their distribution. However, the genetic diversity of populations from distant regions covering the wide distribution range of the genus still remains to be studied. The main objective of this study is to identify and delimit the species of Potamorhina using genetic data as part of a series of studies dealing with the molecular identification of Curimatidae. We used 21 specimens spanning the five valid species of Potamorhina attempting to cover all their entire geographic range, and using Psectrogaster to root the trees. We sequenced partial sequences of the mitochondrial gene cytochrome oxidade C subunit I and analyzed them by genetic distance, maximum likelihood and Bayesian methods. We also used species delimitation analyses through General Mixed Yule Coalescent (GMYC) and Bayesian Poisson Tree Processes models. The generated matrix included 621 bp and did not present any nucleotide saturation signal. The lowest genetic distance appears between P. sauamoralevis and P. altamazonica (0.015±0.007) and the highest genetic distance appears between P. latior and P. pristigaster (0.114±0.034). Our results reveal well-defined clusters with long branches distinguishing the five species of Potamorhina with high bootstrap values (>99%) for each cluster, with the exception of the GMYC analysis that recognized P. altamazonica and P. squamoralevis as only one species. The molecular results did not find new or cryptic species, even when we included samples from distant regions of the Amazon basin, as in the case of P. altamazonica, P. latior and P. pristigaster. These results suggest that the previously reported migration behavior associated with reproduction and feeding of the species of Potamorhina have influenced the gene flow across populations separated over a thousand kilometers across the Amazon basin (e.g., P. latior from Rio Branco and Rio Solimões). One interesting result is related to the position of P. pristigaster as genetically closer to P. laticeps suggesting a putative sister relationship among them. They share the presence of a distinct dark blotch on the caudal peduncle that is unique amongst Potamorhina species. The phylogenetic relationship among them should be tested in future studies.

Key words: Freshwater; Neotropical; Systematics; Taxonomy Financial support: FAPESP, CNPq



### Dr. Richard P. Vari in Venezuela, his contributions to the knowledge of freshwater fishes

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From the late 1970's to the late 1980's, many relevant events occurred in the Laboratory of Ichthyology, at the IZT, Faculty of Science, UCV. Several American scientists, including John G. Lundberg, Jonathan Baskin, Tomas Zaret, Richard P. Vari, Carl J. Ferraris Jr., Barry Chernoff and Scott A. Schaefer, together with laboratory staff, carried out important collecting expeditions, mainly in the Orinoco River basin. Also, they analyzed, cataloged and deposited specimens in the Fish Collection of the MBUCV. Among the colleagues who augmented the knowledge of Venezuela's freshwater fishes during this period, Dr. Richard P. Vari was undoubtedly among the most important. His contributions were mainly focused on Characiformes with emphasis on the families Curimatidae and Anostomidae, and on the genus Creagrutus. In addition, he moved into the Siluriformes by describing new species in the families Auchenipteridae and Cetopsidae. Recently published articles involving Dr. Vari have described new species of the siluriform family Pseudopimelodidae, and in Apteronotidae (Gymnotiformes). His studies on freshwater fishes in Venezuela include 35 species described, in addition to other species cited for the first time for Venezuela. His impact and influence exceeds his individual or group research projects, because he always provided support in literature, specimens at the USNM, and instruction and advice to other researchers, among which I have the privilege to belong.

Key words: Systematics; Taxonomy; Diversity



### What is new in old fish types?

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Ancient (aDNA), also known as historical DNA, is DNA isolated from ancient samples as subfossils, mummies, or museum specimens. The use of ancient DNA in archived specimens helps to resolve queries such as the evolutionary relationships between species, the rescue of extinct populations, and historical taxonomic problems. This new technique reinvents the biological collections, giving new purposes to the museum specimens. Despite the increasing use of the new generation sequencing, the traditional methodologies like Sanger are still an accessible option for aDNA. This contribution reports the experience of extracting and amplifying DNA of 53 type specimens of the Characidae, stored in museums around the word. Two extraction kits (First DNA of Gen-ial and Qiamp micro kit of Qiagen, silica column based) and two facilities, regular and isolated, were tested in the extraction and PCRs processes. The samples yielded a mean of 120 ng/ul of DNA in the extractions and no correlation between amount of DNA and tissue age post fixation was observed. The amount and quality of the extracted DNA seems to be more related with the conservation history and conditions to which the specimens were exposed than to sample age. Both DNA extraction kits worked in extracting DNA, but only DNA extracted with Qiamp micro kit of Qiagen had the sequencing satisfactory. All DNA processed in regular molecular lab facilities were contaminated with amplicons and with positive controls in DNA extraction or amplification process. On the other hand, all DNA processed in isolated spaces (extraction and PCR) worked very well without contamination. So far, 14 samples were amplified, and nine of them generated viable sequences. Even very small samples generated viable DNA sequences, if handled under controlled conditions. The use of silica columns in DNA extraction is better than other methodologies, once it eliminates some impurities as PCR and sequencing inhibitors. Our findings provide good support for the use of short and highly variable regions in the identification of ancient samples.

Keywords: Characidae; Genetype; Sanger methodology; Neotropical fish Financial support: CNPq scholarships PCS (process 141407/2013-1; 406776/2017-2) and LRM (307890/2016-3)



#### Ocean Exploration, Morphological and Molecular Approaches Contribute to Discoveries of Unrecognized Diversity of Tonguefishes (Pleuronectiformes: Cynoglossidae)

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Members of the family Cynoglossidae are small to medium-sized, sinistral flatfishes found worldwide in temperate and tropical seas. The majority of tonguefishes are small-sized species usually reaching adult sizes under 30 cm SL. Tonguefishes are found in a diversity of marine and estuarine habitats ranging from subtidal estuarine waters to deepwater habitats on the outer continental shelf and upper continental slope to about 1500 meters. A small number of species enter freshwater. With 164 valid species recognized in three genera (Cynoglossus, Paraplagusia and Symphurus), the Cynoglossidae is one of the most diverse of the 14 families of flatfishes (Pleuronectiformes). Among the genera, Symphurus is the most diverse (96 species), followed by Cynoglossus (60 species) and Paraplagusia (7 species). During the past 30 years, the recognized species diversity of tonguefishes has increased steadily with new species being discovered in a variety of marine habitats including those in shallow, tropical seas to deepwater habitats at hydrothermal vents. At present, 26 species representing 15.9% of the standing diversity of the family await description. Among these 26 species are 17 nominal species of Symphurus, eight species of Cynoglossus, and at least one species of Paraplagusia. Increases in diversity within this family are being discovered in specimens collected in recent ocean exploration, through re-examination of historical collections of specimens, and in the identification of species complexes and cryptic species using molecular and morphological techniques. Several representative examples of species discovery within each genus are discussed.

Key Words: Species discovery; Flatfishes; Tongue Soles; Biodiversity



### Richard Peter Vari's crucial role in building the Laboratório de Ictiologia de Ribeirão Preto (LIRP), FFCLRP, Universidade de São Paulo (USP), Ribeirão Preto, Brasil

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In 1999, the Laboratório de Ictiologia de Ribeirão Preto (LIRP) was officially inaugurated by the University of São Paulo (USP), with a 260 m<sup>2</sup> area exclusively dedicated to its fish holdings. Of its current fish holdings, 4% are from marine and 96% from freshwater habitats, and most of the lots are from Brazil, including the Upper Rio Paraná, Rio Tapajós and Rio Madeira drainages. It presently contains 15,083 lots and 159,822 specimens, in addition to 33 holotypes, 128 paratypes, 74 tissue samples, 405 C&S specimens, and 42 dry skeletons, almost all from Neotropical Region freshwater habitats. During its approximately 20 years of official existence, many students acquired ichthyological training at LIRP and its fish collection, at least 10 of which now occupying teaching and research positions in major Brazilian public universities. All this, I strongly believe, would not have come to pass without the unflagging and extremely generous support of Richard P. Vari (Smithsonian Institution), from 1986 almost to the time of its demise, in January 2016. LIRP's collection holdings started to be gathered in 1982, with my hiring by the Departamento de Biologia at the University of São Paulo (USP) Ribeirão Preto, SP, Campus. In 2002 Fávio Alicino Bockmann was hired, also becoming a LIRP member and Curator. From 1986 to 1987, I was a Predoctoral Fellow in the Division of Fishes of the National Museum of Natural History, Smithsonian Institution, Washington, D.C., under the advisorship of Richard P. Vari, which I have met some years before, during a visit to the Museu de Zoologia da University of São Paulo (MZUSP) fish collection. That was the beginning of a prolific and long-lasting collaboration between LIRP and the Smithsonian Institution, mostly through our professional relationship, at first, and friendship later on. Suffice to say that between that momentous first encounter in the early 1980's, almost to his demise in 2016, thanks to Richard Vari's efforts and generosity, LIRP received a great and varied quantity of ichthyological collecting and literary material, had six (06) fish collecting joint LIRP/SI expeditions financed (in a total of approximately US\$ 30,000.00) and had at least 10 of its post-graduate Students received at the Division of Fishes of the National Museum of Natural History of the Smithsonian Institution, USA. In a more personal level - although deeply affecting LIRP -, since my predoctoral fellowship under Richard Vari, through him I received a total of approximately US\$ 16,000.00 to finance five (05) research visits to the Division of Fishes of the Smithsonian Institution, totaling nine (09) months of residence in the USA, six (06) of them generously hosted in his own house with his family. This collaboration resulted in the co-authorship of about 10 ichthyological papers - one of them an almost 100 pages long phylogenetic and revisionary study of the Prochilodontidae - and the description of three new fish species. But above all, these almost 30 years of professional collaboration resulted in one of the most important, instructive, inspirational, emotionally satisfying and missed friendships of my life.



### Richard Vari's impact on the development of Colombian Ichthyology

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Colombia is recognized as one of the richest countries worldwide in freshwater fish diversity, in fact based on the available data, Colombia is in second place after Brazil in number of species, currently with 1495 species distributed in five main hydrographic regions (Amazon, Orinoco, Magdalena-Cauca, Pacific and Caribbean). This remarkable Colombian biota has long interested naturalists and scientists and we can track back the history of their study to the first formal scientific descriptions at the beginning of the nineteenth century. Humboldt, Bonpland, Wallace, Steindachner, Regan, Eigenmann, Henn, Wilson, Posada Arango, Fowler, Miles, Dahl, Cala-Cala, are authors that were crucial to the process of documenting Colombian freshwater fishes up to the 70-80's. But it wasn't until the 90's that a new wave of young Colombian ichthyologists started to develop, mainly in association with the fish collection at the Instituto de Ciencias Naturales, Universidad Nacional de Colombia (ICNMHN). During that decade, several of the revisionary studies by Richard Vari published in Smithsonian Contributions to Zoology appeared, and those studies were one of the main sources of reference that facilitated work at the collection, after Rich, with his typical generosity, sent several boxes of those volumes at a time when internet connectivity was in infancy in Colombia. Between 2000 - 2005 as result of his work with some colleagues (one of them Colombian) on Creagrutus and Cetopsidae, Richard published five new species from Colombia and in 2008 was coauthor of the Checklist of the freshwater fishes of Colombia, a paper that highlighted the importance of the Colombian ichthyofauna in the Global and Neotropical context, and addressed important issues in terms of conservation challenges and the need to improve the sampling effort across all continental aquatic systems in Colombia. After almost a decade since that checklist was published, the challenges and tasks pointed out by Richard and coauthors have been attained in several ways. But finally, although Richard's work and relationships in Colombia were not as intensive as in countries as Brazil, Venezuela and Peru, he always was open to discuss ideas, exchange and share information, and to welcome visitors to the fish collection at the National Museum in Washington. These efforts helped launch the careers of several young Neotropical ichthyologists including some Colombians that now are the future of Colombian Ichthyology. For his enormous contribution to Neotropical and Colombian Ichthyology, in 2015 Richard Vari was honored with the ''Golden Fish Award'' from La Asociación Colombiana de Ictiólogos (ACICTIOS) and he will always be remembered for his personal kindness and willing collaboration.

Financial support: Vari's Legacy session (International Symposium on the Phylogeny and Classification of Neotropical Fishes), The Field Museum, Facultad de Ciencias/Pontificia Universidad Javeriana



# Vari's electrifying heritage: the impact on the systematics of the Gymnotiformes

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Rich's journey into the systematics of Gymnotiformes began in 2004, and his legacy will live for decades to come through his collaborators, papers and ongoing projects. His contributions deeply affected the manner in which scientists think and write about Gymnotiformes. His distinct approach to resolving complex taxonomic and phylogenetic issues through comprehensive monographs quickly revealed the forgotten world of the Neotropical electric fishes. Those studies uncovered hidden species-level diversity (e.g., 32+ species of Sternarchorhynchus), unmasked poorly or unexplored anatomical characters (e.g., the caudal skeleton in Electrophorus) and discovered evolutionary patterns known to most of vertebrates but unknown in Gymnotiformes until that moment (e.g., adaptive radiation in Sternarchorhynchus). From 2009 to 2017, Rich and collaborators published 9 papers with 300+ pages describing 34 new species, thereby advancing major hypotheses about the relationships and classification of Neotropical electric fishes. As a researcher, Rich always saw the big picture, keeping one eye focused in the present and another towards the future. Thus, in his later years he strongly encouraged his collaborators and students to incorporate molecules to their research program. The rationale behind his position was to prepare young professionals and students for a worldwide change in the systematics of fishes. Examples of his personal involvement with and support of molecular projects include the genome-wide phylogeny of Gymnotiformes and the phylogeography, molecular taxonomy and phylogeny of the electric eel. He also greatly appreciated and continuously supported ambitious professional endeavors. In doing so, Rich kept working during the last months of his life together with John Kress (NMNH), Naércio Menezes (MZUSP), Carlos Brito (FAPESP) and me to make the thematic project on the inventory and evolution of Gymnotiformes possible. The proposal recently funded through the cooperation of FAPESP and the Smithsonian Institution was his last professional achievement. Among other things, the project will provide a training ground for a new generation of ichthyologists using an integrated methodology in the study of biodiversity. As collaborator and supervisor, Rich was professional, goal-directed, loyal and kind. From weekly chocolate croissants to daily early morning chats on numerous subjects, Rich created the perfect atmosphere in which to work, and always made one feel at home. As for me, I consider myself privileged for having spent 11 years of my life working and learning from an exceptional human being and one of the most influential ichthyoloaists from our time.

Key words: Richard Peter Vari; Neotropical electric fishes; New species; Taxonomy; Phylogeny Financial support: Smithsonian Institution



# A new species of Astyanax Baird & Girard (Characiformes: Characidae) from the rio de Contas basin, Bahia, Brazil

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A new species of Astyanax from the rio de Contas basin, Bahia, Brazil is presented. Astyanax sp. n. can be distinguished from all Brazilian congeners by the presence of small bony processes on all fins of mature males (vs. complete absence of processes on fins or at least absent on dorsal and caudal fins, with the exception of A. hamatilis) and all ossifications of the infraorbital series somewhat reduced, leaving a relatively broad area without superficial bones between borders of ossifications and preopercle (vs. infraorbitals reaching vertical and horizontal limbs of preopercle). The new species can be readily distinguished from A. hamatilis, a species endemic to the rio Paraguaçu drainage, by its conspicuous black and wide midlateral stripe, extending from the rear of the humeral blotch to the end of middle caudal fin rays, not forming a distinct elongated blotch on caudal peduncle (vs. absence of lateral stripe or short stripe and presence of a conspicuous teardrop shaped caudal blotch). Bony processes on all fins of males are reported only for other seven species of Astyanax, known from rivers of Argentina and/or Uruguay: A. chico, A. hermosus, A. ojiara, A. pynandi, A. rutilus, A. stenohalinus, and A. troya. Astyanax sp. n. differs from these species, and from most congeners, by having only three horizontal series of scales from lateral line to pelvic-fin origin (vs. four or more). The new species further differs from congeners of the Astyanax scabripinnis species complex sensu Bertaco & Lucena by having the greatest body depth just anterior to the dorsal-fin origin (vs. body deepest on a vertical around middle or posterior portion of pectoral fin), from congeners traditionally included in the A. bimaculatus species group sensu Garutti by the presence of a vertically elongated conspicuous dark humeral blotch (vs. presence of horizontally elongate black humeral blotch), and from congeners traditionally included in the A. fasciatus species group sensu Melo & Buckup by its low number of branched anal-fin rays (17-20 vs. more than 20) and by having 2-4, usually 3, maxillary teeth (vs. one tooth). The new species represents the second species of the genus known from Brazilian drainages to have bony hooks on all fins and, apparently, does not fit into any "species complex of Astyanax".

Key words: Taxonomy, Northeastern Brazilian drainages Financial support: CNPq



#### A new species of Creagrutus Günther (Characidae: Stevardiinae) from the rio Jamari basin, middle rio Madeira, State of Rondônia, Brazil

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Creagrutus Günther includes stevardiine species with short mandible, premaxilla usually with a cluster of three teeth near symphysis, an oblique primary series of 5-7 teeth and a single tooth anterolaterally, and rectangular fourth infraorbital, participating of the posterior margin of the infraorbital series. Recent collections in the rio Jamari basin, middle rio Madeira, yielded specimens of a new species of Creagrutus. Meristic and morphometric data of 24 specimens of the new species were taken as in Vari & Harold and compared to data from other species taken in the same study. The new species is distinguished from all congeners, except C. beni Eigenmann, by the following combination of characters: humeral spot parenthesisshaped, concave anteriorly, its width homogeneous throughout, extending from one scale ventrally to dorsal midline of body to one scale dorsally to base of last pectoral-fin ray, with no conspicuously darker portion; presence of a light vertical area, about 1.5 scale wide, almost completely devoid of melanophores, along entire anterior and posterior margins of humeral spot; absence of a series of dark spots along middle of flank; presence of a spot of dark pigmentation on the proximal third of median caudal-fin rays, either connected to or isolated from the midlateral stripe, but never distinctly darker or deeper than the midlateral stripe; absence of a band of dusky pigmentation along distal caudal-fin margin; absence of a black spot on dorsal fin; absence of an intense black pigmentation all over body and fins; dorsal fin origin slightly in advance of pelvic-fin origin; premaxillary dentition typical for the genus, in three "series", always with 5 teeth in the primary series, without a distinct gap between first and second teeth; 2-4 maxillary teeth; 2, rarely 3 post-anal scales; absence of a lamellar process in the pore of each lateral-line scale; 36–38 lateral-line scales; 4 scale rows above lateral line; 3 scale rows below lateral line; 8–9 pre-dorsal scales; 9–11 branched anal-fin rays; third infraorbital fully in contact with horizontal arm of preopercle. From C. beni, the new species is distinguished by the presence of chevron-like marks along the middle of the flank (vs. absence); shorter post-orbital length, 34.1–41.5 (vs. 44.6–50.1% HL); 36–38 lateral-line scales (vs. 38–42); and 5 teeth in the primary premaxillary series (vs. usually 6, rarely 5 teeth).

Key words: Neotropical region; Taxonomy Financial support: CNPq (scholarship), Capes (scholarship)



### Integrative approach reveals a new species of Nematocharax (Characiformes: Characidae) from Upper Contas River in the Chapada Diamantina, Brazil

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Distinct species concepts consider different criteria to delimit species, which may produce conflicting species boundaries among recognized lineages. In groups where speciation is recent, defining species limits is not a trivial task due to limited time to accumulate morphological, genetic, and/or ecological differences. Therefore, an integrative taxonomic approach allows combination of several lines of evidence, which result in more robust hypotheses for the existence of different species. This is particularly relevant when dealing with taxonomically controversial groups, as the fish of the genus Nematocharax. This is a putatively monotypic genus in family the Characidae, represented by N. venustus Weitzman, Menezes & Britski, described based on specimens from Jequitinhonha River basin, Minas Gerais State, Brazil. Currently, N. venustus is also known from other coastal drainages of the Northeastern Atlantic Forest ecoregion, including Pardo, Una, Cachoeira, Almada, and Contas river basins. A second species, N. costai Bragança, Barbosa & Mattos, was described from a small tributary of the Lower Contas River, Bahia. However, as morphological differences between the two species were not consistent, N. costai was considered a junior synonym of N. venustus. More recently, analyses with cytogenetic markers, DNA barcoding, and geometric morphometrics supported the presence of distinct evolutionary units in Nematocharax, with the population from the Upper Contas River the most divergent. Morphological analysis also revealed various diagnostic characters for that population. Thus, by integrating morphological and molecular data the population from a headwater of Upper Contas River in Chapada Diamantina, Bahia, Brazil is proposed herein to be a new species. Morphologically, the new species can be readily distinguished from its unique congener, N. venustus, by distinctive sexually dimorphic traits including pelvic fins of maturing and mature males not elongated and without filamentous rays, shorter dorsal-fin height, and shorter anal-fin lobe length. The new species is also distinguished from the congener by its lower body depth, lower number of maxillary teeth (at least in females), and presence of a faint concentration of melanophores along midline of the caudal peduncle, more evident on proximal portion of middle caudal-fin rays, and not forming a dark blotch on the caudal peduncle. As for the molecular data, we used a multilocus approach, which includes fragments of three mitochondrial (16S rRNA, COI, and Cyt b) and three nuclear (histone H3, rhodopsin, and a-tropomyosin) genes. The multispecies coalescent species tree by \*BEAST based on the combined data set (mtDNA and nDNA) strongly supported a basal split between two clades (Posterior Probability = 1), one comprising the new species, and the other including the lineages of N. venustus (considering all basins where it occurs). Thus, the presence of distinctive morphological, morphometric, molecular, and cytogenetic characteristics of the new species, as well as its disjunct and isolated geographic distribution, reinforces the idea that Nematocharax is not a monotypic genus. These results also highlight the importance of the Chapada Diamantina as a highly endemic and geologically complex region.

Key words: Coastal drainages; Integrative taxonomy; Neotropical fish; Species tree Financial support: CNPq (process 443249/2014-8), FAPESB (processes RED0045/2014 and JCB0026/2016), and FAPESP (process 2012/58050-0)



# Richard Vari's contributions to the checklist of freshwater fishes of Suriname

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In 2009, Richard Vari decided to join an effort to produce a checklist of the freshwater fishes of Suriname, mainly because he still had good feelings about the country due to his first tropical fieldwork in 1979 on the pristine Corantijn River in West Suriname. Richard was the most experienced ichthyologist in the team that in 2012 published the Suriname checklist in a special issue of Cybium on the 'Fishes of the Guianas'. The contribution of Richard Vari to the checklist was manifold. First, his 1982 report on the fishes of the Corantijn River still is the most comprehensive listing of the fishes of this large river. Secondly, in his field of expertise, the family Curimatidae, he was able to correct a longstanding misidentification of a curimatid in the collections of Naturalis (Amsterdam and Leiden) and Paramaribo and he described a recently discovered curimatid of the Marowijne River (Vari et al. 2012). But by far the most important contribution of Richard Vari to the checklist was his professional network of specialists in the many groups that together make up the freshwater fish fauna of Suriname: one telephone call or e-mail from Richard would speedily result in an answer of these busy specialists with important comments on the validity and occurrences of species in Suriname. In the last stage of the work, Richard also checked the language of the manuscript, as none of the other authors is a native English speaker. The checklist served as the backbone of a richly illustrated book on the freshwater fishes of Suriname and it still guides ichthyologists in their present research on the fishes of Suriname.

Keywords: freshwater fish fauna of Suriname



# Evolution of the jaw adductor muscles in neoteleostean fishes (Osteichthyes: Actinopterygii: Teleostei)

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The Neoteleostei is a megadiverse group of bony fishes that includes approximately 20,000 species, representing one third of all extant vertebrates. Comparative studies on the soft anatomy of neoteleosts are relatively scarce and the evolution of many anatomical systems within the group is poorly understood. Among the most notable of these systems is the adductor mandibulae, a muscle complex that is the main responsible for the mouth closing in anathostomes and exhibits from two to 13 subdivisions among neoteleosts. In the present study, the adductor mandibulae of representatives of all 41 neoteleostean orders is described, illustrated, and compared with a large amount of data gathered from the literature. We elucidate the homologies of the muscle subdivisions across the Neoteleostei, applying a standardized homology-driven terminology and presenting an extensive synonymy for the nomenclatures employed in previous studies. This achievement allowed the proposition of several new phylogenetic inferences for major neoteleostean groups. Most of these inferences are notably more congruent with previous hypotheses based mainly on osteology than those based on molecular data. Monophyly of the traditional Neoteleostei (i.e., including Stomiiformes and excluding protacanthopterygians) is supported. New synapomorphies are proposed for the Eurypterygia, Batrachoidiformes, Ophidiiformes, and Scombriformes (including Sphyraenidae). Data from the adductor mandibulae is the first morphological evidence corroborating the monophyly of the Anabantaria, a group previously recovered only in molecular phylogenies. Several newly discovered characters are informative for the internal relationships of the Blenniiformes, Gobiiformes, Scombriformes, and Stromateiformes. Myological specializations also support the existence of clades composed by the Gadiformes + Percopsiformes; Callionymoidei + Gobiesocoidei; and Mugiliformes + Atherinomorphae.

#### Keywords: Morphology, Musculature, Phylogenetics

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# Molecular identification of species of *Curimata* (Characiformes: Curimatidae) through DNA Barcoding

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The family Curimatidae contains about 110 species widely distributed from Costa Rica to Argentina. These species are allocated in eight extant genera, including the thirteen species of Curimata Bosc that occurs in both sides of Andes including the Magdalena, Orinoco, Amazon, and Parnaíba basins as well as in the Guianas region. The various Curimata species are important components of the fish biomass in many aquatic ecosystems, and are important in subsistence fisheries. However, no genetic study has been applied to test previous systematic hypothesis of species composition within the genus neither an assessment of the overall genetic diversity of putative lineages possibly existing throughout the Amazon basin and adjacent drainages have been studied. The main objective of this study is to identify and delimit the species of Curimata by genetic data as part of a series of studies dealing with the molecular identification of Curimatidae. In our study, we approached 47 specimens spanning nine out of 13 species of Curimata: C. acutirostris from the Araguaia basin, C. cyprinoides from the Orinoco, Atlantic drainages of the Guianas, Rio Amazonas and Rio Tocantins, C. inornata from the Amazon basin, C. knerii from the Amazon basin, C. mivartii from Magdalena basin, C. ocelatta from the Amazon basin, and C. roseni from the Rio Negro, C. vittata from the Amazon basin and upper Rio Orinoco. We used Potamorhina altamazonica to root the trees. We applied the DNA Barcoding methodology by sequencing partial sequences of the mitochondrial gene cytochrome oxidase C subunit I and analyzed the generated sequences by genetic distance, maximum likelihood and Bayesian methods. We also used species delimitation analyses through General Mixed Yule Coalescent (GMYC) and Bayesian Poisson Tree Processes model. The preliminary species delimitation analyses delimited 11 welldefined clusters representing species. The hidden diversity of which our results found splits of genetic divergence appeared within C. vittata (Ventuari and Tapajós rivers), C. roseni (Nanay and Ventuari rivers) and evidence for absence of genetic divergence appeared within C. inornata/C. knerii and C. cyprinoides/C. roseni. Our results also show that C. cyprinoides may represent a species complex with little or lack of genetic divergence. Overall, these results, in combination with morphological evidence, will provide useful tools that serve as basis for taxonomic and systematic understanding of species boundaries within Curimata.

Key words: Amazon, biodiversity, species delimitation Financial support: FAPESP, CNPq



### A new species of *Eretmobrycon* (Characiformes: Characidae) from the Dagua river basin in Valle del Cauca, Colombia

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*Eretmobrycon varii* n. sp., is described from stream tributaries of lower río Dagua, Valle del Cauca, Buenaventura, Colombia. The new species is distinguished from its congeners by a combination of characters including anal-fin origin, bony hooks on the pectoral and dorsal-fin rays, color in life of the anal and pectoral-fin, head length, number of lateral line scales, predorsal distance, dorsal-fin hypural distance, number of humeral spots, caudal spot, prepectoral distance. This species is, to date, the single known fish endemic of Dagua river basin.

Key words: Characins; Dagua river; Endemic Species

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# The scientific legacy left by Richard P. Vari in Ecuadorian Ichthyological Research

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The orography of Ecuador is the main cause of its megadiversity. In an area of 250,000 km<sup>2</sup> it has 983 species of fish. The presence of the Andes and the different western and eastern ranges between which the watersheds of the Pacific and Atlantic slopes are born have caused the high diversity and endemism of the Ecuadorian ichthyofauna. The presence of such an eminent and renowned ichthyologist as Richard P. Vari has allowed scientists to know and explain the influence of geological events in taxonomic groups such as those he studied in family Curimatidae and specifically in the five species in the transAndean genus Pseudocurimata. The species currently recognized in Pseudocurimata are P. lineopunctata, P. boehlkei, P. troschelii (Günther, 1859), P. boulengeri, and P. peruana. Rich's work permitted establishing a hypothesis of intrageneric phylogenetic relationships that is congruent with a scheme of sequential north to south vicariance events along the western versant of the Andes in northwestern South America. The majority of the species in the genus have discrete allopatric distributions, with some degree of secondary, post-vicariance, dispersal evident in the rivers draining into the northern portions of the Gulf of Guayaguil in the southwest of Ecuador, and possibly in the Rio Santiago system of northwestern Ecuador. It is important to point out Vari's studies carried out in the other cis-Andean genera Curimata, Curimatella, Potamorhina, Psectrogaster, Pseudocurimata, Cyphocharax, Steindachnerina etc. whose presence in the Ecuadorian Amazon has biological and alimentary importance to Ecuadorian ethnic groups. Richard Vari also helped revise the genus Creagrutus, small and diverse characid with cisandine distribution in Ecuador. He and Anthony Harold diagnosed this monophyletic assemblage on the basis of synapomorphies in various osteological and soft anatomical systems. A total of 64 species are recognized in Creagrutus (including 37 new species), 56 of which occur east of the Andean Cordilleras and are reviewed in the paper. In Ecuador there are 8 species including two new species and 5 of these are endemic. The revision of the family Cetopsidae helped to examine additional population samples and also raised questions about the reported geographic distributions for many previously described species. Unraveling these intertwined questions necessitated both an in-depth species-level analysis of the Cetopsinae across its geographic range and an intrafamilial phylogenetic analysis of the Cetopsidae. This study focused on the first of those questions, that of the recognizable species within the Cetopsinae and their geographic distribution. The phylogenetic analysis is the subject of a future publication.

Key Word: Systematics; Taxonomy; Freshwater; Ecuador



### Richard Vari's contribution to ichthyology in Peru

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For Peruvian ichthyology, the influence of Dr. Richard P. Vari was very important and particularly evident from 1980 to 2001 by means of fieldwork in the lowland rivers of the Peruvian Amazon, publications, and the establishment of academic relationships that helped improve the knowledge of several colleagues. His legacy started while he was Rosen's student, conducting research at the American Museum of Natural History, and it continued as he became curator of the Fish Division at the National Museum of Natural History. In 1983 Richard and Susan Jewett went to Peru to collect fishes in Tambopata, Madre de Dios, and after this first successful experience Richard went back to Peru with Jerry Louton in 1984, to collect samples around Iguitos and Pucallpa in the Amazon basin, and in the Río Tumbes, next to Ecuador in the Pacific basin. From 1987 to 1992, lona-term fieldwork,= related to the BIOLAT program, allowed collaboration on the systematic collection of fishes in streams, small rivers and still waters in the Manu National Park. The sampling included dry and wet seasons in the span of seven expeditions. Several thousands of specimens were obtained and shared between Peru (MUSM fish collection) and the United States (USNM fish division) making the material available not only to Peruvian researchers, but to researchers from all over the world. This collaborative work led to the publication of a book: MANU / the Biodiversity of Southeastern Peru, which shows the rich fish fauna of the Manu region, with 210 fish species listed. Richard Vari was the author of taxonomic revisions, including new records, and many new species for Peruvian fishfauna; but. his first paper was the description of a new species of Cheirodon from the Ucayali drainage, in 1980. But the most important single experience was a visit (HO), to the Smithsonian in the spring of 1985, which led to the publication of the first checklist of freshwater fishes of Peru and the revision of the Helogenidae, both works published in 1986. Furthermore, Richard's patience and willingness to do things right may be illustrated by one remarkable paper: the description of the genus Attonitus. This single work had to wait 15 years to get enough specimens, yet the decision benefited the project allowing the description of three new endemic species. Richard Vari's influence in Peruvian ichthyology was more evident since the publication of the first checklist, as the MUSM collection began to be cited, and some young Peruvian students joined the ichthyology department to study freshwater fishes in new areas. These included students like Fonchii Chang (1992), Norma Salcedo (1996) and Max Hidalgo (1997), and recently: Vanessa Meza, Daniela Nuñez and Vanessa Correa. Richard Vari was a researcher that always had the time to reply to student's emails, offer advice, and give words of encouragement. He was not only generous with his time and resources, but also a significant role model for all the researchers that had the opportunity to get in contact with him. He is deeply missed.



#### Description and phylogenetic relationships of a new genus and species of stevardiine fish (Characiformes: Characidae) from Amazon river basin, Peru

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A new genus and species of stevardiine fish is described from the Amazon River basin, Peru. The new taxa are differentiated from the remaining characids by the following combination of characteristics: a terminal mouth, ii,8 dorsal-fin rays; i,7 pelvic-fin rays; presence of two rows of premaxillary teeth, with four teeth in the inner row; the anterior gill filaments of the first gill arch are not modified into a gill gland in adult males; presence of teeth along more than one-half the length of the dentigerous margin of the maxilla; a complete lateral line; presence of several series of modified scales on the lower caudal-fin lobe, which are slightly more pronounced in adult males than in females and form a pocket-like structure; presence of bony hooks on the lower caudal-fin lobe; a dark lateral stripe that extends from the first lateral line scale to the caudal peduncle; the distal portion of most anal-fin interradial membranes is intensely pigmented with dark chromatophores; and absence of a humeral spot. The phylogenetic relationships of the new taxa were examined using a morphological data matrix composed of 532 characters and 73 stevardiines (among other terminal taxa), which was analyzed in TNT 1.5. Characters were analyzed under equal and extended implied weights for comparative purpose, but the final phylogenetic hypothesis was based on the latter method. The results of this cladistic analysis revealed that the new taxa are closely related to a clade including Acrobrycon plus the Stevardiini. Twelve autapomorphies were found for the new genus, which are associated with pectoral girdle, anal and caudal fins, supraneural bones, and coloration pattern. The new genus is named in memoriam of Richard P. Vari.

Key words: Osteology; Cis-Andean fish; Cladistics; Neotropical region Financial support: CONICET, Smithsonian Institution (USNM)

# **Fish Collection**



## The fish collection of the Museu de Zoologia da Universidade Estadual de Londrina

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The Museu de Zoologia da Universidade Estadual de Londrina (MZUEL) was established in 1980 with main goal to disseminate Zoology to school students of Londrina and nearby towns. During the end of the 1980's and beginning of the 1990's, a large scientific project involving researchers of the Departamento de Biologia Animal e Vegetal aiming to study distinct aspects of the Biology of the organism of the Tibagi river basin was responsible to greatly enlarge the collections of the MZUEL, especially through fishes and herpetological specimens. In the end of the 1990's, a researcher with background on Fish Systematics (OAS) was hired to the same department, and led the MZUEL into new scientific projects focusing on fishes. In the same time, two technicians were hired to the MZUEL and helped collect, preserve and prepare specimens, including taxidermy specimens, as well as maintenance of the collections. In the last five years, two other researchers working on Fish Systematics (JLOB, FCJ) were recently hired to the same department and brought extra effort on collecting and cataloging fish specimens. Currently, MZUEL is still engaged in disseminating Zoology to local community, especially contributing in several local exhibits, and it is responsible to maintain the invertebrate and vertebrate collections used in undergraduate and graduate courses in the university. The fish collection of the MZUEL is by far the largest collection in the museum and it has grown exponentially in the last years. The number of catalogued lots grew from 867 in 1993 (when OAS was hired) to about 6.000 in 2012 (when JLOB and FCJ were hired) to a total of nearly 20.000 today. The data of the fish collection is managed using Specify v. 6.6, and is available online through SpeciesLink. MZUEL houses today about 230.000 specimens of fish, catalogued in 18.300 lots representing about 1.200 species. Most specimens are alcohol preserved, but at least 800 specimens are cleared and stained, and 150 were prepared as dry skeletons. 400 MZUEL lots are currently on loan. The most important sampled areas include the Tibagi and Paranapanema rivers (upper Paraná basin), the Miranda river (Pantanal, Paraguay basin), and the Uruguay river. Other smaller but important collections were made in rivers of northeast Brazil (especially in Ceará State), rivers in Acre state (Amazon basin), Juruena river and tributaries (Tapajós basin), Uatumã river (Amazon basin), Southern coastal rivers, and Jeauitinhonha and Contas rivers in eastern Brazil. Holotypes of Hypostomus multidens, Isbrueckerichthys calvus, Isbrueckerichthys saxicola, and Rhyacoglanis paranensis and paratypes of other 48 distinct species described since 2001 are housed in MZUEL. A website of the MZUEL is available at https://sites.google. <u>com/site/museudezoologia/</u>, and includes photographs of most type specimens.

Key words: Ichthyology, Vertebrates, Systematics, Taxonomy Financial support: CNPa, Fundação Araucária, Universidade Estadual de Londrina, CAPES



# Fish collection of the Universidade Federal do Rio Grande do Sul (UFRGS)

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The fish collection of the Universidade Federal do Rio Grande do Sul (UFRGS) traces its origin within the extinct Instituto de Ciências Naturais (ICN), in that time a department attached to the university. For this early period, little information about its operation can be recovered, but the first fish specimens cataloged in the present collection indeed came from a single multi-taxa collection of the ICN. In 1978, the few fishes of that collection were transferred to the Department of Zoology under the care of two ichthyologists: Leda F. A. Jardim and Paulo A. Buckup (up to 1982). One year later, Luiz R. Malabarba joined to these researchers as curator, a position that maintains until today. The collection participated in the Neodat database project and was fully computerized in 1994 with the Muse software. The year 2001 brought several changes to the Ichthyology Laboratory and to the fish collection, which were transferred from the Campus Central near downtown to the Campus do Vale, with a properly designed room. Several field expeditions along the last two decades and the implementation of the software Specify early this century contributed to a considerable increase in the number of lots. In 1997, during the first Symposium on Neotropical Fishes, the collection had 4,694 lots and nowadays has 23,687 lots holding more than 256,000 specimens and 1,884 species, an increase of nearly 1,000 lots per year. In addition, with the advance of DNA in fish research the collection started to preserve sample tissues associated to their respective vouchers. Currently, the tissue collection has 7,698 lots and more than 34,000 specimens with tissue samples, mostly of Neotropical freshwater fishes and some marine specimens, mainly from the Brazilian coast. Fishes from all Brazilian States and 20 other countries are cataloged in the collection, and all data are available in the SpeciesLink web page since 2012. The collection holds 455 lots with cleared and stained specimens and 315 with dry skeletons. The type material includes 28 holotypes and 407 paratypes, all from the Neotropical region and mainly described from southern Brazil. Part of the lots is borrowed to researchers from several Brazilian institutions and other countries as well as donations of tissue samples are provided and usual. Nowadays, the software Specify version 6 is implemented to manage the fish collection of UFRGS and the ichthyologist Juliana M. Wingert acts as the collection manager.

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# INPA Fish Type Collection - source of information on history and diversity of Amazon fishes

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INPA Fish Collection, despite its short existence (32 years), represents and comprises the largest fish collection in the Amazon and probably the second in the country housing Amazon fishes. The rich assembly of data in this collection was originated from very different purposes. As INPA is a federal institution, it is responsible for taking part in many important political moments related to Amazon biodiversity, besides just academic research. Thus, the present summary tries to raise the main sources of fish material currently deposited in INPA and the type material generated from that. In total, the Fish Type Collection of INPA comprises material from 7 orders, 32 families and 328 species described from the Amazon basin. Based on the present survey, we can point out some moments of entrance of fish data of incredible biological relevance such as inventories of impacted areas caused by construction of electric powered dams (Tucurui, Balbina, Belo Monte), and the discovery of new taxa due to these events. For instance, 125 lots have become type material of different species after detailed studies on the fishes collected during the inventories for the construction of the UHE Tucuruí (1974-1985), Tocantins River. Other drainages provided abundant material of new taxa such as Tocantins (147), Negro (141), Xingu (139), Madeira (129), Solimões (116) and Trombetas (111). Similarly, we can point out the importance and relevance of working in Conservation Units and Indigenous Areas, based on expeditions budgeted by federal funds. We can also point out the main contributors to Amazon fish taxonomy in the past 30 years based on the material deposited in the INPA Fish Collection, with a total of 127 authors that described new species and deposited all of 165 holotypes, 992 paratypes and 1 neotype. Besides the type specimens, many types are associated to tissue samples deposited and linked to large databanks such as Genbank and BOLD, available to all scientific and academic community. This rich material has been productively used by academics and scientists to describe and revise new taxa and large taxonomic groups, and by politics, to subsidize the creation of new Conservation Units in the Amazon region, particularly in the Amazonas State, as well as to assembly information of Conservation Status of freshwater fishes in the Brazilian Amazon (ICMBio/IUCN programs). This small contribution, more than showing the protagonism of INPA Fish Collection in the Amazon, should act as an alert for the current governmental lack of resources for new expeditions and collection maintenance and on how we are going to deal with new threatens, and, mainly, with the future of our collections.

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# Fish and tissue collection of Laboratório de Biologia e Genética de Peixes (LBP)

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In 1996 the fish collection of the Laboratório de Biologia e Genética de Peixes (acronym LBP) was created, in the Department of Morphology, Institute of Biosciences, UNESP, Botucatu. In the year 2004, this collection was accredited with IBAMA/CGEN as Faithful Depository of Genetic Component Sample (Published in the Diário Oficial da União on June 24, 2004, Section 1, No. 120, page 108) and Dr. Claudio Oliveira became, officially, the curator of this collection. The collection now has about 24,000 lots of cataloged fish (about 3,000 of them of marine fish and the rest of freshwater fish), containing about 180,000 specimens. Most of those fishes are freshwater species since the main focuses of our research has been doing in the orders Characiformes, Gymnotiformes, and Siluriformes. During the last years a strong effort has been done to get a more representative number of marine fishes in association with other Brazilian collections and researchers. Associated with this collection of animals we have a collection of tissues, preserved in 95% ethanol, destined to genetic research. In addition to representatives from almost every South American country, we have samples from Central America, Africa and a few specimens from other regions. Among the fish in the collection we have 65 samples cataloged as paratypes kept in a fireproof cupboard (15 Characiformes, 28 Siluriformes, 2 Cyprinodontiformes). The tissue collection currently has about 65,000 samples preserved at -20°C. From the infrastructure point of view, we have an available area of about 150 m<sup>2</sup> with steel shelves where fish are stored in glass bottles of 100, 250, 500, 1000 and 3000 ml or in plastic drums of 50 or 100 liters, and a laboratory of 30 m<sup>2</sup> where we carry out sorting and identification of the material recently arrived. As equipment we have several stereomicroscopes, two with image capture, and (-20oC) where voucher tissue samples are stored and also several specimens of whole fish when it comes to very rare or hard to collect material. All of the data in the fish collection is stored in an database that was created in Microsoft Access specifically for this purpose, and the remained data about the collection is stored in tables as document files. The collection has been more and more frequently visited by Brazilian and foreign researchers who come to work with the material deposited here. In addition, in the last three years (2014-2016) we have carried out 82 operations of Ioan/donation, reflecting an intense exchange between our collection and other collections from Brazil and other countries. None of the data in our collection is available for public access but we intent that they should be made available in the near future.

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The fish collection of the "Departamento de Zoologia e Botânica da Universidade Estadual Paulista (UNESP), Câmpus de São José do Rio Preto", São Paulo, Brazil (DZSJRP), is one of the most representative freshwater fish collections of Brazil, hosting about 1,200 species and about 258,000 specimens represented in 21,180 lots. This collection had its origins during the early decade of 1980, from material collected by Valdener Garutti (then professor at the Departamento de Zoologia, UNESP), with a main focus on surveys of the freshwater fishes (mostly species inhabiting streams and small tributaries) from the municipality of São José do Rio Preto and nearby areas. Latter, in 1988, the collection started to have a regular curation made by Francisco Langeani, who was hired as Assistant professor at the Departamento de Zoologia in the previous year (1987). Current cataloging uses Specify 6.6.04. Information about collection in www.splink.org.br. Colletion facilities include: curator office, collection manager office, meeting room, triage space, research lab, and 100 m2 area for fish collection. Specimens deposited include 18 holotypes and 4,923 paratypes, representing 53 species, all from Neotropical region. Most of material is in alcohol, currently serving for systematics, ecological and morphological studies; other material include 1,800 tissue samples and 235 lots of C&S specimens. During the 2015 – 2017 period, 249 lots were loaned and 10 researchers visit the collection.

Key words: Systematics; Ecology; Freshwater; Neotropical; Curatorship Financial support: CNPq (processes 306566/2014-1, 401183/2014-9)



# Fish Department of Natural History Museum at San Marcos University (MUSM)

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The ichthyologic collection of the Natural History Museum of the National University of San Marcos (Fish MUSM) is located at Lima and is the largest in Peru. It shelters around of 600 thousand specimens, in more than 60 thousand lots (all individuals of the same species in one local sample). They are recorded in a catalogue (physical book), our main database, and for now, about 30% has been digitalized. The collection can be easily traced back to 1971, where all specimens of Fish MUSM were collected in different freshwater habitats in Peru (aquatic freshwater coast habitats, highlands and rainforest lowlands). The most important feature related to Fish MUSM is its representativeness, which means both hydrographic coverture (mainly Amazonian) as its diversity composition, including very diverse and valuable taxonomic groups such as those associated with the high Andes (in both sides of the mountains), areas usually difficult to assess. It has also samples from neighboring countries (Ecuador, Colombia, Venezuela, Brazil and Bolivia) and others (Guyana and Panama); and even tough, some fish material comes from the beginning of the 20<sup>th</sup> century, including some species actually considered extinct such as Orestias cuvieri. This Collection has specimens that represent almost all the genera and families of freshwater fishes of Peru. Most of the fish in the collection (99%) are preserved in 70° ethanol, but there is also an osteological collection (dry), clear and stain material (in alycerin), and an incipient tissue collection. One of the most valuable section is the type material collection (approximately 160 lots between holotypes and paratypes), those specimens on which the description of the new species are based on, attributing more prominence to our collection for their significance for Neotropical fish diversity. Geographically, the records comprise 80% of Amazonian species, 90% of Pacific freshwater species and 70% of Andean species, with some few records from marine ecosystems, non-native species introduced and of neighboring basins outside Peru. Because of the high quality and quantity of the fish collection, many research ichthyologists come from several countries to examine the specimens. In recent years, due to the sampling collection efforts in unexplored or even never before explored areas, the scientific collection has grown significantly, with several species not yet described among the specimens collected right now deposited in the Fish MUSM.

Key words: scientific collection, fishes, curatorial.



### Fish collection of the Museu de Zoologia da Universidade Federal da Bahia, Salvador, Bahia, Brazil

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The fish collection of the Museu de Zoologia da Universidade Federal da Bahia (MZUFBA) started in the mid-1970's to accommodate coastal marine fishes from surroundings of Salvador, Bahia. Important initial contributions were provided by Virainia Almeida and Antônio Lima de Britto. Around 30 years later, Rosana Souza Lima increased the representativeness of the collection, mainly with samplings from the rio São Francisco and Paraná basins, reaching around 2,000 lots of fishes catalogued. A new era of expansion began in 2003, when various expeditions aiming to obtain a complete taxonomic and geographic coverage of freshwater species from coastal drainages of the Northeastern Mata Atlântica freshwater ecoregion started. A cataloguing system based on Microsoft Access was implemented and the management is done through the CadZoo program. Most of these sampling expeditions were funded by CNPq and CAPES. Nowadays, the collection has 8,200 catalogued lots (approximately 88,100 specimens), from about 1,000 localities, stored in alcohol and accommodated in sliding sections. The material is mostly identified at specific level, representing 818 species, 420 species of freshwater fishes and 398 marines. The total number of species is possibly underestimated, once large number of lots identified at generic level were excluded of the countings. The collection contains 1,761 specimens of paratypes, representative of 24 species of freshwater fish described and deposited after 2007. High percent of the fish lots is from coastal drainages comprised between the rio São Francisco in the north to the rio Mucuri in the south. Best represented rivers are the São Francisco itself (middle and lower portion), followed by rio Paraguacu, rio de Contas, and rio Itapicuru. Best represented families in terms of number of species in the collection are Characidae, followed by Loricariidae and Callichthyidae. The MZUFBA's fish collection is one of the most representative of the Northeast of Brazil and has the largest amount of fish collected in the Bahia State.

Key words: Taxonomy, Northeastern Brazilian drainages



### Scientific Collection of the Division of Fishes at the Museu de Zoologia da Universidade Estadual Feira de Santana (MZFS)

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A Scientific Collection corresponds to an ordered assembly of dead specimens or body parts fully preserved for taxonomic studies and as a source of evidence of previous research. Among the objectives of the scientific collections can be highlighted: the catalogue of species richness, the availability of material for research, and the permanent record of the biological diversity of an area or a taxon. The scientific collection of the Laboratório de Ictiologia at Universidade Estadual de Feira de Santana (UEFS) was initiated in August 1988, in a small room of the bioterium of the UEFS. With the reform of the bioterium in 1992, the ichthyological collection, at the time with less than 600 lots of specimens, was transferred to a room of the Laboratory of Ichthyology. Since 2005, the scientific collection was definitely been transferred to the Fish Division of the Museum of Zoology of the UEFS (MZFS) [Museu de Zoologia da Universidade Estadual de Feira de Santana]. The collection evolved in quantity, management and cataloging. Gradually the manuscript records migrated to electronic cataloging, with the fundamental support of the Programa de Pesquisa em Diversidade do Semiárido (PPBio - Semiárido). The record in the database containing information relating to lot of specimens is being done regularly. This process allows the rapid exchange of information, making the database available on the internet through the speciesLink at CRIA database that integrates primary data from scientific collections. The fish division of the MZFS currently has 17,100 lots registered in its files. Among these, 16,757 have already been made available for consult through the speciesLink, corresponding to 1285 species (or morphospecies) in 577 registered genera. Most lots are of the specimens of fish populations of the Northeast region, followed by fishes of the Southeast, North and South. The most representative family for continental fish is Characidae, followed by Cichlidae and Erythrinidae. For marine fish, we can highlight Sciaenidae, Carangidae and Serranidae. Among these groups there are a large number of specimens that have not vet been identified or determined only as morphospecies and that may become new species when investigated more accurately. Thus, the importance of the biological collection of the Fish Division of the MZFS is emphasized, as well as the need to take part in projects and of the support of development institutions, thus guaranteeing resources for constant updates of the collection.

Key words: Cataloging, diversity, representativeness, updating of the collection Financial support: PPBio do Semiárido; CNPq (process 406532/2013-3)



# The zoological collections at the Instituto Nacional da Mata Atlântica - INMA

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The zoological collections organized at the Instituto Nacional da Mata Atlântica exist since 1940 and are now composed by vertebrates as fishes, amphibians, reptiles, birds and mammals. Small collections of insects and crustaceans are under organization. A didactic collection provides support in various educational activities which are already being held at the INMA, and completes the Collections Sector [Setor de Coleções- SeCol]. After the establishment of the INMA in 2014 it was possible to include researchers granted by the Programa de Capacitação Institucional – PCI – a partnership between the ministry MCTIC and research council CNPq. Actually three doctors act as researchers in the zoological collections at INMA. The formation and interchange of taxonomists is advancing as these researchers develop partnerships and collaborations. All the vertebrate collections are available at "SpeciesLink" of Centro de Referência em Informação Ambiental - CRIA database. In July 21<sup>st</sup>. 2017 the catalogued material under fish collections corresponds to 12700 lots, conserving 93979 specimens, distributed in 34 orders, 140 families, 438 genera and 920 species. Among these, 120 lots need further specific identification. Fifty nine specimens correspond to primary and secondary types. Most specimens are of freshwater fishes (corresponding to 10927 lots) captured at aquatic environments specially at Northeastern Mata Atlântica ecorregion. The maior river basins represented in fish collections are Rio Doce (1538 lots); rio São Francisco (1426 lots); rio Itapemirim (1192 lots); rio de Contas (892 lots); rio São Mateus (577 lots); rio Santa Maria da Vitória (528 lots); rio Itaúnas (519 lots) and rio Reis Magos (504 lots) and remaining river basins represented by less than 500 lots. The geographical precedence is mainly from the states of Espírito Santo (7700 lots), Bahia (3716 lots); Minas Gerais (488 lots) and Rio de Janeiro (399 lots). The fish collections occupy part of a 46 square meters room, with 326 linear meters of shelves, being in its limit of capacity. We reinforce the importance of collections for storage of biological specimens sheltered in an adequate place for development of investigation and research activities. In this sense the zoological collections at the INMA remains problematic, as the pavilion with collections stored are victimized by seasonal flood of the nearby rio São Pedro. We recommend the convenience of immediate transfer of all the collections to a better safe place free of inundations and arranged in larger space, in order to assure its secure increment. Zoological collections are libraries of life and it is our responsibility to take care of them. We need to keep them live as representing a fundamental space of research formation as well as scientific and cultural education.

Key words: Atlantic forest; fish collections; curatorial; Neotropical Financial support: CNPq (process 1993/2846-8)



### The Fish Collection of the Núcleo em Ecologia e Desenvolvimento Socioambiental de Macaé (NPM), Universidade Federal do Rio de Janeiro, Brazil

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The Fish Collection of the Núcleo em Ecologia e Desenvolvimento Socioambiental de Macaé (NPM), Universidade Federal do Rio de Janeiro (UFRJ), Brazil, was established in 2008. The original goal of the collection was to foster studies on taxonomy, phylogeny, biogeography and ecology of the richly diversified biota of the northern portion of the State of Rio de Janeiro, which in the last decades has been significantly impacted by human activities mostly related with the urbanization and industrial growth resulting from economic activities associated with the offshore oil exploration at the Campos Basin. The NPM also supports the formation of undergraduate and graduate students of the Biology Undergraduate Course and the Graduate Program of Environmental Sciences and Conservation (PPG-CiAC), which are both offered in the Núcleo em Ecologia e Desenvolvimento Socioambiental de Macaé (NUPEM/ UFRJ). Currently the NPM holds 4000 lots, distributed in 60 orders, 209 families and 871 species of marine and fresh water fishes. The Chondrichthyes is represented by 54 species in 20 families and eight orders, while the Teleostei is represented by 817 species in 189 families and 42 orders. The collection has a strong regional focus, since a substantial portion of the specimens deposited was collected in both marine and continental environments in the State of Rio de Janeiro and adjoining areas. However, since its inception in 2008, the Fish Collection is growing exponentially in terms of number of specimens, taxonomic diversity, and geographic coverage. It now includes specimens from nine Brazilian states and 36 countries in four continents. Almost all specimens catalogued at NPM, including those from other countries, were collected by the staff (students and professionals) associated with the collection in different scientific projects and expeditions, but significant exchanges with other national and international collections have also been made and include, in a few cases, the repatriation of specimens. The NPM also holds a significant collection of deep-sea fishes from the Campos Basin, but other deepsea portions of the Brazilian Exclusive Economic Zone are also relatively well represented. A tissue bank associated with the fish collection provide additional support for taxonomic, phylogenetic, phylogeographic and demographic studies. In addition to supporting scientific activities, the NPM is also integrated with several outreach projects that promote the diffusion of the biological and conservation knowledge in different segments of the local society. Additional information on the NPM holdings is available at www.splink.cria.org.br.

Key words: Icthyology, Systematics, Taxonomy Financial support: CAPES



# The "Acervo Ictiológico do Museu Paraense Emílio Goeldi (AICT-MPEG)": past and present

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The "Acervo Ictiológico do Museu Paraense Emílio Goeldi (AICT-MPEG)" comprises around 35,000 lots containing roughly 340,000 specimens preserved in alcohol, dry skeletons and cleared and stained specimens stored in glicerine. The type collection, currently, is composed by 270 lots of type material, being 37 holotypes and 233 lots of paratypes. There are representatives of nearly 1750 species, the vast majority from the Amazon Basin, with species of Chondrichthyes, Sarcopterygii and with more emphasis on Actinoperygii. The oldest records date back from 1889 by H. Merwarth from Pará State. However, ichthyological research only truly started in 1932 by Carlos Estevão. Also, of great importance for the institution were the studies of Antônio Estevão de Oliveira, Sprague Myers, Alba Maranhão, Inah Silveira, Rodolfo von Ihering and Paulo Sawaya who organized samples of the regional ichthyofauna. Between 1945 and 1980 the collection had no significant contribution. In the begining of the 80's, collections made by Ronaldo Barthem and Michael Goulding started a new period, culminating with a new collection building in 1992. From 1994 to 2003 Dr. Horácio Higuchi (than the curator) and Ronaldo Barthem started the digitalization of the AICT-MPEG finalizing the process with 5,750 lots. In 2003 curatorial procedures underwent renewal by Dr. Wolmar Wosiacki and initiates modifications and improvements in the logistics infrastructure and curatorial protocols. A partnership with Petrobras in 2010 allowed the expansion of the collection with the construction of an additional building to house marine specimens from north coast of Brazil. Also in 2010, the migration of the database from the Access platform to the Specify platform, specific to zoo collections, was initiated along with the implementation of a protected database management system on the "Núcleo de Biogeoinformática (NBGI)" of MPEG. Currently the AICT-MPEG has two researchers (the curator and Dr. Alberto Akama) and two curatorial assistants.

Key words: Collection, Fishes, History Financial support: CNPq (300940/2015-7; WBW)



# Fish Collection: Coleção Ictiológica da Universidade de Brasília (CIUnB)

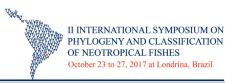
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The Coleção Ictiológica da Universidade de Brasília (CIUnB) was founded in October 2006, due to the need to store fishes from research in the central region of Brazil. The curator of the collection is the biologist Dr. Pedro De Podestà Uchôa de Aquino (pedropua@gmail.com). Currently, the collection has 1,488 lots with 25,770 individuals. These specimens are distributed in 15 orders, 48 families, 142 genera, and 310 species. From these lots, 83 species (six orders, 19 families, and 56 genera) presented preserved material for DNA analysis (i.e., tissue samples), a total of 1,348 individuals. The lots are stored in compacting cabinets in an acclimatized room of 9.96 m<sup>2</sup> in the Department of Zoology of the Universidade de Brasília. There is a lot with three paratypes of the species Itualanis goya Datovo, Aquino & Langeani, 2016. The collection also has specimens of endemic and endangered species, such as the pirá-brasília (Simpsonichthys boitonei), an emblematic species from Brasília. The collection has mainly representative fish samples of the headwaters of the Cerrado river basins. The material is a testimony of the freshwater fishes coming from the headwaters of the Tocantins-Araguaia basin (Paranã and Maranhão rivers), upper Paraná basin (São Bartolomeu and Corumbá rivers), East Atlantic basin (Jequitinhonha river), São Francisco basin, and upper Madeira (Guaporé and Corumbiara rivers). The management is done through the Microsoft Excel program where all lots are georeferenced. The standardization of the spreadsheet follows the proposed by the speciesLink project with the purpose of making information available and access to the integrated database of collections. The collection is visited by researchers and students of several courses of Brazilian universities; also doing donation, exchange and loan of lots for other Brazilian collections and museums. The importance of this material is justified by the lack of information for the group, as well as its biogeographic location (region of headwater rivers captures) and the need for conservationist actions (strong deleterious human pressure).

Key words: Ichthyology collection; Central Brazil fishes; Cerrado, headwaters Financial support: CAPES (process BEX 9437/11-8)



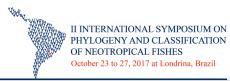
# The fish collection of the Laboratório de Ictiologia Sistemática do Departamento de Ecologia e Biologia Evolutiva (LISDEBE) of the Universidade Federal de São Carlos

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The Laboratório de Ictiologia Sistemática do Departamento de Ecologia e Biologia Evolutiva (LISDEBE) of the Universidade Federal de São Carlos (UFSCar) houses a fish collection of Brazilian rivers resulted from near 40 years of research about the Neotropical fish diversity. The research in taxonomy of freshwater fish aroups initiated in the seventies by Dr. Julio C. Garavello and was developed in collaboration with other ichthyologists. These studies focused in the taxonomy of the Anostomidae (genera Leporinus and Schizodon) and Loricariidae (Hypoptopomatinae and Hypostominae subfamilies), but also include studies in Characidae, Parodontidae, Doradidae, Trichomycteridae, Pimelodidae, Heptapteridae and Poeciliidae. Besides the taxonomical research, the ichthyological collection provides basis to studies about fish diversity in different river basins of Brazil, allowing to access quality information about fish composition and distribution in Neotropical freshwaters. There are actually (July 2017) 6972 lots of fishes digitalized, representing near half of the entire material of the collection. Near 78% of the collection already digitalized is represented by material collected in the upper rio Paraná basin (rio Grande, rio Tietê, rio Paranapanema and rio Paranaíba drainages). Recently, field surveys in some type localities allowed the collection of topotypes of species described from the rio Grande and rio Tietê basins and motivated the begin of a collection of tissue samples for molecular analysis accompanying the preserved whole specimens. In addition to the great amount of ichthyological material from the upper Paraná basin, the LISDEBE fish collection also houses lots of specimens collected in other Brazilian rivers. Most part of these lots are in process of digitalization. Of the amount already digitalized, near 7% comprises lots of the Amazon basin (including material of the rio Araguaia-Tocantins basin, rio Capim, rio Aripuanã, of the rio Madeira basin, and rio Teles Pires, of the Tapajós basin). Near 12% of the digitalized material comprises isolated rivers of the Brazilian eastern (including rio Itapicuru, Bahia, rio Jequitinhonha, rio Doce, rio Paraíba do Sul, rio Ribeira de Iguape and isolated coastal rivers of São Paulo state). Material of the Paraná-Paraguai, chiefly from Pantanal rivers, basin and rio Iguacu represents near 2% of the digitalized lots. The fish collection also includes 47 lots of paratypes of recently described species. To the near future is planned the migration of the databases to the software Specify (currently the data are in Excel spreadsheet) and provide the data online.



# Ichthyological Collection of the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupélia) of the Universidade Estadual de Maringá (UEM)

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As part of the Center of Research in Limnology, Ichthyology and Aquaculture (Nupélia) (http:// www.nupelia.uem.br, https://www.facebook.com/Nupelia/), the Ichthyological Collection of Nupélia was created in 1990 with the initial aim of keeping specimens of the center of research. Over time, it has expanded its size, importance and scope. Currently, it houses comparative material from different localities, paratypes of several new species, as well as voucher specimens. It is housed in a building of about 700 m<sup>2</sup>, with an infrastructure and specifications necessary for collection storage, laboratory activities, air conditioning and a combo network for telephony and logic. All material is available to the scientific community. Loans, exchanges and other transactions have been carried out with different institutions from Brazil and other countries. Most of the collection is preserved in 70°GL ethanol, but a set of cleared, stained and preserved in glycerin specimens is gradually increasing, as well as a tissue bank for molecular analysis. The geographic representation of the Collection is Neotropical, with emphasis on the ichthyofauna of the rio da Prata basin, but several lots from other Brazilian basins also have been deposited in the collection. Almost 20,000 lots of more than 2,000 species, more than 350 genera and 63 families of fish are in our database and many are yet to catalog. The Collection is accredited to the Genetic Heritage Management Council (CGEN) since 2006, and it is also part of the Network of Biological Collections of the Paraná State, Taxonline (http:// taxonline.bio.br/index.php). Since 2010, the Ichthyology Collection also houses a collection of ichthyoplankton from the main Brazilian hydrographic basins, with stages of development of several fish species of our ichthyofauna, being one of the few specialized laboratories in Brazil. Their data have been incorporated into the Ichthyology Collection and allowed the disclosure of the study of eggs and larvae of freshwater fish. Due to non-computerization of the collection data, only an estimate of its size can be provided, a rating of about 290,000 eaas, 370,000 larvae and 50,000 juveniles. The larvae and juveniles are members of 35 families, encompassed by nine orders. This collection, in addition to serving as a reference for the development of projects in the region, in recent years it has served researchers from different institutions of various Brazilian states and forums in Brazil, who seek advice on the development of their projects.

Keywords: Biodiversity, Fishes, Freshwater, Ichthyoplankton, Neotropical

Financial support: UEM/Nupélia and government funding agencies, such as CNPq, CAPES and Fundação Araucária



### The Ichthyological Collection of the Museu Nacional, Universidade Federal do Rio de Janeiro

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Founded by Dom João VI, King of Portugal, in 1818 during his stay in Brazil, the Brazilian National Museum (Museu Nacional) is one the oldest museums in the New World. Ichthyological research in the Museum dates back to the end of the 19th Century, when Alípio de Miranda Ribeiro began his notorious career as a professional scientist. Alípio dedicated most of his life to collection building in the Museu Nacional until his death in 1938. His main contributions to Brazilian Ichthyology included the monumental publication titled Fauna Brasiliensis and the establishment of the ichthyological collection that currently bears the MNRJ acronym, a task continued by his son, Paulo de Miranda Ribeiro, who published a catalog of type specimens. During World War II, the Fish Collection benefited from work carried out by the North American ichthyologists George S. Myers and William A. Gosline. Myers served as special professor of ichthyology at the Museu Nacional in charge of the extensive Survey of Brazilian Marine Fishes of Commercial Importance, while Gosline was later hired temporarily to work at the fish collection. Myers seems to have been influential on the work of Haroldo Travassos, who continued publishing descriptions of new species until his death in the late 70's. Former curators also included Gustavo W. A. Nunan and India B. Moreira. Cataloguing of the MNRJ Fish Collection formally started in 1939, and computer-based cataloging and management began in 1993 using the MUSE software with online access since then, first through the NEODAT project, and now through the Sistema de Informação sobre a Biodiversidade Brasileira – SiBBr. During the last two decades, the collection had a five-fold increase and now exceeds 50 thousand lots and about 600.000 specimens, encompassing all major Brazilian freshwater drainages and coastal marine waters, as well as oceanic islands, deep-water samples from the continental slope, and representatives from all continents. It also includes dry-mounted exhibit specimens (over 600 lots), skeletal materials, cleared and stained specimens, and over 12,000 tissue samples for DNA studies. The type-specimen collection currently contains 7,061 type specimens, including 456 primary types. The Collection, which is housed in a 588 m<sup>2</sup> facility, is under the responsibility of three curators, assisted by a full-time technician, and several graduate and undergraduate students, volunteers, and post-doctoral associates. Annually, close to 30 researchers visit the collection and approximately 38 inter-institutional loans are fulfilled. The collection is a major research asset available to the UFRJ Biological Science Graduate Program in Zoology. Associated research facilities include digital radiography equipment and a molecular laboratory.

Key words: Museum, Collection management, Preservation, Natural History, Cultural heritage Financial support: CNPq, FAPERJ, CAPES



### Ichthyological collection of the Universidade Federal do Rio Grande do Norte: a relevant assemblage of fishes from the semiarid Caatinga and adjacent areas

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Brazilian fish collections encompass a massive assortment of Neotropical region and their majority are located in the Southern and Southeastern regions of the country. Both studies and collections regarding Northeastern ichthyofauna have been growing in the last years with increasing importance for the Ichthyological Brazilian Society (Sociedade Brasileira de Ictiologia). The ichthyology collection of the Department of Botany and Zoology of the Universidade Federal do Rio Grande do Norte (UFRN) was permanently deployed in 2011 and it is currently under the responsibility of Dr. Sergio Lima. Even though it is recent, the collection has gradually developed over the last few years due to inclusion of collected specimens from projects of the Laboratório de Ictiologia Sistemática e Evolutiva, and donations of other institutions. The oldest lots date from 1992 and the most recent from 2017, and its rate of growth has increased mainly from 2009 and, today, the collection encompasses about 4,450 lots with more than 49,500 specimens preserved in ethanol 70% or 96% (for molecular studies). There is also a wide collection of tissue samples preserved in standard anhydrous ethanol for molecular analyses, summing up to 3,876 duly registered samples. The collection comprises the groups Actinopterygii and Chondrichthyes, and most of them are freshwater species from Brazilian Eastern coastal basins: Atlantic Forest areas, chiefly Serra do Mar (Rio de Janeiro to Santa Catarina states) and Brazilian Northeastern coastal basins; reef species from Northeastern Brazilian coast and oceanic islands; and it has been highlighting itself as one of the most representative assemblage of Caatinga's fishes. It is worth mentioning that it also holds a significant sample of the ichthyofauna from drainages involved in the São Francisco river interbasin water transfer project and the MATOPIBA agricultural frontier (Maranhão, Tocantins, Piauí and Bahia state borders), both areas under severe anthropogenic impacts in the Caatinga and Cerrado, respectively. The UFRN fish collection possesses some type-material of recently described species (e.g. Acyrtus pauciradiatus Sampaio, de Anchieta, Nunes & Mendes 2004, Bathygobius brasiliensis Carvalho-Filho & de Araújo 2017, Hypostomus sertanejo Zawadzki, Ramos & Sabaj 2017, Ituglanis agreste Lima, Neves & Campos-Paiva 2013, Parotocinclus seridoensis Ramos, Barros-Neto, Britski & Lima 2013, and P. cabessadecuia Ramos, Lima & Ramos 2017). There are also some threatened species according to the Brazilian red list, such as Anablepsoides cearensis (Costa & Vono 2009) (CR), Apareiodon davisi Fowler 1941 (EN), Hippocampus reidi Ginsburg 1933 (VU), Kryptolebias brasiliensis (Valenciennes 1821) (CR), K. gracilis (Costa 2007) (CR), Malacoctenus brunoi Guimarães, Nunan & Gasparini 2010 (VU), Megalops atlanticus Valenciennes 1847 (VU), Mycteroperca bonaci (Poey 1860) (VU), Parotocinclus spilurus (Fowler 1941) (EN), Rhamdiophis krugi Bockmann & Castro 2011 (VU), and Taunayia bifasciata (Eigenmann & Norris, 1900) (VU). Currently, the collection has been going through a process of displacement to the Museum of Morphology of UFRN, where they will be organized in shelves by their catalogued number and systematics. The material is available for the academic community and loans have been undertaken to some Brazilian institutions, however it has not been implemented in any digital platform yet.

Key words: Northeastern Brazil, Regional representation, Actinopterygii



### Forty years of the Ichthyological Collection of Universidade Federal da Paraíba (UFPB)

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The Ichthyological Collection of the Universidade Federal da Paraíba (UFPB) was initiated in 1977 by Graciela Cannella and Ricardo de Souza Rosa (current curator), in the Bioloay Department of UFPB, and it is completing forty years of existence. Currently, it is located in the Department of Systematics and Ecology, Campus of João Pessoa, and is considered the second largest regional collection in number of species lots and possibly the most representative collection in number of fish species of Northeast Brazil. The collection was accredited in 2001 as an official depository of samples of the Brazilian genetic patrimony. It is divided into two collection rooms, one containing the Myxine, Petromyzontia, Chondrichthyes and marine and estuarine Osteichthyes, and the other, the freshwater Osteichthyes. The specimens are treated according to standard curation procedures, most being preserved in 75° GL ethanol; double-distilled glycerin with thymol crystals is used for cleared and stained materials, and dry storage for preserved skeletal pieces. The collection records are in the process of being computerized on the Specify platform, and in 2018 they will be available for online access. The collection contains 11,290 registered lots, including 187 type-specimens, 12 of which are holotypes. Freshwater fishes comprise 5,233 cataloged lots, corresponding to 283 species distributed in 139 genera, 42 families and eight orders. Marine and estuarine fishes comprise 6,057 cataloged lots, corresponding to 409 species, distributed in 250 genera, 56 families and 31 orders. The aeographical representation of samples covers predominantly northeastern Brazil, but also includes other Brazilian regions and foreign countries (Argentina, Canada, Colombia, United States, Japan, Paraguay, Uruguay and Venezuela). As a reflection of its dominance in the Neotropical region, taxa of the order Characiformes predominates in the collection. The most representative families are Characidae, with 1,541 species lots, followed by Loricariidae, with 628 species lots. The UFPB Ichthyological Collection has been continuously serving as a repository and source of materials for systematic research and scientific publications, especially regarding the ichthyofauna of the Brazilian northeast region.

Key words: Systematic Collections; Biodiversity; Northeast Brazil Financial support: CNPq



# Fish collection of the Universidade Federal de Mato Grosso, Cuiabá (CPUFMT)

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The fish collection of the Universidade Federal de Mato Grosso (CPUFMT) is housed in the Department of Biology and Zoology, Cuiabá, Brazil. It includes mainly freshwater neotropical fishes (but also marine/estuarine representatives) from nine Brazilian states. The collection also include fossils. Savanas (Cerrado), Amazonian headwaters and Pantanal wetlands are the most represented biomes in the collection. Up to 4.500 lots are in on-line database (and an estimative of about 10.000 still awaiting inclusion) encompassing 17 orders, 53 families, 263 genera and about 762 species (a total of 55.712 specimens). Type specimens includes 8 paratypes. Since 2011 the collection belongs to the species link project (http://splink.cria.org. br/manager/detail?setlang=pt&resource=CPUFMT).

Key words: Systematics; Taxonomy; Fish colletion; Mato Grosso

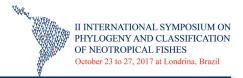


# The Fish Collection of the Museu de Ciências e Tecnologia da PUCRS

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The fish collection of the Museu de Ciências e Tecnologia of the Pontifical Catholic University of Rio Grande do Sul began in 1967, concomitant with the founding of the Museum. Collection data used to be recorded in catalogue books until 1988, when data began to be computerized with the software MUSE. Computerization allowed the collection to be made available on the Internet after 1991, through the participation in the NEODAT project. Currently, all new materials are immediately catalogued and managed with the software Specify, and the online data can be accessed through the museum (http://webapp.pucrs.br/colecoesMCT) and the speciesLink (http://splink.cria.org.br/) websites. The first loan of fishes to a Brazilian institution (specimens of Sciaenidae) was shipped in 1979, to the University of Santa Ursula, Rio de Janeiro, and the first loan to a foreign institution was sent in 1982, to the Virginia Institute of Marine Sciences, USA (specimens of Cynoglossidae). Specimens in the collection began to be cited in Brazilian journals in 1972 and in foreign publications in 1987. The MCT-PUCRS fish collection is among the largest in Brazil, with more than 52 thousand lots cataloged, totaling about 510 thousand specimens in alcohol, 3,704 specimens cleared and stained for osteological studies, and 5,565 tissue samples for molecular analyzes kept in ethanol at -20°C. Most of the lots cataloaed are freshwater fish from Brazil, especially from south and southeast rivers drainages and the basins of the São Francisco, Amazonas, and Paraná-Paraguay rivers. Since the first collecting expeditions were made in the coast of Rio Grande do Sul and Santa Catarina states, there is a good representation of marine species of this region. The collection holdings contain 82% of the currently threatened species of the Rio Grande do Sul State, including 24 of the 26 threatened species of annual fishes (Rivulidae), and 36% of the threatened species officially listed for Brazil. The most highly represented family in the collection is Characidae (27% of the total), followed by Loricariidae (14.5%), and Cichlidae (7%), with a significant representation of Gymnotiformes. The collection currently occupies five rooms in the museum building basement, totaling approximately 280 m<sup>2</sup>, one of them exclusively to house type-specimens. There are 297 primary types – 292 holotypes and five neotypes – and 18,154 paratypes belonging to 642 species. High resolution images of the primary type specimens are available on the museum website. Visitors are offered a room with stereomicroscope, internet access, and all other necessary equipment and tools. Visits must be previously scheduled with one of the curators and logged on the website (http://www.pucrs.br/mct/colecoes/formulario/).



# The Fish Division of the University of Michigan Museum of Zoology

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The University of Michigan Museum of Zoology (UMMZ) is home to one of the largest and most diverse collections of fishes in the world. The collection contains 197,388 cataloged lots and over 3.5 million specimens. 406 fish families are represented in the collections. Neotropical fishes comprise 15,168 cataloged lots and more than 265,000 specimens. 2,087 nominal species are represented in the collections from Central and South America. Type specimens of Neotropical species are represented by 464 cataloged lots. Major Neotropical taxonomic strengths in the collections are characiforms, siluriforms, cyprinodontiforms and cichlids. The Fish Division collections were built by UMMZ curators such as Carl L. Hubbs, William A. Gosline, Reeve M. Bailey, Robert R. Miller, Gerald R. Smith and William L. Fink. Collections made by UMMZ graduate students from the U.S. and South American countries have contributed greatly to the geographic and taxonomic diversity of the collections. Notable among these student collectors are Frederick Cichocki, James S. Albert, Paulo A. Buckup, Brian S. Dyer and Andrea T. Thomaz. Exchanges of specimens between the UMMZ and Central and South American institutions have provided not only valuable accessions, but also opportunities for scientific collaboration. The Fish Division curatorial staff and the collections have served as outstanding educational resources for students, postdoctoral and sabbatical researchers in their studies of Neotropical fishes. Since 1980 more than 800 publications on Neotropical fishes have acknowledged the UMMZ for specimens and/or information used in the published research. Collaborations in digital technology and information exchange between the UMMZ and Central and South American museums (MUSE and NEODAT) paved the way for presentday online digital data collection and distribution. The UMMZ has relocated its collections and staff from the Alexander G. Ruthven Museum to a new facility, the Research Museums Center, but our commitment to excellence in collection curation, teaching and service to the ichthyological community remains unchanged.

Key words: UMMZ, Neotropical, collection, NEODAT



### Central and South American fish holdings of the Royal Ontario Museum, Canada

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The Royal Ontario Museum (ROM) was formed in 1914 starting with approximately 150 lots of preserved and mounted fish specimens. By 1950, over 17,000 lots of fishes had been catalogued into the ROM collection, mostly from Canadian freshwater and marine expeditions. With expansion in curatorial staff over the next 50 years, rapid collection growth occurred. By the year 2000, over 80,000 lots had been catalogued into the collection with significant collections of freshwater and marine fishes from North America, the Caribbean and Indo-Pacific coral reef fishes. The ROM fish collection currently houses over one million specimens in approximately 103,000 catalogued lots. There are 7,000 species represented, including 155 holotypes and 1,250 paratype lots. The collection has specimens from all continents and oceans, with particular strengths in Canadian, Central and South American freshwater fishes, and Indo-Pacific coral reef fishes. The collection is actively used in research programs by ROM Curators and their students in the Department of Ecology and Evolutionary Biology at the University of Toronto. The South and Central American freshwater fish collection rapidly expanded during the past decade. In 2008, Hernán López-Fernández became fish Curator, specializing in fishes from a region that had been poorly represented in the ROM fish collection. Current Neotropical research and collection growth at the ROM focuses on biodiversity discovery, evolutionary, ecological and conservation studies with emphasis on the family Cichlidae, among several others. Since 2008, the ROM Neotropical freshwater fish collection increased by approximately 9,100 lots of fishes, and holds one of the three largest collections of freshwater fishes from Guyana, one of the most diverse countries of South America. The ROM fish collection houses 1,324 primary and secondary type specimens of Neotropical fishes. The research program involves collaborations with colleagues in Canada, the United States, Guyana, Suriname, Brazil and Uruguay; as well as loans and exchanges with various museums in North and Latin America. At least 80 publications were based on or used the ROM collection since 2008. Research in Guyana and other South American countries rapidly increased the role of the ROM ichthyology collection as a centre for research in Neotropical freshwater fish diversity, conservation and evolution. The most remarkable collections from Guyana include those from the upper Mazaruni River basin. The upper Mazaruni contains a unique fish fauna with nearly 80% of species new to science and possibly 95% rate of endemism. In addition to specimen collections, the ROM holds the largest frozen fish tissue collection in Canada, with over 22,200 samples with vouchers housed in the alcohol collection. The Neotropical freshwater fish tissue collection exceeds 14,600 samples from Guyana, Venezuela, Uruguay, Suriname, Brazil, Bolivia, Ecuador, Perú, México, and Costa Rica, making it one of the most extensive collections of its kind. The ROM tissue collection is heavily used for research in molecular systematics, population genetics and conservation by both ROM personnel and researchers from institutions worldwide.



# Fish collection of State University of Piaui: insertion in academic projects and importance to ichthyology

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Regional collections are characterized by the small number of records and may hold specimens of undersampld areas. Its importance resides on the possibility of furnishing detailed local data for biological, systematic, biogeographic and conservation studies. The small fish collection of the university campus Alexandre Alves de Oliveira, hosted by the Universidade Estadual do Piauí (UESPIPHB) has samples of freshwater and marine species collected in the lower course of the Parnaíba river and adjacent estuarine area, and in the Timonha and Ubatuba rivers, in northeastern Brasil. Here we describe the regional context and characteristics of the UESPIPHB Fish Collection and discuss aspects related to the relevance of the regional fish Collections in the Brazilian university structure. Collections were carried out in the municipalities of Teresina, Boa Hora, Cabeceiras do Piauí, Buriti dos Lopes, Piracuruca, Porto, Parnaíba, Cajueiro da Praia and Chaval mainly through research projects sponsored by Fundação de Amparo a Pesquisa do Estado do Piaui (FAPEPI) and Petrobras Ambiental in the states of Piauí, Maranhão and Ceará. Fish were sampled with gillnet, beach seine, cast net, and hand net. All specimens are stored in 70% alcohol. Large specimens are in plastic drums of 50 liters. Most samples consist of small and medium-size specimens stored in glass jars. The material has been the basis for scientific undergraduate student projects (PIBIC), and graduate projects at the specialization and master level. The collection has been visited by students of fundamental public schools as part of science extension projects. Its maintenance has enjoyed the support of a full-time professor, as well as undergraduate and gradute students. The collection was started in 2012, and since 2015 its data have been available on the internet through SpeciesLink (http://splink. cria.org.br/manager/detail?setlang=pt&resource=UESPIPHB), in an agreement with Centro de Referência em Informação Ambiental, CRIA. The collection data is managed through the Speciesbase database in Microsoft Access. The fish collection contains a total of 4,169 specimens, out of 62 families, 172 species of fishes arouped in 723 lots. Characidae is the family with the greatest number of records, 134, followed by Cichlidae, with 55, and Caranaidae, 46. Lots are arranged in numerical sequence and since May 2017 they were transferred to a new room and building, which facilitates the access to visitors and researchers.

Key words: Diversity; Caatinga; Taxonomy; Neotropical Financial support: FAPEPI, Petrobrás Ambiental, UESPI



# The fish collection of the "Coleção Zoológica Norte Capixaba" (CZNC): a small and growing collection of fishes from northern Espírito Santo state, southeastern Brazil

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The "Coleção Zoológica Norte Capixaba" was created in march 2012 to receive animal specimens collected at northern Espírito Santo state (ES). The collection is part of the "Programa" de Pós-Graduação em Biodiversidade Tropical" (PPGBT) from Universidade Federal do Espírito Santo (UFES) and is composed by Ichthyological and Entomological divisions. The oldest fish specimens were collected for ecological studies by LFD at São Mateus river drainage in June 2009 (CZNC473, Clarias geriepinus), just after his arrive at UFES. The fish collection was started as part of the postdoctoral project of LFSI about survey and biogeography of fishes from the same river drainage in November 2011, and was recognized by the Brazilian government in October 2013. The collection occupies an area about 30 m<sup>2</sup> in an exclusive collection heated room (18°C) with shelves, cabinets, barrows and freezers, and have an associated laboratory. The collection staff counts with a curator (LFSI), one researcher/auxiliary curator (LFD), at least 12 undergraduate and graduate students, and, since November 2016, with one project technician two days a week. The fish collection is totally informatized with the software The Artedian 2.8 running under MS Access 2003, using traditional phylogenetic organization of the families, but is not online available yet. CZNC contains about 2,300 lots with around 35,000 fish specimens preserved in ethylic alcohol 70%, plus about 10 c&s lots, eight dry skeletal parts, and 360 lots of samples preserved in ethylic alcohol 96% under -18°C. All the 190 species present in the collection are from Brazil and about 96% of the lots are from the freshwater or estuary regions of the three river drainages from northern ES (Itaúnas, São Mateus and Barra Seca), including nationally (Epinephelus itajara, Hippocampus reidi) or regionally (Brycon ferox, Gramma brasiliensis) threatened species. Remaining specimens are from coastal river drainages from ES, southern Bahia or northern Rio de Janeiro states, from Atlantic Ocean at ES or upper Paraná river drainage. The collection keeps links with several Brazilian fish collections (mainly from southeastern and south Brazil) for donations, exchanges and loans and intends to expand its relationships to other national and international collections. Projections indicates the collection will reach 10,000 lots before 2022 and new facilities will be needed, as well new financial support, which currently is based mainly at LFSI's postdoctoral projects.

Key words: Regional collection; Freshwater; Estuary; Atlantic Rainforest

Financial support: Fundação Estadual de Amparo à Pesquisa do Estado do Espírito Santo (FAPES, procs. 53132203/2011, DCR, and 68856598/2014, PROFIX to LFSI); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, procs. 558246/2009- 5, PELD to LFD; and 350672/2011-3, DCR to LFSI); and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, PROFIX to LFSI)



# Collection of Fishes from the Museo Nacional de Historia Natural del Paraguay (MNHNP), Paraguay

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The Collection of Fishes from the Museo Nacional de Historia Natural del Paraguay, Paraguay, is a Scientific Collection holding specimens of fishes from Paraguay and other countries. The Paraguayan Museum of Natural History is a Governmental Organization dependant of the Secretaría del Ambiente, Republic of Paraguay, a Secretariat directly relied to the executive power to manage and enforce environmental laws. The aim of this paper is to divulge to the scientific community the significance of the Collection of Fishes and the role of the National Museum to the protection and conservation of Paraguayan biodiversity. The scientific collection began in the end of the 1970's and early 1980's as a National Biological Inventory Project of Paraguayan Governmental Organizations in cooperation with local and foreign researchers and volunteers. Since this first step and from the formation of the National Museum of Natural History, which occurred in the middle of 1990's, important projects have been developed with his own personnel and local and international researchers. Successful projects related to fishes and other groups of plants and animals were developed in cooperation with other museums and organizations, as the Swedish Museum of Natural History and Conservation International. It is the most important scientific collection of fishes from Paraguay and actually holds more than 4,000 catalogued lots, including type material, mainly in the form of liquid preparation. However, it maintain about 12,000 uncatalogued material from Paraguay and other countries, as Italy and the United States of America. The Ichthyology Section reached its peak of Ioaning activity in the end of 1990's period, with Museums and Universities from Brazil and United States of America, and suffered a strong decrease in its activity during the 2000's period. The Museum has an significant responsibility in the conservation and protection of wildlife, fisheries, and national parks acting as a consultant organ to the Secretariat related to these areas. In addition, is a local leader in the formation of young researchers having important collaboration with local Universities. Also, since its formation, was one of the first organization supporting science in the country with the publication of original research related to the field in his Boletín del Museo Nacional de Historia Natural del Paraguay, still publishing these days in the format of peer reviewed scientific journal. Today, the Ichthyology Section is strive to the accommodation of the entire collection, laboratory and administrative area; implementing the use of open source software to manage and reveal his holdings; and continuing with collecting expeditions in the field to expand his collection to reach its vision of being a regional reference to the scientific community.

Key words: Governmental Organization; biodiversity; Inventory



## Fish collection of the Museo de Zoología of the Universidad de Costa Rica (UCR)

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The fish collection of the Museo de Zoología of the Universidad de Costa Rica (UCR) began in 1962, from material collected by the ichthyologists William A. Bussing (USA) and Myrna I. López (Costa Rica). The first specimens were originally collected with the purpose of being used in investigation and teaching. In 1966 a systematic ichthyological sampling plan for all Costa Rica began, which promoted and consolidated the collection as such. Nowadays, the UCR fish collection comprises more than 250000 specimens, in about 18200 lots, including material from 16 countries (about 80% from Costa Rica) and about 1792 species. The material from Costa Rica represents about 73% (1433) of all reported/expected species for the country (about 1963). The collection includes 7420 type specimens (3 holotypes and 7417 paratypes). Most of the acquis is preserved in 65-75% ethyl alcohol and there are about 250 cleared and stained specimens and 40 dried skeletons, additionally, there about 10800 lots of eggs and larvae from marine fishes (both Pacific and Caribbean, mainly from Costa Rican coasts), representing more than 100 species. The collection is organized phylogenetically by orders and families, and alphabetically by genera and species. All the material is distributed into 16 metal shelves occupying a physical area of 150 m<sup>2</sup>, located in the main campus of the Universidad de Costa Rica in San José. As part of the products derived from the analysis and review of the specimens deposited at the collection, multiple scientific and divulgative publications are included (more than 250 publications). Recently (2015), an accessory collection of otoliths and tissues, was initiated, this with the aim of complementing the extant information and to promote future applied works; they are composed by more than 500 otholit pairs (from 60 species) and 1750 tissue samples (from 160 species). The collection and specimens data are available for consultation in digital format directly contacting those responsible for the collection.

Key words: Freshwater fishes; Marine fishes; Central America; Neotropical region Financial support: No applies



# Fish collections in Uruguay: more than a hundred years of history

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The main ichthyological collections currently active in Uruguay are those from Museo Nacional de Historia Natural (Ministerio de Educación y Cultura, Acronym MHNM) and Facultad de Ciencias (Universidad de la República, Acronym ZVCP). MHNM collection history began in the XIX century. The Museum was funded in 1837 and has passed through many cycles alternating intense and rich research activity and stasis. Unfortunately, specimens worked by D.A. Larrañaga were not catalogued and are lost. The probably older fish lots comes from late nineteen century due to the work of C. Berg. Today the Museum preserves 4000 catalogued lots plus several hundred still uncatalogued; 28 are holotypes, 21 paratypes, and five syntypes. Most records come from Uruguay (83%). Freshwater fish families represent 83% of the collection; the most common are Characidae (34%), Rivulidae (14%), and Cichlidae (12%); the hydroaraphic basins represented are the Uruguay river (44%), Río de la Plata (36%), Laguna Merin (16%), and Atlantic Ocean small drainages (4%). Among marine families, Sciaenidae presents the highest number of records (7%). On the other hand, for ZVCP the older lots come from 1940 decade, due to the research activities of R. Vaz-Ferreira (Facultad de Humanidades y Ciencias at that time). Despite it shorter life and a period of abandonment (from the late seventies to the late nineties), this collection has currently 13513 lots catalogued; 97% freshwaters fishes and 96% from Uruguay. The collection holds 14 holotypes and 15 paratypes, all of freshwater species. The most represented freshwater families are Characidae (34%), Cichlidae (15%), and Loricariidae (9%); the hydrographic basins represented are the Uruguay river (44%), Laguna Merin (20%), Río de la Plata (18%), and Atlantic Ocean small drainages (8%). Scigenidae is also the most recorded group (24% of records) among marine families, followed by Engraulidae (11%), Batrachoididae (8%), and Myctophidae (6%). The main challenge for both collections still relies on the need to have permanent technical positions as a key issue for its curation and development.

Key words: La Plata basin, Laguna Merín basin, Southwestern Atlantic



# The fish collection of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) of Mar del Plata, Argentina

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The fish collection began in 1970, in the ex Instituto de Biología Marina (currently INIDEP) under the direction of Dr. Fernando Cervigón, eminent Spanish ichthyologist established in Venezuela. The samples come mainly from the research cruises carried on by the INIDEP but there are also those delivered by fishing vessels crew and recreational fishermen. The specimens are fixed in water with 10 % formaldehyde and then preserved in a solution of 75 % ethyl alcohol and 25 % distilled water. The collection acronym is INIDEP. The principal origin area is the Southwestern Atlantic continental shelf between 34° - 55° S and adjacent areas, but it also contains samples from South Georgia and South Shetland Islands, Southern Ocean. The data are digitized and they have been entered into the Sistema Nacional de Datos Biológicos (http://datos.sndb. mincyt.gob.ar/), Ministerio de Ciencia, Tecnología e Innovación Productiva de la Nación. The holotype and 16 paratypes of Bathyraja cousseauae Díaz de Astarloa & Mabragaña, 2004, the holotype and 9 paratypes of Dipturus argentinensis Díaz de Astarloa, Mabragaña, Hanner & Figueroa, 2008 and 1 paratype of Myliobatis ridens Ruocco, Lucifora, Díaz de Astarloa, Mabragaña & Delpiani, 2012 make all part of the collection. The collection contains 376 species of 164 Families representing 64 % of the total number of species presently known as occurring in the abovementioned Southwestern Atlantic sector. The collection contains 58 species of Antarctic origin (members of the Families Nototheniidae, Bathydraconidae, Chanichthyidae, Liparidae, among others), they constitute 21,3 % of the species mentioned by Gon & Hemstra for the Southern Ocean. On the continental shelf and adjacent areas 41 of those species have been captured, the rest (16 species, corresponding to 5 Families) come from the Southern Ocean.

Key words: Argentine Sea; Southwestern Atlantic; Southern Ocean



# The Ichthyological Collection at the Museu de Zoologia da Universidade de São Paulo, Brazil

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The birth of the Ichthyological Collection at MZUSP dates back to the late 19th century, predating the founding its current institutional affiliation. The growth of the collection was modest but steady in its early years until the 1920's. Growth was stalled during subsequent decades, but the situation changed dramatically in the 1960's. That was when curators Heraldo Bristki e Naércio Menezes were hired and the field program EPA started, triggering eight years of intense collecting of Amazonian fishes. Important additions to marine fish holdings happened in the late 1960's and early 1970's, with the hiring of curator José Lima de Figueiredo and the expeditions of the oceanographic vessel 'Prof. W. Besnard' along the Brazilian coast. By the middle of the 1970's, the collection comprised in excess of 200,000 specimens, an order of magnitude larger than its pre-1960 figures. In 1969, the museum was incorporated into the Universidade de São Paulo and the three fish curators then in the staff became Professors, starting their careers as academic advisors at the undergraduate and araduate level. Continuous research activity by those professors and their students resulted in continuous inflow of material to the collection. In this period, the Fish Sector of MZUSP achieves international relevance and establishes long-term partnerships with foreign institutions. In the 1980's, a landmark addition was the incorporation of a large collection of Amazonian fishes built by M. Goulding. In the same period, Osvaldo Oyakawa is hired as Technician and Collection Manager. Informatization and databasing of the collection begins in the following decade. Among various projects active in the years 1990's and 2000's, PRONEX, generated enormous amounts of ichthyological material from different localities in the country. Other important projects were Calhamazon and ReviZEE. Mário de Pinna was hired as Professor and Curator in 2000 and expands the scope of the collection to other continents, especially Africa and Asia. Recent hirings include Michel Gianeti (2010) as Collection Technician and Aléssio Datovo (2014) as Professor and Curator. Large amounts of material from Amazonian drainages resulted recently from the SACI Project. In the course of its 130-year history, the Ichthyological Collection at MZUSP has grown to include more than 120,000 lots and 1,5 million specimens (5,000 types), now fully computerized, belonging to 350 families, 1,800 genera and 9,000 species. Ca. 80% of lots are from freshwater species and mostly from Brazil. The collection receives ca. 85 research visitors per year and sends large amounts of material on loan to other institutions (ca. 120 invoices/year and 12,000 lots currently on loan). Those figures make the Ichthyological Collection at MZUSP the largest in Latin America and one of the most important in the world for neotropical taxa.

Keywords: Curatorship, Ichthyofauna, Neotropical



# A new system of organization of samples for molecular studies in the fish collection of MZUSP

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Over 122 thousand lots are catalogued in the fish collection of the Museum of Zoology of the University of São Paulo (MZUSP), the largest fish collection in Latin America. Traditionally devoted to biodiversity and systematic studies based mainly on morphology, the physical and informational organization of the collection was optimized according to that tradition. Recently, the collection was expanded to incorporate tissue samples or whole fishes fixed in ethanol for molecular studies. Today, thousands of DNA samples of many different taxonomic groups are deposited in the freezers of the institution. However, a great part of this material was stored without standardized procedures: i) not catalogued as such and/or ii) heterogeneously preserved or organized. Consequently, much of that material was difficult to access by the scientific community. In order to change that situation, a new method of sample management has been designed and implemented since October, 2016. The basic principles of this system are: the absolute correlation sample-voucher (individuals as operational units); the partial independence from the "main collection" (lots as operational units); and the optimal costbenefit relative to physical space, lab material and staff efforts. Our system accommodates both fragments and whole specimens. Three kinds of containers are used to preserve these samples, depending on their size: 5 ml eppendorfs, 15 ml falcon tubes and 50 ml falcon tubes. Every fragment (and correspondent voucher) or individual receives an standardized ID (number preceded by the acronym MZict) and is stored in the freezer following the MZict numerical sequence, into boxes suitable for each kind of container. Internally, each container has a label with its MZict number. Externally, containers are labelled with color-coded MZict, MZUSP catalog, and field numbers in stickers on top and lateral surfaces. Each MZict code is also cross-referenced with the original lot in the main collection database in Specify, which also indicates the type of vial containing each sample. The physical organization of the samples can be promptly checked with a "map of eppendorfs" without the need to refer to the Specify database, so that positioning mistakes are visually evident to users. Over 2100 samples are already entered in the new system, belonging to the orders Siluriformes (45% of the samples), Characiformes (37%), Labriformes (7%), Cyprinodontiformes (6%), Cypriniformes (4%), Gymnotiformes (2%), Anguilliformes, Clupeiformes, Gasterosteiformes, Petromyzontiformes, Tetraodontiformes, and Synbranchiformes (less than 1% each). Space efficiency was at least 33% superior over previous arrangements. Our system of organization is an efficient alternative for collections with large backlogs of uncatalogued molecular samples of heterogeneous nature, especially when those samples comprise both whole specimens and tissue samples.

Key words: tissues; sample management; Specify; DNA; genetics; Neotropical; curatorship Financial support: (HRV) CNPq 150304/2017-0; (TSL) Fapesp 2017/07284-5; (ADS) FAPESP 2016/19075-9; (MCCP) FAPESP 2015/26804-4, 2014/11397-1, 2013/18623-4, 2016/25467-7; CNPq 308962/2013-3



# Coleção Biológica do Instituto Oceanográfico da Universidade de São Paulo "Prof. Edmundo F. Nonato" (ColBIO): the collections of ichthyoplankton obtained from Brazil

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The Coleção Biológica do Instituto Oceanográfico (ColBIO) "Prof. Edmundo F. Nonato" was initiated in 2012 and represents now adays one of the largest collections of marine ichthy oplankton from the western South Atlantic. Initially, the purpose of the ColBIO was to aggregate and organize the collections obtained during the several oceanographic expeditions on board of the research vessels hosted at the Instituto Oceanoaráfico of the University of São Paulo (IO-USP). Most part of the samples is composed by specimens collected by the Brazilian Research Vessel Prof. W. Besnard between 1968-2006 – over 8,200 oceanographic stations –, mainly off the Southern and Southeastern Brazil coast. However, the ColBIO also hosts samples obtained from off the Central, North and Northeastern coast of Brazil and oceanic islands during several research and environmental projects. The lots are being catalogued with the use of the software SPECIFY 6 to manage species and specimen data for biological research collections. Each lot contains information about the project name and research on board, field station, research vessel, gear and size of mesh, coordinates, depth, date and time; and some of them also includes data of salinity, temperature of water at surface and temperature of water at depth of sampling. The ichthyoplankton collection, currently with 45.680 lots, is organized taxonomically, with 21.8% of the lots identified to the species level, 12.8%, to the genus level, 60.6%, to the family level, and only 4.7%, to the order level. The lots include specimens from 27 orders, 126 families and 208 species, being the Perciformes and Clupeiformes the most common, corresponding to 34% and 14% of the total, respectively. The ColBIO is also fully equipped with stereomicroscopes, computers and high-resolution photography equipment to process and acquire images. So far, 150 specimens were photographed and the images were also incorporated to the database. Throughout the years, ColBIO has been used mostly by students and professors of the IO-USP in publications and academic works, but now it is time to make it available for the entire scientific community. The next goal is to make the database available on-line for searches. Visitors interested in helping to identify the specimens and develop research projects are very welcome.

Key words: Fish larvae; Western South Atlantic; Marine Environment, Oceanography Financial support: FAPESP (Process 2014/15168-7), PETROBRAS (4600249986 e 4600237181), FUNDESPA, CNPq Universal (Process 433050/2016-0)



# Contributions of The Núcleo de Pesquisa em Ictiologia da UFAC, Campus Floresta for the extreme Western Brazilian Amazon ichthyofauna research

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The Amazon drainage is one of the most diverse in fish species richness, but the ichthyofauna of some peripheral regions as the extreme Western Brazilian Amazon are poorly know. Since the Núcleo de Pesquisa em Ictiologia da UFAC Campus Floresta (LBA-I) implantation there was a significant increment in the ichthyofaunal knowledge of an almost not sampled Amazon region, with improvement on the list of species for Acre state and their geographical distribution and data available in the Coleção Sinóptica de Peixes do Vale do Alto Juruá (CSPVAJ) and the ichthyologic collection of the Museu de Zoologia da UNICAMP (ZUEC). In 2014 the LBA-I starts the first ichthyology researches emphasizing the identification of the commercial fishes sold in the Cruzeiro do Sul, AC, municipal market and the species consumed in the Reserva Extrativista Riozinho da Liberdade, AC (RESEX) totalizing 60 identified species, some of those exemplars became the first records of the LBA-I collection, therefore, since 2015, the LBA-I dedicated its efforts to develop the CSPVAJ both in wet and dry storage, to training undergraduate students in collect, identify, recognize and learning the importance of the taxonomical, museological and curatorship works and to contribute with renowned Brazilian scientific collections like ZUEC and Museu de Zoologia da Universidade de São Paulo (MZUSP), with collected material. Until the present the LBA-I participated in two expeditions in partnership with USP, and UFSCAR-Sorocaba to the Parque Nacional da Serra do Divisor where for the first time an ichthyologist's team sampled 1800 specimens from 18 different localities. Until now 25% of the samples were identified totalizing 37 species, some of which never registered in previous studies for the Juruá River and its tributaries. All the material will be deposited in the MZUSP ichthyologic collection that presents only one register from this region. The second expedition was performed in 2016 in collaboration with ZUEC that collected 1435 exemplars from 14 different localities in the municipalities Cruzeiro do Sul and Mâncio Lima, Acre state and Guajará, Amazonas state totalizing 71 species. In addition, all the further and later samples collected by the LBA-I until 2016 from the current institutional project "Peixes do Vale do Juruá, AC: levantamento da ictiofauna dos Rios Juruá, Moa e Liberdade", totalizing 6151 exemplars, were deposited in the ZUEC what render this collection the most representative for the extreme Western Amazon ichthyofauna, with 7586 exemplars, 93 identified species and accurate locality registration from 2015. So the contribution of the LBA-I to this collection represents 70% of all records for the Vale do Alto Juruá since 1973.

Key words: Freshwater fishes; Inventory; collection; Western; Vale do Alto Juruá Financial support/ Acknowledgment: FAPAC, CNPQ, ICMBIO, PNUD and UFAC



## Neotropical fishes at the Biodiversity Research and Teaching Collections of Texas A&M University

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The Biodiversity Research and Teaching Collections (formerly Texas Cooperative Wildlife Collection; TCWC) at Texas A&M University, College Station, maintains the largest collection of vertebrates in the state of Texas (USA) divided across four sub-collections: (1) Collection of Fishes; (2) Collection of Reptiles and Amphibians; (3) Collection of Birds; and (4) Collection of Mammals. The Collection of Fishes at Texas A&M currently maintains ~784,300 specimens, representing 3,880 species, 1,535 genera, and 360 families of fishes, distributed across 52,095 catalogued lots. Only a handful of these specimens are nomenclaturally important, including five holotypes and one neotype. At present, the Collection of Fishes at the BRTC includes over 15,000 specimens of freshwater and marine fishes from the Neotropics, including South America (11 countries), Central America (6 countries), and the Caribbean (5 countries). Highlights of this modest collection of neotropical fishes include: (1) ~4000 specimens of freshwater fishes from the Orinoco drainage (Venezuela); (2) ~1000 specimens of freshwater fishes from the Brazilian Amazon (Pará state); and (3) ~100 batoids representing nine families from freshwater and shallow coastal marine areas (Atlantic and Pacific) of South America. These specimens were collected through the research activities of faculty, staff, and students associated with the Department of Wildlife and Fisheries Sciences at Texas A&M University, are searchable online via VertNet and Fish Net 2, and are largely underutilized by the scientific community.



## Freshwater fish collection of the Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH-P), Colombia

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The freshwater fish collection of the Instituto Humboldt (IAvH-P) was founded in 1971 in Bogotá and then moved to its current location in Villa de Leyva in 1995. The IAvH-P collection is one of the largest ichthyological repositories in Colombia with more than 16000 catalog lots entirely systematized in the Specify sofware, representing around 2000 unique sampled localities across all main river systems (Amazon, Orinoco, Magdalena-Cauca, and Caribe and Pacific versants) and all political departments of the Colombian territory. In terms of taxonomic coverage of the native Colombian ichthyofauna, almost 990 species are represented which accounts around 66% of the total number of recorded species for this country. The IAvH-P collection also holds specimens from other Neotropical countries (Brazil, Costa Rica, Ecuador, French Guiana, Guyana, Panama, Peru, and Venezuela) and an important number of type specimens of more than 60 nominal species, with 13 of them represented by their respective holotypes. Recently, the electronic version of the catalog of type specimens of IAvH-P was made accessible online along with high resolution photographs of these specimens. Catalog information of records at IAvH-P is available in the Darwin Core format through the Colombian node of the GBIF, the SiB Colombia. Currently, we are developing an online database to search and access the catalog data and associated multimedia content (photographs, radiographs, links to published DNA secuences, and files of bibliographic references) in a single website.

Key words: Catalog of types; Ichthyological collection; Darwin Core; Online database; Specify



# The fish collection of the Laboratório de Ictiologia de Ribeirão Preto (LIRP), FFCLRP, Universidade de São Paulo (USP), Ribeirão Preto, São Paulo, Brasil

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The collection holdings started to be gathered in 1982, with the hiring of RMCC by the Departamento de Biologia at the University of São Paulo (USP) Ribeirão Preto, SP, Campus. Later, in 1984, a full time and exclusive LIRP dedication Biologist, Hertz F. Santos, was also hired. From 1986 a 1987, RMCC was a Predoctoral Fellow in the Division of Fishes of the National Museum of Natural History, Smithsonian Institution, Washington, D.C., under the advisorship of Richard P. Vari. That was the beginning of a prolific and long-lasting collaboration between LIRP and the Smithsonian Institution, mostly through R. P. Vari, that gave, since them to almost until his recent demise, great and unflagging support to LIRP's infrastructure and collection building efforts and also student training. In 2002 FAB was also hired by the Departamento de Biologia at USP Ribeirão Preto, SP, Campus, having become co-responsible by LIRP and co-Curator of its fish collection since them. Until 1997, the fish collection received mainly fishes collected in southeastern Brasil, mostly during joint collecting expeditions with the Division of Fishes of the Smithsonian Institution. Between 1998 and 2004, LIRP was a member of the PRONEX-97 Project "Conhecimento, Conservação e Utilização Racional da Diversidade da Fauna da Peixes do Brasil" (FINEP/CNPq), Coordinated by Naércio A. Menezes (MZUSP), and the Thematic FAPESP BIOTA Project "Diversidade de Peixes de Riachos e Cabeceiras da Bacia do Alto Rio Paraná no Estado de São Paulo, Brasil / Fish Diversity of the Headwaters and Streams of the Upper Paraná River System in State of São Paulo, Brazil", Coordinated by RMCC. Consequently, there was massive expansion in both LIRP's infrastructure and fish holdings. Therefore, in the second semester of 1999, LIRP was officially inaugurated by the University of São Paulo (USP), with a 260 m<sup>2</sup> area exclusively dedicated to its fish holdings. Between 2015 and 2017, LIRP's fish collection (http://splink.cria.org.br/manager/detail?setlang=pt&resource=LIRP), on the average, received eight (08) visitors and loaned 38 lots per year. Of its current fish holdings, 4% are from marine and 96% from freshwater habitats, and most of the lots are from Brasil, Upper Rio Paraná, Rio Tapajós and Rio Madeira drainages. For cataloging purposes Specify 6.5.05 is used, for a total number of 15.083 lots and 159.822 specimens, 15.069 and 159.798 of which from the Neotropical Region, respectively, in addition to 33 holotypes, 128 paratypes, 74 tissue samples, 405 C&S specimens, and 42 dry skeletons, almost all, but not exclusively, from the Neotropical Region.

Key words: collection building, USP, LIRP, Brasil Financial support: CNPq, CAPES, FAPESP, MCT and Smithsonian Institution



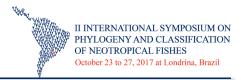
# The fish collection of the California Academy of Sciences

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The Department of Ichthyology houses one of the largest and most important research collections of its kind in the world. There are more than 200,000 cataloged lots containing roughly 1.2 million specimens, with thousands of backlogged specimens still to be processed (mostly from tropical marine habitats). There are representatives of nearly 11,000 nominal species of fishes in the collection, representing more than a third of those known to science. The strengths of our holdings lie principally in marine fishes of the western and central Pacific, California, and freshwater fishes from South America and southeastern Asia. Our older historic collections from North America and the large number of type specimens are of particular importance to the ichthyological community. Our type collection contains over 37,000 specimens in over 6,000 lots, of which almost 2,000 are primary type lots. The type database consists of names that were published using CAS, Stanford University (SU), or Indiana University (IU) specimens as primary types (holotype, syntype, lectotype, or neotype). Most of the Indiana collection was acquired by the Academy in 1928, although part of the collection, including some types, were given to the Carnegie Museum and later transferred to the Field Museum of Natural History. The entire Stanford collection came to the Academy in 1970. The collection database is hosted at http://www.calacademy.org/scientists/ichthyology-collections, and includes photos and x-rays of our almost 2,000 holotypes.

Key words: Ichthyology, Vertebrates, Systematics, Taxonomy Financial support: California Academy of Sciences, National Science Foundation



# The regional collection of fishes in western drainages of Venezuela

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The Colección Regional de Peces (CPUCLA) was formed in 2002 with the support of the Faculty of Agronomy and Scientific Council of Lisandro Alvarado University (UCLA) in Barquisimeto (Lara, Venezuela). The primary objective of the collection is to generate and protect samples of fish species and records of coastal fluvial ecosystems, as well as the creation of databases and the promotion and development of research, extension and divulgation for the management of hydrobiological resources. The main line of research is the biogeography, integrity and conservation of the continental ichthyofauna. The geographic coverage includes the coastal rivers to the Caribbean Sea, Lake of Maracaibo and western plains of the Orinoco. Currently the collection protects wet samples of fish and macroinvertebrates (insects and crustaceans) and environmental records generated in standardized samplings. To date, the collection covers 80% of the watersheds and rivers in the Western Caribbean Province of Venezuela and practically all the fish species in this biogeographic entity, in addition to having extensive records of the ichthyofunas in other regional drainages. Currently the collection there are more than 5000 numbers (~ 40,000 fish specimens, 135 species). The collection has contributed to the development of interinstitutional projects with national scope and grade practices and postgraduate theses, thus generating information on fish biodiversity and integrity in river ecosystems, the risk category for the ichthyofauna (Red Books) and the relevance of protected areas for conservation. Currently, the collection develops academic editorial series (Rivers at Risk of Venezuela and Coastal Fishes of Venezuela) that are supported by the digital platform (www.ucla.edu.ve/museopeces) for their free and continuous access. It is planned to set up a regional laboratory for river biomonitoring and consolidate its training and divulgation projects. However, it is now essential to obtain resources to maintain the collection, prevent deterioration and ensure the protection of its biological samples.

Key words: Fish collection; Coastal freshwater fishes; Conservation, Venezuela



# Colombian Fish Biodiversity, an integrative insight from the Neotropical collection perspective and the Darwin Core standard

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Colombia is a country that has the privilege of harboring an enormous biodiversity, being one of the countries with the greatest fish richness in the world. However, the known numbers that support and represent this diversity originate mostly from scale lists that provide information on the number of species distributed in the national territory and from occurrences published in the Darwin Core (DwC) standard through Colombian Biodiversity Information System (SiB Colombia), a GBIF node. One of the best sources of information on richness and biodiversity in a territory are biological collections, which have been concentrating their efforts along the years to collect and store biological records on a periodic basis. Our effort focused on taking 85,000 species occurrences from 11 Colombian ichthyological collections and making a thorough methodology for cleaning both taxonomic and geographic information and thus obtaining a high quality of fish biological data. The process consisted of six steps: adequacy to the DwC standard, cleaning of names and dates, cleaning of geographic information, point-radius method for georeferencing, taxonomic cleaning and taxonomic validation by river basins. Finally, all data sets were published through the Integrated Publishing Toolkit of SiB Colombia and GBIF, and are available online. This initiative is presented as a useful and adoptable pilot model in order to increase the knowledge of the actual, and in many cases unknown, biodiversity present in the different biological collections of the country. The data published throughout this process represent the highest quality at the taxonomic and geographic level, and will serve not only as an input for decision making but as a real representation of the Colombian fish biodiversity, revealing the taxonomic status of these collections and thus showing the curatorial needs that they require. This endeavor also served as input for the update of the Checklist of the freshwater fishes of Colombia and it is the backbone for the nascent Catalog of fishes of Colombia.

Key words: Biodiversity; Freshwater; Neotropical; Taxonomy; Open Data

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# Mini-Symposia



## An overview on the study of Cheirodontinae (Characiformes: Characidae)

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The Cheirodontinae, as we know today, is a group of minute characid fishes with adult specimens ranging from 25-40 mm in standard length, and also nearly seven miniature species reaching sexual maturation under 20 mm. They are found in lentic to stagnated waters in most river drainages from Costa Rica in Central America to Argentina in South America, including Trans-Andean basins in the western slopes of Chile and Colombia. Doubts about the monophyly of the Cheirodontinae and some of its genera existed since its creation, when Eigenmann himself, and following taxonomists, have frequently questioned the artificial nature of the group. The monophyly of the Cheirodontinae was first tested by Malabarba in the first cladistic analysis regarding the group including 21 taxa and 72 characters. Based on Malabarba's results the subfamily was found to be monophyletic in a more strict sense than those of Eigenmann and Géry, containing only 15 genera. Additionally, for the first time, the subfamily, as well as several clades that resulted in tribes (Cheirodontini and Compsurini) and genera, were well supported by synapomorphies that could be tested now in further analysis. In this work, we present an overview on the studies concerning the subfamily's diversity, biology, anatomy and systematics on the last 20 years, since Malabarba's work. Cheirodontinae includes now 19 genera and 70 species, and since that time the monophyly of the subfamily, its tribes and genera has been tested by many authors resulting in incongruities between molecular and morphology-based hypotheses. Sharing the same database of cheirodontine sequences, molecular-based hypotheses consensually agree in Spintherobolus as sister group of all other characids and Prodontocheirodon pi as sister group of all remaining cheirodontines. In the present molecular-based concept, Cheirodontinae is divided in four tribes, Protocheirodontini, Pseudocheirodontini, Cheirodontini and Compsurini, and most of the genera stablished by morphology are now paraphyletic. Considering this scenario we present a morphology-based phylogeny for 78 species of Cheirodontinae built on 278 characters including external morphology, osteology, miology and ultrastructure of spermatozoa. The method of parsimony implemented in the software TNT using "implied weights" and "parsimony ratchet" algorithm set to 100 iterations and 100 replications was used. As outgroup, 23 other characids were included with Brycon pesu in the root. Cheirodontinae and its genera were found to be monophyletic and arouped in three tribes: Cheirodontini, Compsurini and Pseudocheirodontini. Beyond systematic studies, we conclude that although most cheirodontines are not rare once they are commonly listed in ichthyofaunal inventories, studies investigating aspects of reproduction and trophic dynamics are so far restricted to a small number of species, only 12.8% of all subfamily. A single behavior study was published for the representatives of Cheirodontinae, however the presence of insemination and studies on secondary sexual characters was investigated in nearly 38.6% of all species.

Key words: Systematics; Taxonomy; Freshwater; Neotropical Financial support (FCJ): CNPq (process 453850/2014)



#### Macroevolution and morphological diversification of anostomoid fishes as revealed by new comprehensive molecular and morphological phylogenies

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The approximately 150 fish species in the characiform family Anostomidae exhibit remarkably diverse head and jaw morphologies, varied diets and disparate color patterns, while three closely related families (Curimatidae, Prochilodontidae and Chilodontidae) appear to have diversified much less in external morphology, diet and coloration, despite possessing many species. Does anostomid ecomorphology truly exceed that of their close relatives, or have the other portions of the radiation diversified along less apparent morphological dimensions? Using ultraconserved elements-based phylogenomics, multilocus Sanger-sequencing, and a densely sampled osteological supermatrix, we inferred phylogenies spanning approximately 65% of the species in the superfamily. Thought substantially congruent, these phylogenies also indicate some intriguing systematic discordances between the datasets, particularly with respect to the placement of the enigmatic family Chilodontidae, and the position of the superior-mouthed fishes of subfamily Anostominae within Anostomidae. By linking those phylogenies to geometric morphometric data, we reconstructed the diversification of head, jaw and gill arch shape in these fascinating fishes and fit alternative models to their evolutionary history using MVMORPH and other approaches. All four families clearly occupy distinct morphospace regions in the head and gill-arch datasets, and each likely sits upon at least one unique adaptive optimum, suggesting that the early history of the radiation involved morphological exploration and establishment of the four distinct bodyplans currently recognized as families. After that initial diversification, patterns of evolutionary lability and constraint differed considerably among families. Anostomid morphology evolved guickly and without constraint in the head and jaws, and if the molecular phylogeny is correct, this family repeatedly evolved species with terminal, inferior and superior mouths. Anostomid gill arches also diversified relatively rapidly, but with a strong pull towards a morphological optimum that resulted in a more muted diversification in that character system. Curimatids and chilodontids, on the other hand, evolved new morphologies more slowly, but with almost no constraint in gill arch morphology. As a result, their standing aill arch diversity approaches or exceeds that of the Anostomidae despite the latter family's higher rate of evolutionary change. The most powerful constraints were inferred among the universally detritivorous and largely allopatric species of Prochilodontidae, suggesting that these highly specialized species are ecological equivalents occupying an exceptionally strong adaptive peak. Overall, while Anostomidae did indeed evolve the greatest external diversity of the four families, the rest of the superfamily evolved substantial internal diversity. As foreshadowed by the numerous systematically informative characters peppered throughout the bones of the gill arches in Rich Vari's landmark osteological treatises on Curimatidae and Chilodontidae, much of their true beauty lies within.

Key words: Characiformes, Disparity, Ultraconserved Elements, Systematics, Morphometrics Funding: NSF Grant DEB-1257898 and FAPESP 2011/08374-1



### Advances on the systematics of Pseudopimelodidae (Siluriformes: Pimelodoidea)

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Pseudopimelodidae is a family of catfishes with seven genera (Pseudopimelodus Bleeker, 1858; Batrochoglanis Gill, 1858; Lophiosilurus Steindachner, 1889; Cephalosilurus Haseman, 1911; Microglanis Eigenmann, 1912; Cruciglanis Ortega-Lara & Lehmann, 2006; Rhyacoglanis Shibatta & Vari, 2017) and 48 species. Its distribution includes the trans-Andean region of Colombia and Ecuador, and the main hydrographic basins of Cis-andean region (Maracaíbo, Orinoco, Amazon, São Francisco, La Plata, and coastal drainages of Guyanas, Surinam, and Brazil). Species of this family is easily recognized by a combination of morphological characters: head and mouth wide, eve covered by skin, short maxillary barbel, pectoral-fin spine well developed, serrated on anterior and posterior margins, and body with dark blotches, at least when juveniles. Analyses based on molecules and gas-bladder morphology support the close relationship of Pseudopimelodidae with Pimelodidae within Pimelodoidea, and the family monophyly was recognized with osteology. The relationships of genera are partially resolved, but Lophiosilurus and Cephalosilurus deserve more attention. Different analyses point out that both genera are sisters, but the relationships among Cephalosilurus species are unknown. In order to fill this gap, all species of Cephalosilurus are analyzed with a matrix of 44 morphological characters and 18 terminals, using implied weighting (k = 7.4118) and traditional search in TNT. The strict consensus tree (CI = 0.55; RI = 0.74) shows Lophiosilurus alexandri as sister group of Cephalosilurus fowleri, both species endemic to the Rio São Francisco, and this clade sister of (C. nigricauda (C. apurensis, C. albomarginatus), with species from the Orinoco basin (C. apurensis) and coastal rivers of the Guyanas (C. nigricauda and C. albomarginatus). The monophyly of this clade is supported by the following characters: postcleithral proccess short, broadly covered by opercular membrane, caudal fin emarginated, and presence of lateral trabeculae on internal T-shaped gas-bladder septum. The results highlight Lophiosilurus and Cephalosilurus as non-monophyletic groups. Because Lophiosilurus is the oldest name, all species of Cephalosilurus must now be rearranged accordingly: Lophiosilurus fowleri (Haseman, 1911), L. albomarginatus (Eigenmann, 1912), L. nigricauda (Mees, 1974) and L. apurensis (Mees, 1978).

Key words: Catfishes; Cepahlosilurus; Lophiosilurus; new combinations Financial support: CNPq Research Productivity fellowship (Process 304868/2015-9)



### Phylogeny of Loricariinae (Siluriformes: Loricariidae) – updates on morphology-based analyses

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Despite being recognized as a monophyletic group for a long time by different authors, internal relations within Loricariinae genera still lack an agreement. Loricariinae comprises approximately 300 hundred species in around 28 genera. Since the first unpublished attempt to analyze the group cladistically using morphology (1997) until more recent molecular analyses (2016), new taxa have been described bringing more challenges to a more conclusive resolution to intergeneric relationships. The former morphology-based analysis came up with two major clades, named Loricariini, with the genera Metaloricaria, Spatuloricaria, Rineloricaria, Loricaria, Planiloricaria, Crossoloricaria, Apistoloricaria, Loricariichthys, Pseudoloricaria, Limatulichthys, Hemiodontichthys and Reganella, and Harttiini with Harttia, Farlowella, Sturisoma, Sturisomatichthys, Lamontichthys and Aposturisoma. The cited molecular analysis also came up with two major clades: Loricariini, with basically the same genera above plus Farlowella, Sturisoma, Sturisomatichthys and Lamontichthys, and Harttiini, with one single genus, Harttia. In addition to that, recent revisions of different genera of Loricariinae brought new insights and more accuracy on hypotheses of relationships. As the most inclusive hypotheses of relationships, morphological/1997 and molecular/2016, showed different results, we aim to conduct a phylogenetic analysis incorporating new available taxa. The present contribution is part of a large project, still in progress, that has, as the main goal, to consolidate the morphological tendencies in the subfamily and to understand the relationships of new taxa within Loricariinae. Based in our preliminary analyses so far under PAUP, using ordinary Heuristic Search, the two major clades (Loricariini + Harttiini – senso morphology) originally found came up again as the principal result. The clade representing Harttiini was shown to include other genera besides Harttia, differently from the molecular results available so far. Fonchilloricaria, however, concur with same basal position shown in the molecular analysis: Fonchiiloricaria came as an independent branch, sister group to the remaining Loricariini. More analyses though are going to be conducted (under TNT and PAUP again) in order to clarify if these two large evolutionary lineages are morphologically consistent.

Keywords: neotropical fishes, diversity, morphology



### Phylogenomics of the order Characiformes using ultraconserved elements

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The megadiverse ostariophysan order Characiformes includes over 2100 species in Africa, Central and South America ranging in size from miniature Neotropical tetras to the huge African tigerfish. Recent molecular phylogenetic studies have focused on the inter- and intrafamilial relationships of several characiform lineages, which previously had received only morphological attention. Notwithstanding these advances, the position of many characiform families remains uncertain, as do their intergeneric relationships. These uncertainties demonstrate the need to reexamine all characiform families via robust molecular datasets and phylogenies. Next-generation sequencing and phylogenomics allow for unprecedented resolution of relationships within many groups across the Tree of Life. One such method uses ultraconserved elements, which can be found across the genome and are, as the name implies, highly conserved across a broad taxonomic range. These elements are flanked by variable nuclear regions, which can be enriched, sequenced, and analyzed using phylogenetic methods. Using a probeset capturing approximately 2500 ultraconserved elements, we present a new family-level molecular phylogeny for the order Characiformes containing a high-taxon sampling. The 75% complete matrix have 1327 loci with 345.179 bp spanning 356 terminal taxa. Our results return a well-supported topology that is congruent with previous multilocus and genomic datasets. The preliminary analysis returns the monophyly of the clade Citharinidae+Distichodontidae as sister to a larger clade containing the Characoidei+Siluriformes. A linearized topology contains Crenuchidae and successive clades such as the African families Alestidae+Hepsetidae and another large clade with Erythrinidae sister to Cynodontidae and this family sister to Hemiodontidae+Serrasalmidae. These two later families appeared to be sister to the clade containing Parodontidae sister to Anotomoidea (Anostomidae (Prochilodontidae (Chilodontidae+Curimatidae))). In the other side of the tree, Ctenoluciidae is sister to Lebiasinidae and this clade sister to a large clade that has successive lineages such as Chalceidae, Iguanodectidae, Acestrorhynchidae, Bryconidae, Gasteropelecidae, Triportheidae and Characidae. Internal resolution of Characidae permits the recognition of several new arrangements of clades. The genera Amazonspinther and Spintherobolus are the sister group of all remained Characidae. The Stevardiinae appeared sister to the clade B and these two clades sister to clade C. We discuss novel relationships and compare our results with previous molecular and morphological hypotheses. Most notably, the well-supported position of many families in early branches of the tree provides more robust hypotheses of ancient diversification resulted from the split of Gondwana.



#### Phylogenetic relationships within the South America fish family Hemiodontidae (Teleostei: Ostariophysi: Characiformes)

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The neotropical characiform family Hemiodontidae consists of 31 species commonly known as voadores, ubaranas, jatuaranas or piaus-banana. Hemiodontids are distributed throughout the basins of the rivers Amazonas, Orinoco, and Paraná-Paraguay, and the rivers of the Guyana, Suriname, and French Guiana. A hundred and seventeen morphological, mainly osteological, characters were described and analyzed for 38 terminals (32 hemiodontid species, excluding H. jatuarana and H. langeanii, not available, and including three supposed new Hemiodus species, and 6 taxa from other characiform groups), aiming to test the monophyly of the Hemiodontidae and of the currently recognized genera, and also to develop a hypothesis of the phylogenetic relationships among its species. The phylogenetic analysis was performed using Winclada v. 1.7 TNT (Nixon) to run TNT v.1.1 (Goloboff, Farris & Nixon). All characters were considered non-additive and received equal weights. To find the most parsimonious tree, a heuristic search was performed using new technologies (ratched, with 200 iterations, and drift, with 50 cycles), random addition sequences, 1,000 replicates and maximum retention of 10,000 trees; the tree was rooted between Crenuchidae and orher examined taxa; from the equally parsimonious trees a strict consensus tree was constructed. Biogeography hypotheses were generated after superimposing the known areas of distribution for each of the hemiodontid species analyzed to its respective branch. Forty two most parsimonious trees were found (361 steps, CI=0.42, RI=0.74). The monophyly of the Hemiodontidae and of the genera were confirmed; the recovered relationships among genera were: [((Anodus + Micromischodus) + Hemiodus) + (Argonectes + Bivibranchia)], contrary to an anterior hypothesis (Langeani) with Hemiodus as most related to Argonectes and Bivibranchia. Among Hemiodus species, the resolution was not complete and two politomies remained, one within the H. auadrimaculatus species group and another within the clade (H. argenteus, H. orthonops, (H. microlepis, H. parnaguae, (H. "longo" + H. "xingu")). Hemiodontidae ancestor seems to have originated in the lowlands of the Amazon river basin. After a first cladogenetic event spliting the Bivibranchiinae, with Argonectes and Bivibranchia, and the Anodontinae, with Anodus and Micromischodus, and the Hemiodontinae, with Hemiodus, the most inclusive species inside each of the major resultant subclades occur in the upper portions of the rivers, draining the Brazilian and the Guyanas shields, whereas the most basal ones are widespread along main river channels in the lowlands. This pattern is clearly seen in the two Hemiodus sister groups of species, one with species widespread along main river channels in the lowlands, and the other with species restricted to smaller areas in the upper portions of the drainages; same pattern could be also seen in the Bivibranchiinae. Hemiodontidae hypothesis presented here brings to discussion an example of group that initially diversified in the lowlands but secondarily invaded the shield areas, contrary to the main pattern for South America where "upland shield groups represent ancient distribution, since lowland foreland basin systems are new topographic landscapes relative to shield areas" (Lima & Ribeiro).



#### A phylogeny of the armored catfishes, with emphasis on the Neoplecostominae-Hypoptopomatinae clade (Siluriformes: Loricariidae): integrating phenotypical and molecular data

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Despite loricariids have been recognized as a group for more than a century, the taxonomic arrangement of its subfamilies is still unstable, particularly regarding the Neoplecostominae and Hypoptopomatinae. The composition of the former, in addition, has changed dramatically during the past century. Charles Regan in 1904 classified the less than 200 loricariid species into five subfamilies, recognising the Hypoptopomatinae as a group, and erecting the Neoplecostominae to include only Neoplecostomus. Bill Gosline in 1947 added a subfamily to the then 400 species classification, and expanded the Neoplecostominae to include 12 genera. Subsequently, Isaäc Isbrücker in 1980 rearranged the ca. 600 loricariid species into six subfamilies but again placed only Neoplecostomus in the Neoplecostominae. It was not until the present millennium, that Jon Armbruster in 2004 concluded, based on phylogeny using morphological characters, that some genera were indeed related to Neoplecostomus to form a more diverse subfamily. More recently, phylogenies based on DNA sequences found that Neoplecostominae and Hypoptopomatinae form a clade, corroborating morphology-based analyses. None of the molecular studies, however, recovered both subfamilies as monophyletic and sister-groups to each other, as previously recognized. During the last decade, five dissertations by the present authors have been completed on the phylogenetic relationships of the Hypoptopomatinae or Neoplecostominae, using either morphological or molecular characters. The results of those dissertations along with additional DNA sequences from species potentially important taxonomically were combined to form a total evidence data matrix, with the aim of searching for a more reliable phylogeny and stable classification for the clade Neoplecostominae + Hypoptopomatinae. The data matrix includes 153 taxa, 331 phenotypic characters from osteology, arthrology, and external morphology, and a molecular dataset from genes 16s, cytB, co1 (mitochondrial) and f-reticulon, rag1, and rag2 (nuclear). The concatenated matrix was analyzed under both optimality criteria of Maximum Parsimony, using a combination of New Technologies in TNT, and Bayesian Inference in MrBayes. Each partition (phenotypic characters and each individual genetic marker) were also analyzed separately to test their phylogenetic signal. Our results corroborate previously discovered relationships among loricariid subfamilies: (Lithogeninae (Delturinae (Loricariinae (Hypostominae (Neoplecostominae + Hypoptopomatinae), and some of the previous relationships in the two last subfamilies. This study intends to present a new classification for Neoplecostominae and Hypoptopomatinae, and provide diagnoses for the subfamilies and genera. Additionally, a revised compilation of morphological characters from various sources will be made available to future studies seeking a universal use of such a dataset.



### Phylogenetic systematics of the Driftwood catfishes (Siluriformes: Auchenipteridae): a combined morphological and molecular analysis

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The Auchenipteridae, a Neotropical family of inseminating catfishes, have an extensive and confusing taxonomic history with divergent proposed phylogenetic hypotheses. Several studies developed since 1766 generated controversial results about the composition of the family and its interrelationships. The composition of some genera remains controversial, mainly those of the Centromochlinae, due to the conflicting phenotypic analyses and absence of phylogenetic comprehensive reconstruction. In this study, a phylogeny of the Auchenipteridae is reconstructed with a large-scale taxon sampling based on combined, morphological and molecular datasets. The estimated phylogeny included most species of Auchenipteridae (114 species), as well as multiple members of siluriform families as outgroup (31 species of Ariidae, Aspredinidae, Cetopsidae, Diplomystidae, Doradidae, Mochokidae, and Pimelodidae) to embrace the diversity of forms within related catfishes. As the first large-scale phylogeny of Auchenipteridae, comparison between taxa included information from both morphology (261 characters) and mitochondrial and nuclear molecular markers (3490 characters) from five genes: col, 16S, rag2, myh6, and sh3px3. Trees were generated under different optimality criteria (Maximum Parsimony, Maximum Likelihood, and Bayesian Inference) and also with separate partition data to test the congruence of the hypotheses and the respective phylogenetic signal. A new classification for the family was inferred based on the MP reconstruction and is herein presented to bring the taxonomy more in line with the new phylogenetic hypothesis. The strict consensus tree corroborates the monophyly of the superfamily Doradoidea, the family Auchenipteridae, and its two subfamilies, Centromochlinae and Auchenipterinae. The classification increases the number of tribes in Auchenipteridae from two to nine, based on evidence of major groups within Auchenipterinae: Liosomadoradini, Trachelyopterini, Asterophysini, Auchenipterini, and Ageneiosini; and in Centromochlinae: Gelanoglanini, Gephyromochlini, Centromochlini, and Glanidini. On the other hand, Centromochlus, Glanidium, and Tatia were recovered as paraphyletic. To maintain the classification monophyletic, new genera are being described with a nomenclatural reorganization of the species of Tatia and Centromochlus. In addition, Glanidium leopardum is regarded as a distinct valid genus, resurrecting the available name of Gephyromochlus leopardum. Finally, the phylogenetic position of the intriguing monotypic genus Spinipterus was tested for the first time with a complete codification including osteological characters through a CT-Scan analysis and molecular data, revealing its closest relationship with Trachelyopterus. An estimate of the origin of the family, subfamilies, and genera are also proposed, as well as important divergence events of the family in the geological history of South America.



#### Phylogenetic relationships within the Characidae (Teleostei: Characiformes) using ultraconserved elements

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Characidae is the fourth largest family of bony fishes with ~1150 species after cyprinids, gobiids, and cichlids, respectively. The enormous species diversity derived from the complexity of body forms, dentition, and coloration provide a range of possible specializations to adaptation in distinct and variable Neotropical environments. However, evolutionary mechanisms involved in the characid diversification remain unclear, mainly due to the lack of a robust intergeneric and interspecific phylogeny. Conflicting hypotheses from morphological datasets containing incomplete taxon coverage or from multilocus datasets using Sanger sequencing approaches remain untested. Herein, we applied a phylogenomic method using ultraconserved elements (UCEs), highly conserved regions with variable flanks, which can be enriched, sequenced, and analyzed using phylogenetic methods. Our ingroup contained 366 terminals in a tentative to cover all hypothesized characid lineages. Outgroup contained all remaining 23 characiform families. We assembled reads in contigs, assigned them to each UCE loci and aligned them through softwares in the Phyluce package. We ran a maximum likelihood tree and 50 bootstrap replicates in RAXML. The 75% complete matrix had 1487 loci with 597.783 bp. The phylogeny resulted in strongly supported clades and largely agreed with previous molecular hypotheses. Amazonspinther and Spintherobolus form a clade sister to three major lineages: clade C sister to clade A + clade B. The clade A, or Stevardiinae, presented practically the same reconstruction that a recent molecular hypothesis with Eretmobryconini, Xenurobryconini, Glandulocaudini, Argopleura, Hemibryconini, Stevardiini, Lepidocharax+Planaltina, Creagrutini (including Caiapobrycon), and Diapomini (including Aulixidens, Boehlkea, Parecbasis and Rhinopetitia). Clade B contains Aphyocharacinae (including Cyanogaster) sister to Cheirodontinae and other clade with Bryconexodon sister to Exodon+Roeboexodon that, in turn, is sister to Characinae plus Tetragonopterus (including Jupiaba meunieri). The complexity of the relationships increases dramatically inside clade C. First to diversify is a new characid genus and, then, Trochilocharax. We found successive sister lineages such as Deuterodon clade (including Coptobrycon, Myxiops, Probolodus, Stygichthys and others), Paracheirodon clade (including Brittanichthys), the Rose Tetra clade (Hyphessobrycon micropterus, H. rosaceus, H. haraldschultzi, and others), Moenkhausia clade (including M. xinguensis, M. jamesi group, Hasemania, Nematocharax, and several Hemigrammus). Then, our results returned a clade with three species of Jupiaba plus Hemigrammus ora, and a clade with Ectrepopterus, Moenkhausia lepidura and Stethaprioninae. The phylogeny also evidenced Gymnocorymbus sister to the Hemigrammus clade (H. unilineatus, Pristella, M. collettii group and others), the Tyttobrycon clade (including Tucanoichthys+Priocharax), Jupiaba clade (J. poranga, J. abramoides and Astyanax anterior), the Hyphessobrycon clade (H. compressus, Macropsobrycon, and others), the H. agulha group, the Erythrocharax clade (including H. notidanos), and a clade with a new genus, Dectobrycon, Phycocharax, and other species of Hyphessobrycon. The phylogeny also returned Hollandichthys+Rachoviscus, the Thayeria clade, the Bario clade sister to Hemigrammus ocellifer group (including Bryconella and Parapristella), the Rhoadsiinae, the clade Astyanacinus+Grundulus, Ctenobrycon+Psellogrammus, and the large clade with numerous lineages of Astyanax (including Oligosarcus). Finally, we discuss various morphological congruencies that might contribute to better define monophyletic lineages and discuss putative rearrangements in the current and problematic classification of Characidae in light of the phylogenetic evidence.

Key words: Neotropical fishes, Next-gen sequencing, Phylogeny, Tetras Financial Support: FAPESP 2014/26508-3, 2016/11313-8, CNPq and NSF



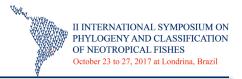
# Systematics of the Banjo Catfishes (Siluriformes: Aspredinidae): A comprehensive approach untangling a continental radiation of Neotropical freshwaters

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The Aspredinidae is a continental radiation composed of 45 valid species distributed in 13 genera. This group is found throughout major drainages of South America including: Magdalena, Maracaibo, Orinoco, Amazon, São Francisco, and Paraná-Paraguay. Aspredinids are guite peculiar catfishes, and their monophyly is well supported by many synapomorphies. This family is diverse in terms of size and range from miniaturized fishes (e.g., Micromyzon akamai, 16 mm SL), to medium-sized species (e.g., Aspredo aspredo, 380 mm SL). Aspredinid genera are found in shallow backwaters of small to large rivers or in deep riverine waters the latter exhibiting morphological adaptations for this stygian environment. Unusual, are the aspredinine genera Aspredinichthys, Aspredo, and Platystacus that live in the benthic tidal zone of brackish waters from Venezuela to Northeast Brazil. Aspredinidae was traditionally divided into three subgroups: Aspredininae, Bunocephalini, and Hoplomyzontini the last two tribes composing the subfamily Bunocephalinae. Alpha taxonomic revisions of Aspredininae and Hoplomyzontini established much of the current classification and composition of genera. In a previous phylogenetic study based on morphology, the monophyly of Bunocephalinae was rejected but agreement exists in supporting hoplomyzontins and aspredinines as monophyletic groups. A recently submitted molecular phylogeny of Aspredinidae, based on five gene fragments (mitochondrial: 16S and COI; nuclear: RAG1, myh6 and SH3PX3) partially support previous phylogenetic results using morphology and most genera are supported as monophyletic groups except for Bunocephalus. This phylogeny is based on an extensive sampling of 114 terminals that represent 31 species in 12 genera, and collaborators and I propose a suprageneric classification that re-evaluates ranks and composition of current intrafamilial groups. This work suggests a classification with three subfamilies: Hoplomyzontinae, Bunocephalinae and a new subfamily to carry Pseudobunocephalus; Bunocephalinae being subdivided into tribes Aspredinini and Bunocephalini. In this analysis, phylogenetic positions of Acanthobunocephalus and Bunocephalus chaimazelus were uncertain. The present work adds a morphological partition of 185 characters to currently available molecular data analyzed in combined approaches using both equal weight Parsimony and Bayesian inference. These combined analyses support most of the previous study using molecular data but supports Acanthobunocephalus as sister to Pseudobunocephalus and recognizes Bunocephalus chaimazelus as a new monotypic genus. A node-dated phylogeny using a single fossil calibration point on the MRCA of Auchenipteridae plus Doradidae, suggests that Aspredinidae began its radiation in the early Paleocene and most genera and Aspredinini were established in the Oligocene. This dated phylogeny reinforces the idea that most aspredinid bauplans were long-established and may have been in a relative morphological stasis since. In terms of biogeography, clades examined in this study, distributed on both sides of the Andes (Bunocephalus and Xyliphius) suggest a separation associated with the rise of eastern Cordillera at about 11 Ma. Estuary-dwellers of Aspredinini may have invaded this habitat at sometime between 23-50 Ma, which may be associated with habitat availability of marine continental incursions in the northwest portion of the continent.

Key words: Classification, Total evidence, Biogeography, Evolution, Morphological characters Financial support: CNPq (#PDE), CAPES (#PNPD), Fapergs (#PQG)



### Combined phylogeny and subfamilial classification of the Characidae (Ostariophysi: Characiformes)

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This is the most comprehensive phylogenetic analysis of the Characidae to date and the first large-scale hypothesis in the family combining molecular and morphological data. A morphological dataset of 262 examined species coded for 520 characters is combined with a molecular dataset of eight markers mostly composed of published DNA sequences. Almost 100 of those characters are new definitions. A total of 473 species are analyzed by parsimony under equal and extended implied weighting. The final hypothesis was chosen using a stability criterion and it was found by weighting molecular characters with the average homoplasy of entire blocks of data (genes). Results are rather congruent with previous molecular phylogenies of the family. The Characidae, restricted to the species lacking a supraorbital, are obtained as monophyletic with four main clades composed, respectively, of the Spintherobolus and relatives, an expanded Stethaprioninae including the tribes Gymnocharacini, Probolodini, and Stethaprionini, the Stevardiinae, and a clade composed of the Aphyocharacinae, Characinae, Cheirodontinae, Tetragonopterinae, and Exodon and relatives. Also, a "stem" Characidae is found, as formed by the Eocene-Oligocene genera †Bryconetes and *†Paleotetra*. The classification of the Characidae still need much data and work to get an overall stable classification and monophyletic genera but, given the congruence of these results with previous molecular hypotheses and considering that the main direction in the generation of new information most probably will face towards molecules, this subfamilial classification could be presumed to be stable by the near future.

Key words: Classification; Taxonomy; Cladistic; Morphology



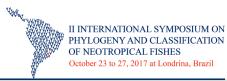
### Systematics of the Lebiasinidae (Teleostei: Characiformes): general overview and perspectives from molecular data

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The systematics of the Lebiasinidae has been unnattended since the end of the 1970s, after a fruitful period of publications spanning from the late 19th century. Among those are included detailed anatomical, revisionary and systematic studies resulting in the first proposal of the family as non related to the Erythrinidae, contradicting the general view of close relatedness among both groups; the recognition of Copeina and Copella as distinct from Pyrrhulina; and the synonymy of several generic names under Nannostomus, leading us to present taxonomic arrangement of the group. From the 1980s, most contributions on the family involved species descriptions, with exception of a "tentative" phylogenetic and biogeographic study of Nannostomus, and the phylogenetic corroboration of Erythrinoidea, a group of predacious Characiformes, once again placing lebiasinids as close relatives to Erythrinidae. During the last 15 years, the interest of ichthyologists on the group intensified the rate of publications and species descriptions, besides the recognition of Derhamia as a distinct genus from the upper Mazaruni. In addition, several phylogenetic contributions approached the relationships among the genera in the family, the interrelationships within each genus, and also brought additional evidence the refuting a monophyletic Erythrinoidea, and suggesting the Lebiasinidae as closer to the Ctenoluciidae. In the present contribution each of those hypotheses is revisited, and compared to preliminary phylogenetic hypotheses based on molecular data. As expected, both datasets largely disagree, especially regarding the position of Nannostomus, which is proposed as sister to Lebiasina, instead of being a member of the Pyrrhulininae. On the other hand, contrarily to the morphological study, the molecular dataset seem to corroborate most pairs of "species" previously proposed to the group, most of which is represented by up to four independent lineages. Our preliminary results also refute Derhamia as a Pyrrhulininae, as also proposed by morphological studies. Instead, Derhamia hoffmannorum would be nested within Lebiasina (including Piabucina as its synonym). The close relationship among (Copeina, (Copella and Pyrrhulina)) are corroborated, but an increment on the representation of Pyrrhuling is expected, to understand if the evolution of several shared characters between the small sized species of the P. australis group with members of Copella are a result of homoplastic evolution related to the small body size, or evidences to the paraphyly of Pyrrhulina.

Key words: Systematics; Pyrrhulininae; Lebiasininae; Freshwater; Neotropical Financial support: FAPESP and CNPq



#### A fossil-calibrated, phylogenomically constrained phylogeny for Hypostominae catfishes (Loricariidae) and its biogeographical implications

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Phylogenetic analysis of an 810 exon (172,794 bp) dataset spanning 41 Hypostominae species and 29 genera has, for the first time, yielded strong support for a monophyletic group of nine mostly Guiana Shield (GS) distributed genera: Avalithoxus MS, Exastilithoxus, Lithoxancistrus, Lithoxus, Neblinichthys, Paralithoxus, Paulasquama, Pseudancistrus, and 'Pseudancistrus'. To explore the biogeographical ramifications of these results, a more taxonomically comprehensive (185 species, 45 genera) five-gene (4,101 bp) dataset was constrained to the above topology and time calibrated using the late Paleocene (58 my) callichthyid fossil †Corydoras revelatus and a Loricarioidea maximum-age prior of 120 my inferred from previous studies. This analysis estimated that the GS-clade originated in the middle Oligocene (~28 my) and that the two earliest-branching lineages within the GS-clade, each representing divisions between predominantly eastern and western GS lineages, both developed in the late Oligocene (~26 my) when geologic evidence suggests that modern GS surfaces 300-450 meters above sea level (m asl) first underwent vertical, nondeformational uplift. Both major GS subclades comprise lineages that have respectively diversified across highlands of the western GS (e.g., Exastilithoxus, Lithoxancistrus, Neblinichthys, Paulasquama) and lower elevation uplands of the eastern GS (e.g., Paralithoxus, Pseudancistrus). Temporal and biogeographical patterns within these subclades are consistent with cladogenesis having been influenced by the Pliocene/ Pleistocene breakup of the proto-Berbice paleodrainage and contemporaneous erosion of other trans-highland biogeographical barriers. A second phase of geologic uplift of GS surfaces now approximately 150 m asl is also known to have occurred during this period. Overall, there is close correspondence between geologic evidence for the timing and pattern of GS uplift and drainage evolution and the diversification of fishes across GS drainages. Correspondence is further corroborated by dates for several trans-Andean vicariant nodes that closely align with geologically inferred dates for Andean uplift. Larger regional influences on and from the GS shield fish fauna are mostly inferred from the eastern GS subclades, both of which contain lineages that have dispersed into tributaries of the lower Amazon, including one lineage that has dispersed into the northern Brazilian Shield. This Guiana–Brazilian shield connection is explored in the context of other Hypostominae lineages that exhibit a similar pattern.

Key words: Guiana Shield; suckermouth armored pleco; geologic uplift; vicariance Financial support: NSF OISE-1064578, NSERC Discovery, Canada Department of Fisheries and Oceans



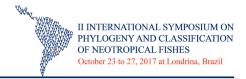
#### A new classification of Beloniformes based on a total evidence analysis

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Beloniformes is a diverse group that includes ricefishes, halfbeaks, needlefishes, sauries, and flying fishes. Previous molecular phylogenies suggest that some morphologically defined beloniform families are not monophyletic, including the Belonidae and Hemiramphidae. In the present study, we performed a total evidence analysis using both Maximum Parsimony and Bayesian analyses of 2029 characters, of which 123 are morphological and 1906 are base pairs from one mitochondrial (cytb – 259 bp) and two nuclear genes (RAG2 – 402 bp and TMO-4C4 – 245 bp). The sample of 56 taxa is composed of species of all recognized Beloniformes genera, two fossil species from the Oligocene, †Cobitopsis acutus from Puy de Dôme, France, and *†Hemiramphus jerzyi* from the Carpathians in Poland, and three species of Cyprinodontiformes as outgroup. The Atheriniform Labidesthes sicculus was used to root the trees. The results support previous hypotheses of non-monophyly of Belonidae and Hemiramphidae and suggest the recognition of seven families. Ricefishes (Adrianichthyidae) are sister group of the remaining Beloniformes. Sauries (Scomberesocidae) are nested within the Belonidae and should be recognized as belonging to that family. The internally fertilizing halfbeaks (Zenarchopteridae) are the sister group of the Belonidae, which together form the superfamily Belonoidea. The fossil *†Hemiramphus* jerzyi is closely related to *†Cobitopsis* acutus, both early diverging lineages of Exocoetoidea, which also includes the remaining halfbeaks and the flying fishes. Halfbeaks do not form a monophyletic group and should be split into three families: *†Cobitopsidae* (*†Cobitopsis*), a new family Hyporhamphidae (Hyporhamphus, Melapedalion, Arrhamphus, and Chriodorus) and Hemiramphidae sensu scricto (Hemiramphus, Oxyporhamphus, Euleptorhamphus and Rhynchorhamphus). Flying fishes (Exocoetidae) remain as monophyletic and are the sister group to Hemiramphidae sensu scricto. A comparison between Maximum Parsimony and Bayesian analyses showed similar and congruent tree topologies for family composition. The major difference between those analyses is the position of halfbeak genus Rhynchorhamphus, which was recovered as sister to the clade Hemiramphidae + Exocoetidae (Bayesian) or as an early diverging lineage within Hemiramphidae (MP). Rhynchorhamphus had never been tested in a phylogeny before, so a more conservative approach was adopted in keeping it in Hemiramphidae. Therefore, results of the combined analyses of morphology and molecules are consistent and support previous molecular analyses. Based on those results, a new classification for Beloniformes is provided, where all families are monophyletic and diagnosed by morphological synapomorphies.

Keywords: Systematics, Phylogeny, Needlefish, Halfbeak, Flyingfish, Ricefish, Saurie



#### Higher-level Phylogeny of Characiform Fishes (Teleostei: Ostariophysi)

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The order Characiformes comprises a diverse group of fishes with 24 families, roughly 520 genera, and more than 2400 species. Representatives of this order are widely distributed in freshwater habitats in southwestern United States, Mexico, Central and South America, and Africa, reaching its higher diversity in the Amazon basin. The order has a long fossil record, with fossils dating from the Oligocene. Some fossil forms related to African taxa are also known from Europe. The positioning of some fossil taxa dating from Cretaceous, including marine ones, is highly controversial, and may belong to basal Ostariophysi. In the last two decades there was a burst of studies on the phylogeny of the order or its subsets, employing morphological or molecular characters. However, there is little consensus between them, and except for a few groups, the topologies found in these studies are very discrepant. In addition, except for two studies that tackled specifically higher-level phylogeny within the order, most studies focused on the questionably monophyletic Characidae and as a by-product generated phylogenetic hypotheses for the order. In the present study, we pursued a more balanced sampling, including representatives of all currently recognized families, including the recently described Tarumaniidae, and of all the characid subfamilies. Additionally, we included several taxa considered as incertae sedis in the Characidae, or with position controvertial. This approach, totaling 115 species, has the objectives of testing the monophyly of all characiform families, of hypothesizing the limits of the family Characidae, and of determining the relationships among these families. A preliminary matrix with 410 characters was already analyzed. These characters were derived from the morphology of scales, swimbladder, digestive tract, and from osteology. The phylogenetic relationships recovered by this analysis will be presented, and similarities and differences between previous hypotheses based on morphological and molecular data will be addressed.

Key words: Systematics; Freshwater; Neotropical; Otophysi



#### The Trichomycterus hasemani species-group as a new genus and subfamily of Trichomycteridae (Siluriformes: Loricarioidea) with a taxonomic review

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The Trichomycterus hasemani species-group is composed by the miniaturized trichomycterids (maximum 18.6 mm SL) that have been traditionally proposed as exemplifying the non monophyly of Trichomycterinae, however a conclusive systematic and corresponding nomenclatural action has never been presented up to now. A phylogenetic analysis based on 100 morphological characters including 33 species from all Trichomycteridae subfamilies was conducted based on a maximum parsimony analysis. The results indicate that the Trichomycterus hasemani speciesgroup represents a new genus and subfamily of Trichomycteridae. The new subfamily is the sister aroup to the clade (Vandelliinae (Tridentinae Steapphilinae)) by sharing five unambiguous characters (maxilla expanded distally and strongly curved posteriorly; dentaries forming almost a straight line in dorsal view; anterior process of the posttemporo-supracleitro absent; coronoid process located posterior to Meckel's cartilage; interopercular arc present). The monophyly of the new taxa is supported by nine synapomorphies (axis of autopalatine transverse; anterior cartilage of autopalatine with medially directed process; posterior process of autopalatine bifurcated; autopalatine cartilage hypertrophied anterolaterally, extending towards maxilla; first pectoral basal radial elongate with a small posterior process; second pectoral basal radial elongate and parallel to first; mesethmoid cornua forming anterior straight line; round and short vomer; and presence of short process on posterior ceratohyal). The relationships within the new taxa demonstrated that "T." anhanga is the sister species of the clade ("T." hasemani "T." johnsoni) that shares two unambiguous synapomorphies (neck of mesethmoid broad and preopercle compact). A taxonomic review of the species included in the new subfamily is provided and four species are recognized. "Trichomycterus" anhanga, from the Aripuanã and Arapiuns basins, is diagnosed by the reduced latero-sensory system, number of branched pectoral-fin rays, absence of pelvic fins and girdle, reduced jaw and pharyngeal dentition, number of interopercular odontodes, autopalatine shape, position of dorsal fin, and number of pleural ribs. "Trichomycterus" hasemani, from the Amazon, Orinoco, and Essequibo basins, is diagnosed by details of color pattern and position of anal fin. "Trichomycterus" wapixana is proposed as a junior synonym of "T." hasemani. "Trichomycterus" johnsoni, from the Amazon and Paraná-Paraguay basins, is diagnosed by the presence of longitudinal lines on the flank and a median ventral stripe. A new species from the Casiguiare canal in Venezuela is described based on the presence of mentonian barbels.



### Towards a comprehensive phylogeny and classification of thorny catfishes (Siluriformes: Doradidae)

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The first cladogram of relationships among the genera of Doradidae (thorny catfishes) was first published in the 1998 Phylogeny and Classification of Neotropical Fishes. In his historical overview of Neotropical catfishes, Mário C. C. de Pinna summarized the synapomorphies identified and analyzed by Horácio Higuchi in his 1992 doctoral dissertation on the family. Higuchi (1992) recognized 70 valid doradid species (plus one undescribed) distributed among 30 valid and three undescribed genera. Though unpublished, Higuchi's work opened new avenues of investigation for a family that had not been comprehensively revised in nearly 70 years (i.e., Eigenmann, 1925). Since Higuchi's thesis, a surge in doradid research has described 22 new species (including one fossil) and three new genera. Furthermore, the classification of Doradidae is now underpinned by two new comprehensive phylogenetic analyses, one based on molecules (Arce et al. 2013) and the other on morphology (Birindelli, 2014). Additional morphological and molecular studies have advanced hypotheses on the phylogenetic placement of Doradidae within Siluriformes. Such work has transformed doradids into a robustly informative group for evaluating the evolution, phylogeography and ecology of freshwater fishes on a continental scale. We synthesize published taxonomic and phylogenetic data on Doradidae, and report on ongoing studies involving morphology, fossils and next-generation sequencing (exon-based phylogenomics).

Key words: Systematics; Taxonomy; Freshwater; Neotropical; Biogeography; Fossils Financial support: NSF DEB-1257813 (iXingu Project), NSF DEB-1541491 (GoLife program)



### Phylogenetic relationships among pristigasteroid fishes (Clupeiformes: Pristigasteroidea)

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The Pristigasteroidea is a superfamily of Clupeiformes that includes nine genera and 36 species of mostly coastal/estuarine, medium sized, schooling fishes with a pantropical distribution. Members of the Pristigasteroidea show a surprising array of morphological specializations, including distinct modifications in anterior vertebrae and ribs, the presence of postcoelomic swimbladder diverticula, and strongly keeled pectoral girdles. Herein, we present a new hypothesis of phylogenetic relationships for 21 terminals representing all genera of the Pristigasteroidea and most species of Pellona, Ilisha, Opisthopterus and Odontognathus based on 188 morphological characters. The outgroup includes 22 terminals representing all currently recognized subgroups of the Clupeiformes, as well as representatives of Ostariophysi (Hollandichthys, Opsariichthys, Kneria, Chanos), Alepocephaliformes (Talismania, Searsia), Salmoniformes (Osmerus, Salmo), Osteoglossomorpha (Hiodon) and Elopomorpha (Elops, Albula), in a total of 54 terminals. The data matrix was constructed in Mesquite 3.2, and parsimony analyses were performed in PAUP\*4.0. Eight (8) most parsimonious cladograms (L=831; CI=0.301; RI=0.744) were obtained after 10.000 replicates under Stepwise Addition (Random Addition Sequence) followed by Branch Swapping (TBR). The root was placed in the branch between a monophyletically-constrained Elopomorpha and remaining clades. Monophyly of Pristigasteroidea is supported by 29 transitions, including the presence of a prominent dentition associated with a single tooth plate extending over the dorsal surface of basibranchials 1 to 3, tooth plates fused with hypobranchials 1 and 2, predorsal bones vertically oriented or slightly inclined anterodorsally, Baudelot's ligament originating at the first and second vertebra, absence of interlobar notch, and bifurcated pelvic bone. In the strict consensus tree, Pellona ditchela and P. harroweri are included in a basal trichotomy with a clade containing remaining pristigasteroids, which are related as follows: ((Pellona castelnaeana, Pellona flavipinnis) (((Ilisha kampeni (Ilisha melastoma, Ilisha striatula)) ((Ilisha elongata, Ilisha megaloptera) ((Ilisha amazonica, Pristigaster) (Ilisha africana (Opisthopterus tardoore (Raconda ((Chirocentrodon, Neoopisthopterus) ((Opisthopterus dovii, Pliosteostoma) (Odontognathus compressus (Odontognathus mucronatus, Opisthopterus equitorialis))))))))). Pellona, Ilisha, Opisthopterus and Odontognathus are not monophyletic and a new classification is proposed. The evolution of key clupeiform characters, such as the gongyloid cartilage, cartilage chevrons at the tips of epicentrals, and abdominal scutes are also discussed in light of the phylogenetic hypothesis.

Key words: Clupeomorpha; Taxonomy; Otocephala Financial support: FAPESP



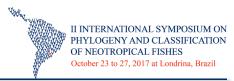
### History of biogeography of Amazonian fishes: from Agassiz to Vari and beyond

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Several current paradiams and controversies about Amazonian fish biogeography are rooted in principles dating from the second half of the XVIII to the first half of the XIX centuries. The present work establishes a relationship between current biogeographical ideas and their old predecessors, on the basis of a chronologically-oriented historical continuity analysis. The entire tradition of Amazonian biogeography takes place within the evolutionary paradigm, although its founder Louis Agassiz was himself an anti-evolutionist. The origin of Amazonian biogeography is Agassiz's travel journal of the Amazon. That document makes it clear that the author did not consider species movements as an explanation for biogeographical patterns found. Later, Carl Eigenmann helps to spread the dispersionist tradition as a model for biogeographical explanations in fish distributions, a phase which lasted until the late XX century. A major shift occurs with the contributions of Stanley Weitzman and Richard Vari, who associated the temporal framework of phylogenetic hypotheses with distribution patterns, underscoring the predictive power of vicariant biogeography. The present-day paradigm begins with the work of John Lundberg and attempts to incorporate geomorphological information into biogeographical hypotheses. Since its foundation in 1868, truly revolutionary shifts in the biogeography of Amazonian fishes have only occurred in the last three decades with the advances of the historical biogeography. One of the more recent developments is the idea that hydrographic basins are highly dynamic historical units (i.e., reticulate areas which received portions of their biotas at different ages, under the influence of different events), a notion already implicit in Eigenmann's work but which has only recently become a premise for biogeographical studies. As a result, geomorphological processes seem to be more important in understanding Amazonian fish distribution than present-day basin divides. Another recent development is a change of emphasis to process biogeography, most notably on the basis of Bayesian inference.

Key words: Composite areas; Historical Biogeography; Vicariant Biogeography Financial support: FAPESP (processes 2011/23419-1; 2016/07246-3)



#### A synthesis of morphological evidence for the phylogeny of Siluriformes, with a new hypothesis, a broad-scale evolutionary scenario and a revised classification (Teleostei: Ostariophysi)

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We present the results of a phylogenetic analysis based on a summation of all the comparative morphological evidence available for the order Siluriformes. Data include characters from both published and unpublished sources, as well as evidence originally proposed. Terminal taxa were selected according to prior hypotheses about their relative position within respective families or associated clades in order to maximize both taxonomic density and phylogenetic scattering. Taxa with uncertain familial placements were included a priori. Previously-proposed characters are reassessed as to their homology assumptions, descriptive consistency and taxonomic distribution. Myological characteristics are revised in especial detail in order to disentangle a convoluted history of discrepant homology assumptions and associated terminology in previous literature. Results of this part of the study are summarized in a synonymy of siluriform facial musculature with a revised nomenclature. A total of 102 taxa and 502 characters are organized in a matrix analyzed by parsimony and Bayesian methods, with the aid of programs TNT and MrBayes, respectively. Results corroborate the classical position of Diplomystidae as sister aroup to all other siluriforms, with Cetopsidae and Loricarioidea as next successive sister groups. Other relevant hypotheses include the position of Amphiliidae as sister group to Sisoroidea, and both close to Loricarioidea; the monophyly of a group composed of Claroteidae, Ariidae (including Ancharius), Austroglanididae, Ictaluridae, Cranoglanididae and Lacantuniidae; the position of Malapteruridae as sister aroup to Auchenoalanididae; an assemblage composed of Chacidae, Plotosidade, Siluridae and Clariidae (with Heteropneustes); and the traditional Doradoidei (Auchenipteridae, Doradidae, Mochokidae). A new phylogenetic classification of the Siluriformes, including revised diagnoses for all familial and suprafamilial taxa is proposed. A comparison between our results and proposals based on molecular data reveal several incongruences. Reasons for such disagreements are discussed and characters that do not fit our favored hypothesis but which agree with molecular clades are identified and discussed.

Key words: catfish, phylogenetic analysis, morphology, molecules and morphology, parsimony, Bayesian, classification

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#### A reappraisal of the systematics of Neotropical freshwater stingrays (Chondrichthyes: Potamotrygoninae): a complex tale of morphological conservatism and cryptic diversity

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Neotropical freshwater stingrays are currently ranked as a subfamily in the family Potamotryaonidae, as the sister-group to the Styracurinae (the Chupare stingrays, Styracura pacifica and S. schmardae), a phylogenetic relationship strongly based on molecular analyses. Potamotrygoninae are unique among fossil and extant chondrichthyans for their extraordinary diversification in freshwater habitats. Currently 32 valid potamotrygonin species are recognized, all but 5 of which are in Potamotrygon; but at least 6 additional species of this genus have been discovered and are being described (3 are now in press), and Paratrygon is a recently revised species complex, not a monotypic species as previously thought. Paratrygon and Heliotrygon are viewed as sister groups both on morphological and molecular grounds. The nested position of Plesiotrygon within Potamotrygon, pointed both by molecular and morphological analyses, is still subject to controversy, considering that species of these two nominal genera show clearly distinct and non-overlapping diploid chromosome numbers. Even though species present a highly conservative morphology, current trends to recognize species based on differential diagnoses, in tandem with more concerted collecting efforts, will soon elevate the diversity in the subfamily to over 50 species. Currently recognized species are considered distinct lineages, even though some widespread species were not amenable to subdivision up to date based on morphological characters, such as Potamotrygon orbignyi and the Amazonian Potamotrygon motoro. Certain species are hypothesized to form groups based on morphological characters, such as the Potamotrygon scobina, P. orbignyi, and P. motoro groups, as well as the black stingray group of endemic species draining the Brazilian Shield. To further investigate these groups, comparative studies of morphological complexes are underway, contributing data to a species-level phylogeny. Morphological and molecular phylogenetic signals may be significantly at odds, as the latter support the independent evolution of morphological features within separate basins, a premise strongly contradicted by morphological data.

Key words: Potamotrygonidae, diversity, classification, phylogeny



#### Phylogeny of Engraulinae and taxonomy of Anchoviella Fowler, 1911 (Clupeiformes: Engraulidae)

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Engraulinge, a subfamily of Engraulidae, includes eleven genera and about 90 species with marine, estuarine and freshwater habits, distributed in South, Central and North America, except Encrasicholina and Engraulis, the first one restricted to the Indo-Pacific, and the second, with a global distribution. Anchoviella, one genus of Engraulidae that belongs to the New World Anchovies group, is currently represented by 17 described species, but according to many authors, most species of the genus are poorly described and in need of revision. The monophyly of Engraulinae is supported by four synapomorphies related to the general morphology of the body, including the pattern of the sensory cephalic canals and characteristics of the caudal skeleton and branchial arches. The present study had two main goals: i) analyze the phylogenetic relationships within Engraulinae based on morphological data; ii) perform the taxonomic revision of Anchoviella based on the analysis external and internal morphological characters. The monophyly of Engraulinae and Engraulini was not herein corroborated, as well as the monophyly of most of the genera within the subfamily, except Anchovia, that was included in a monophyletic clade with a strong Bremer support. Proposed as the most derived genus within the Engraulinae in previous studies, the genus Encrasicholina was herein included within one of the major clades of the family, together with species of Stolephorus, Engraulis and Anchoa. Pterengraulis atherinoides is the more derived taxon within the Engraulidae, together with Lycengraulis grossidens and Anchoa spinifer, also included within the more derived taxa of the family. Moreover, one major clade with species belonging to Amazonsprattus scintilla, Anchoviella and Anchoa was obtained, corroborating the hypothesis of a close relationship among Amazonsprattus scintilla and Anchoviella and among Anchoa and Anchoviella. The taxonomic revision of Anchoviella revealed the existence of 13 valid species and two undescribed freshwater species. However, according to the results obtained through the phylogenetic analysis undertaken, only three among these species should be maintained within the genus, while the other species would form a new genus including Amazonsprattus scintilla.

Key words: Systematics; Neotropical; Anchovy; Manjuba Financial support: Fapesp (process 2011/06830-0)



#### A new genus of a small "milkfish" from the Areado Formation, São Francisco basin, Early Cretaceous of Brazil (Teleostei: Gonorynchiformes: Chanidae)

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Gonorynchiform fishes present a rich fossil record ranging from the Early Cretaceous (Barriasian-Valanginian) to the Early Miocene, as well as having a few dozen living representatives. The order is currently divided into two major clades: Suborder Gonorynchoidei, which includes the families Gonorynchidae and Kneriidae, and Suborder Chanoidei, encompassing a single family, Chanidae. Chanidae, with a single recent species, the Indo-Pacific Chanos chanos, and several fossil taxa is diagnosed on the basis of several apomorphies. Despite being a well delimited group, Chanidae still includes some problematical taxa, such as Dastilbe moraesi Silva Santos, 1955, described from the Aptian (Early Cretaceous) of the Areado Formation, São Francisco basin, Brazil, a species which is currently considered to be a junior synonym of the type species of its genus, Dastilbe crandalli Jordan, 1910, known from the Crato Member of the Santana Formation, Aptian, northeastern Brazil. A large amount of D. moraesi has been examined for this study, revealing many new features, some of which previously misinterpreted in literature. To elucidate its phylogenetic relationships, D. moraesi was incorporated into a modified character matrix for Gonorynchiformes. The parsimony analysis generated three shortest trees, which are consistent in revealing that D. moraesi is not only distinct from D. crandalli, but also belongs to a distinct monophyletic subgroup, the tribe Chanini, forming a sister pair with Chanos, which is closely related to Tharrhias. For the nomenclature to reflect the topologies obtained, it is necessary to erect a new genus for D. moraesi.

Key words: Fossil; Systematics; Brazilian Shield



#### Broad-scale phylogenomics provides unprecedented resolution for the primary freshwater fishes in the order Characiformes

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Fishes of the order Characiformes (tetras, piranhas and allies) constitute an exemplary case to study intercontinental evolutionary biogeography given their contemporary ubiquity in two major Gondwanan landmasses (Africa and South America) and freshwater confinement which makes trans-Atlantic dispersal unlikely. Although recent analyses using genome-wide data tested characiform monophyly and examined their phylogenetic position among other primary freshwater ostariophysans (e.g., catfishes, knifefishes, and carps, barbs and allies), conflict prevails and many major lineages and families lack robust placement. Herein, we examined 1,027 exon markers and 208 characiform species representing all Neotropical and Paleotropical families of characiforms. Phylogenetic analyses were conducted using a variety of methods, including concatenation and species tree analyses. We also applied a recently proposed methodological approach, named Gene Genealogy Interrogation or GGI, that uses topological tests to ask which genealogical history among a set o predefined alternatives each gene supports with highest probability. We used the GGI approach to address some stubbornly unresolved aroups in Characiformes: the monophyly of the Paleotropical aroups (currently in Citharinoidei and Characoidei), the branching order at the root of Characoidei, and the relationships among lineages in the superfamily Anostomoidea. Our results resolved with high confidence the monophyly of all families, and provide well support for the relationships at both shallow and deep time-scales. We provided strong support for the non-monophyly of the Paleotropical families, and the monophyly of three major clades among the suborder Characoidei: (1) Alestidae and Hepsetidae, (2) Erythrinidae, Cynodontidae, Hemiodontidae, Serrasalmidae, Parodontidae and the superfamily Anostomoidea, and (3) a clade comprising the remaining families of Characoidei. A simultaneous analysis based on 500 characiform species and the nuclear recombination gene (RAG1) confirmed the phylogenetic placement of the recently described family Tarumaniidae as the sister aroup of the family Erythrinidae. Our results provide a new phylogenetic framework to test further biogeographic and diversification scenarios associated with the evolutionary history of characiform fishes in the Paleotropical and Neotropical regions.



#### Addressing intractable groups in the Fish Tree of Life using genomewide Gene Genealogy Interrogation

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Much progress has been achieved in disentangling evolutionary relationships among species in the Tree of Life, but some taxonomic groups remain difficult to resolve despite increasing availability of genome-scale data sets. Recently, we introduced a new practical approach, named Gene Genealogy Interrogation (GGI), that uses constrained tree searches in combination with topology tests to account for gene tree estimation error and its downstream effects in coalescent analyses. We initially showed its efficacy in resolving controversial relationships within the largest freshwater fish radiation (Otophysi) based on newly generated DNA sequences for 1,051 exons from 225 species. Here, we address other difficult nodes in the Fish Tree of Life based on a large-scale phylogenomic dataset consisting of 1,105 exons, which was assembled via in silico mining of 305 genome- and transcriptome-enabled fish species. In addition to revisiting Otophysi with the new dataset, other difficult nodes addressed include the base of teleosts, protacanthopterygians, base of acanthomorphs, sister group of percomorphs, and atherinomorphs. Our GGI results show that, while phylogenetic resolution for major protacanthopterygian and acanthomorph lineages remains contentious, the majority genes favor a single tree topology in all other cases. GGI provides a powerful approach to identify incompatible signals in large data sets and to discern between estimation error and actual biological conflict explaining gene tree discordance.

Key words: Phylogenomics; recalcitrant groups; Fish Tree of Life Financial support: RBR: DEB-147184, DEB-1541491; GO: DEB-1457426 and DEB-1541554



### Riverscape genetics: applying phylogeographic models to understand divergence in coastal Brazilian fishes

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The movement of organisms in spatially structured landscapes is affected by constraints imposed by geographic and physical properties of the environment, and by the response of the organisms to this environment (i.e., ecological requirements). Freshwater environments, especially rivers, are known for imposing stronger movement constraints in comparison to terrestrial and marine environments. These constraints are affected by the isolation of different river drainages and the properties of a river, such as shape and water flow. Because of these unique characteristics of riverine landscapes (i.e., riverscapes), understanding of neutral demographic processes in these environments is still lacking in comparison to other environments. This research aims to help fill this knowledge gap by advancing the understanding of the effects of riverine environments on neutral demographic processes. I combined simulated and empirical data to ask how riverine basins over different spatial (i.e., local and regional) and temporal (i.e., past) scales interact with organisms to promote the observed patterns of genetic diversity in freshwater fishes. The Brazilian coastal drainages are the ideal area for this study because they represent a series of isolated basins that were cyclically connected and disconnected because of Pleistocene sealevel changes, leading to a great diversity of endemic fishes. I use here one to multi-species comparisons applying a combination of different sequencing technologies (i.e., mtDNA and ddRADseq) with GIS techniques and statistical approaches. The results first demonstrate that paleodrainage during Pleistocene sea-level retreat is the main factor explaining population genetic differentiation in one taxon (Characidae: Hollandichthys). Then, I test how riverine landscapes and their physical properties during past time periods structure this species genetic diversity within drainages. Finally, I used a comparative approach with four taxa of tetras (Characidae: Mimagoniates microlepis, Hyphessobrycon boulengeri, Hollandichthys and Bryconamericus microcephalus group) to elucidate whether sea level changes in coastal areas affected the freshwater community as a whole, or if responses were species-specific depending on the taxon ecology. The work presented here advances knowledge pertaining to the evolution of freshwater fishes, particularly those of the Neotropics. Overall, by exploring relevant hypotheses in order to identify processes that structure genetic variation within and between basins and species allowed to distinguish the evolutionary mechanisms operating at different spatial and temporal scales, providing insights into patterns of genetic diversity in freshwater fishes, especially along the coastal Brazilian basins.

Key-words: Characidae, Coastal basins, Freshwater fish, mtDNA, paleodrainages, sea-level changes, SNPs Financial support: NSF (DEB-15-01301)



### Myology and systematics of bony fishes

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Some of the most remarkable studies on fish skeletal musculature were published in the late 19th and early 20th centuries. For most of the 20th century, however, myology was widely neglected by most fish systematists, at the same time that improved clearing and staining techniques fomented a new era of osteological studies. As a result, only 6% of all known morphological synapomorphies proposed for the major lineages of bony fishes are from the skeletal musculature. Such a modest proportion derives not only from a generalized scarcity of myological studies but also from the many uncertainties surrounding the identity of various muscles. This scenario is gradually changing as homologies of some of the most intricate myological complexes are being unraveled and an increasing number of phylogenetic studies incorporate muscular characters into their analyses. Systematics of bony fishes may accordingly benefit from these achievements. Myological data are proven to be crucial for the understanding of the phylogenetic allocation of several recalcitrant groups, such as the Synbranchiformes, Notacanthiformes, Alepocephaloidei, Gobiesocoidei, and Sphyraenidae. Muscular specializations unambiguously support the monophyly of traditional taxa that have been either poorly diagnosed—e.g., Elopiformes and Ophidiiformes—or dissolved by molecular phylogenies—e.g., Neoteleostei, Siluriphysi, Stromateiformes, and Scombriformes. On the other hand, myology provides the first myological character states supporting some clades recovered only by molecular analyses. Original hypotheses of relationships are also advanced primarily on the basis of shared myological specializations, such as the putative monophyly of a perciform lineage including the Centropomidae, Latidae, Moronidae, Polynemidae, and Sciaenidae. Additionally, some of the major bony fish clades-namely Osteichthyes, Actinopterygii, Neopterygii, Teleostei, Osteoglossocephala, Osteoglossomorpha, Ostariophysi, Characiphysi, Eurypterygia, and Ctenosquamata—are now known to share unequivocal, previously unknown myological synapomorphies. These findings portray the immense potential of the muscular system for the fish systematics and demonstrate how morphology is still far from a satisfactory exploration by ichthyologists.

Keywords: Morphology, Musculature, Osteichthyes, Actinopterygii

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### Phylogeny and classification of Neotropical cichlids twenty years later – a macroevolutionary perspective

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Classifying organisms, establishing their evolutionary relationships and clarifying their timeline of divergence are central goals of systematics studies. Combined, these goals are the foundation for understanding the evolution, biogeography and ecology of clades. With an estimate exceeding 8000 species in dozens of families, the Neotropics represent a staggering challenge to systematists. The First Symposium on Phylogeny and Classification of Neotropical fishes in 1997 prompted the current research program in systematics of Neotropical fishes. At that time, the chapters of Kullander, Farias et al. introduced the first morphological and molecular phylogenetic analyses, respectively, of Neotropical cichlids. Twenty years later we have a much stronger knowledge of the higher-level phylogenetic relationships of Neotropical cichlids. New methods of analysis are also providing a nuanced understanding of species-level diversity and hinting at the complexity of mechanisms underlying speciation in riverine cichlids. Field exploration in ever more remote areas continues to reveal unknown taxa and the classification of Cichlinae, although still unstable and in need of revision, has seen valuable improvements. Importantly, cichlids are becoming a model to understand the adaptive evolution and historical biogeography of Neotropical riverine faunas. They offer a relevant complement to evolutionary studies of cichlids centered on the African Rift Lakes. The expanding fossil record of cichlids, including several Neotropical taxa, has taken center stage in a vigorous debate about the age of cichlids and its implications for fish evolution and historical biogeography. Because of the age and modern morphology of recently discovered fossil cichlids (mostly from the Argentinean Eocene), their insertion in a phylogenetic framework is essential to discern the timeline of evolution and diversification of cichlids. Despite these advances, various challenges remain in Cichlinae systematics, pointing towards areas for future research in cichlids and other Neotropical freshwater fish families. In our view, the advancing our understanding of Cichlinae phylogeny, classification and evolution will require: 1) developing a comprehensive and expandable phylogenetic hypothesis based on both molecular and morphological data. Partly underway, this work needs improvement and should lead to an eventual species-level phylogeny of the subfamily as the foundation for: 2) phylogeny-based classifications that explicitly test the identity of taxonomic units and the nature of their relationships, and use standard criteria to define taxa at the species and genus level; 3) creating a solid framework to incorporate the fossil record into reconstructions of the evolutionary relationships and time of divergence of Neotropical cichlids. Together, these objectives provide the foundation to: 4) analyze the patterns, processes and evolutionary timeline of morphological, ecological, and behavioral diversification of cichlids, and 5) clarify the direction and timing of diversification and dispersal events that resulted in the current distribution of cichlids across the Neotropics. Perhaps more importantly, 6) this agenda of study should expand into other families of Neotropical fishes whose origin and diversity are inextricably intertwined with that of cichlids. This is an essential collective goal because our systematic studies of Neotropical fishes should lead to a cohesive knowledge of the evolutionary and biogeographic history of the largest vertebrate assembly on the planet.



### Phylogeny and classification of Neotropical cichlids: Ubi sumus? Quo vadimus?

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The cichlids (Stiassnyiformes: Cichlidae), with an estimated 2456 nominal and 1698 valid species, make up the second or third largest vertebrate family, and is the only perciform family of importance in freshwaters. Seven hundred and eighty-four nominal and 539 valid Recent species are registered for the Neotropical region. Only minor, mainly nomenclatural advances were made on the phylogeny and classification of Neotropical cichlids since the first revision in 1840, and again not much since 1997. Reviewing phylogeny and classification of these fishes is best made at separate levels. I) Species diversity: 157 new Recent Neotropical cichlid species were added after 1997 (about 30% of total), but a portion represents synonyms, questionable species, or incomplete descriptions, and revisions are few. It is not expected that the number of known species will increase significantly. Application of DNA barcoding may replace morphological species taxonomy in non-systematic biology but has limitations. Examples from coastal southern Brazilian and Uruguayan cichlids demonstrate limitations of both morphology and molecules. II) Genus level diversity: Currently, 79 genera of Neotropical cichlids are recognized, each containing 1–91 species (average about 7). Twenty-seven genera (about 30%) are monotypic. Twelve genera have more than 10 species. The systematic and nomenclatural concept of genus, formalized by Artedi (1738) denotes a group of species sharing distinctive characteristics (presumably synapomorphies). There is no limit to the number of species in a genus but there are considerations on the information content particularly because the generic and specific names together make up a species name, and because the genus is part of the hierarchical classification of organisms. III) Classification and phylogeny: Compared to classification and phylogeny attempts in the late 1990s, very little change is expected. Within-cichlid phylogenies have not changed radically since 1997; they are just more or less bad or good as before. The major phylogenetic achievements since, have been with the long understudied Central American cichlids, and the major novelty among South American taxa was the association of crenicichlines with geophagines. Application of conventional sequencing and reliance on selected gene fragments is likely to continue to provide repeatable but not necessarily optimal phylogenetic hypotheses. Phylogenetic "classifications" based on dating and trees tend to emphasize disjunction, whereas categorization based on characters emphasizing relationship provide more informative classifications. Nomenclatural revisions just changing ranks or increasing monotypy are not really advances. Examples show how phylogenetic information can be accommodated without compromising the classification information (the 2017 version of the Neotropical cichlid phylogeny). The real question to ask those days, however, is whether producing "new taxa", phylogenetic hypothesises and "classifications" is meaningful when we have resources, machinery and computers to explore non-invasive anatomy, population genetics, gene functions, and more. Organismal biology has developed far beyond what could be imagined in the 1990s. Why are we still counting fin-rays? And what's wrong with Stigssnyiformes?

Key words: Systematics; Taxonomy; Morphology; Genetics; Congruence; Freshwater; Cichlidae; Labriformes Financial support: (SOK) MEC/MCTI/CAPES/CNPq/FAPs, n°71/2013; (HRV) CNPq150304/2017-0, Fapesp 2013/17056-9, 2011/14630-0; (CASL) MEC/MCTI/CAPES/CNPq/FAPs n° 71/2013



### Phylogeny and classification of the catfish family Pimelodidae (Siluriformes: Siluroidei: Pimelodoidea) in 2017

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The siluriform superfamily Pimelodoidea (Sullivan et al., 2006, 2013) includes five divergent Neotropical lineages: Pimelodidae, Pseudopimelodidae, Heptapteridae, Phreatobius, Conorhynchos. Monophyly of each polygeneric family is firmly supported by morphological and molecular data. Family Pimelodidae, currently with 30 genera and 111 species, is united by several unique nucleotide and morphological synapomorphies. This review of pimelodids is based on molecular phylogenetic results of Lundberg et al. (2011, 2012, 57-64 species, >1,190 informative characters), morphology-based results of Rocha (2012, 64 species, 145 characters), plus work in progress that will advance combined data analysis and revise classification. Within Pimelodidae, the near-basal neopimelodine clade contains all but three genera and is supported by perfect Bayesian posterior probability, unambiguous nucleotide synapomorphies and a synapomorphic aortic canal below the Weberian centra. Steindachneridion, Phractocephalus and Leiarius are near-basal and species-limited genera. Molecular data arrange these three paraphyletically apart from neopimelodines, whereas morphology recovers them as a monophyletic phractocephaline clade sister to neopimelodines. Within the neopimelodine clade most genera are confidently placed among four well-supported subclades: sorubimines, calophysines, pimelodines and exallodontines. The sorubimines include at least Sorubim, Pseudoplatystoma, Sorubimichthys, Brachyplatystoma, Platynematichthys, Hemisorubim, Zungaro and Hypophthalmus. Monophyly of the sorubimines is supported by perfect Bayesian posterior probability, plus unambiguous nucleotide and morphological synapomorphies. The interrelationships of sorubimine genera are incompletely resolved except for Tribe Brachyplatystomini (Brachyplatystoma, Platynematichthys). Molecular and morphological data congruently support a CPE clade uniting the calophysines, pimelodines and exallodontines. Below the root of CPE, other small, yet divergent, taxa are placed, some tentatively and with alternative resolutions between datasets. Among these are "Pimelodus" ornatus, a species complex long known to be distinct from typical Pimelodus, and the obscure Duopalatinus emarginatus and Bagropsis reinhardti from the São Francisco Basin. Also, the recently discovered Pimelabditus moli from Surinam is placed by molecular and some morphological data close to "Pimelodus" ornatus. However, Rocha's morphological data resolved Pimelabditus as a basal lineage among calophysines. A third contentious group is Platysilurus + Platystomatichthys that molecular data weakly resolve among sorubimines, but morphology places close to the CPE clade. The calophysine group contains at least Calophysus, Aquarunichthys, Luciopimelodus, Pinirampus, Pimelodina, Megalonema and Cheirocerus. Monophyly of the calophysines is supported by perfect Bayesian posterior probability and high bootstrap scores, unambiguous nucleotide and morphological synapomorphies. Internal relationships among the calophysine genera differ between studies, including discordance between molecular resolution of Megalonema and Cheirocerus as sister taxa versus their remote separation in the morphological tree. The pimelodine group contains Iheringichthys (here including Bergiaria), Parapimelodus and a paraphyletic mix of Pimelodus species groups such as the "maculatus," "blochii" and "pictus" groups. Several nominal species of Pimelodus are excluded from the pimelodine group, but the resolution among its remaining members is generally concordant between the molecular and morphology-based trees. The exallodontine group contains Exallodontus, Propimelodus, generically misplaced species such as "Pimelodus" altissimus and "Duopalatinus" peruanus and divergent undescribed species. Monophyly of the Exallodontus group is established by molecular and morphological features, and their emerging internal relationships foreshadow revisions of generic delimitations.

#### Key words: Neotropics, freshwater, systematics

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### Phylogeny and biogeography of the miniature catfishes Scoloplacidae (Siluriformes: Loricarioidea)

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The Neotropical Scoloplacidae is distinguished from other Siluriformes by the presence of a conspicuous autapomorphy: a shield-shaped rostral plate bearing numerous large and recurved odontodes. Eight synapomorphies, four of these uniquely derived, diagnose the Scoloplacidae as monophyletic. Within Loricarioidea scoloplacids is the sister group of astroblepids plus loricariids. This monogeneric family is endemic to South America and known from the Amazon and Paraguay-Paraná basins. At the present time six species of Scoloplax are recognized, all of these inhabit small water courses with flooded areas in substrates with leaves and debries. Scoloplacids are miniaturized catfishes with the largest known specimen measuring just under 2 cm SL. Because of their low activity, occurrence in small streams, Scoloplax are potentially a good model for biogeography. This study is part of a larger investigation on the Biogeography and Evolution of this family in which nuclear and mitochondrial genes are used across populations throughout their area of range. The only published phylogenetic study of Scoloplax is the morphology-based work of Schaefer in 1990. Two additional species were described by Rocha in 2008, 2012, and additional data have been gathered in the past 10 years, including tissue samples from many localities. Here are shown the results of phylogenetic analyses using the COI and 16s mitochondrial genes and a total evidence analysis. 53 samples were sequenced, including 7 outgroup taxa (Nematogenys inermis, Callichthys callichthys, Megalechis thoracata, Corydoras sp., Astroblepus sp., Delturus parahybae, Neoplecostomus paranensis) and 46 Scoloplax representing S. dicra, S. baskini, S. empousa, S. dolicholophia, S. distolothrix, but not S. baileyi. A total evidence cladogram using Bayesian methods shows S. dicra as sister group of the other Scoloplax. In this large aroup two clades were recovered, one containing S. baileyi as sister group of S. empouse and S. distolothrix, and another with S. dolicholophia and S. baskini (Aripuana, Purus(Negro)). Scoloplax empousa is structured into populations Ivinheima (type locality) and Pantanal. Within S. distolothrix populations were structured as (Araguaia (Upper Xingu, Lower Xingu)). The other clade, S. dolicholophia is the sister group of S. baskini, which is well-structured in three populations with Aripuanã and Purus as sister group of the Negro population. A Bayesian analysis to test the time divergence (not including S. baileyi) shows two large clades, one containing S. dicra as sister group of S. empousa (paraphyletic with S. distolothrix), and another with S. dolicholophia and S. baskini. The S. empousa population from Ivinheima is sister group to S. distolothrix and S. empousa from Pantanal. This time tree indicated the family's divergence about 90 mya and about 53 mya the separation of the two large clades. The separation of S. baskini from Aripuanã (type locality) from the Purus was about 14 mya, and this group from Negro population was about 30 mya, indicating a large time difference. Morphological analyses also indicated differences between these populations, possibly indicating that they are cryptic species. More genes are being sequenced as well as more populations being sampled for a better understanding of family evolution in the Neotropics.



#### The diversification and evolution of gymnotiform electric fish: integrating alpha-taxonomy, phylogeny, biogeography, and electric communication signals at the continental scale

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The gymnotiform electric knifefish offer unrivaled opportunities for understanding the mechanisms of reproductive isolation and speciation – primarily because they generate and sense dual-function mate attraction/object detection signals that are easy to record and quantify, and that are well understood from the neurobiological perspective. However, incomplete alpha taxonomy and the paucity of phylogenetic reconstructions have greatly restricted progress toward a synthetic understanding of gymnotiform evolution. During the two decades since the First International Symposium on the Phylogeny and Classification of Neotropical Fishes, the number of described gymnotiform electric knife fish has increased at a steady pace from 100 to 240 + species. On the other hand, well-resolved and comprehensive molecular phylogenetic reconstructions of the order are only recently becoming available. Using examples from the family Hypopomidae (with emphasis on the genus Brachyhypopomus), we explore how a unification of data from alpha-taxonomy, phylogeny, biogeography, ecology, and electric signals can illuminate patterns of diversification, geographical expansion, and habitat occupancy. We begin at the continental scale by examining geographical and habitat correlates of electric signal diversity among 28 described and 11 asyet undescribed species – distributed from Uruguay to Costa Rica. We demonstrate evidence for reproductive character displacement of signals – manifest as divergences in the number of phases in their electric signals among sympatric sister species pairs – and argue that these divergences are associated with speciation. Next, at the local scale, for a well-studied assemblage of Brachyhypopomus from the lowland upper Amazon we explore the extent to which co-occurring sympatric species are reproductively isolated, and examine phylogenetic patterns in both their far-field detectable electric signals as well as the geometric complexity of the electric fields close to the signaler's body. We then review the extent to which signal diversity may be shaped by biotic selective pressures related to the coexistence of other electrically signaling fish, versus abiotic selective pressure related to electrolocation (object detection) performance of signals in different microhabitats. Next, we review the use of ancestral character state reconstruction to explore the evolution of gill morphology and hypoxia tolerance. Hypoxia tolerance is an important factor in the occupation of floodplain ecosystems, and a potential pathway for ecological speciation across sharp dissolved oxygen gradients. We conclude by reviewing general patterns of diversification and sensory ecology in the gymnotiform electric fish, and provide a roadmap for research on gymnotiforms over the next two decades - one that will lead to a more integrative understanding of diversification that may be applicable to other Neotropical fish aroups.

Key words: Electric Organ Discharges (EODs), Reproductive isolation, Reproductive character displacement, Sensory ecology, Speciation

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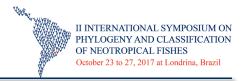
### Otomorphs (= otocephalans or ostarioclupeomophs) revisited

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The understanding of teleosts and of their classification have undergone numerous major changes during the last 170 years. Diverse reasons may be responsible for this, such as the speciose nature of the group, its highly diversified morphological structures, different approaches in studying the fishes, various interpretations of characters and their homologies, or a combination of some or all of these factors. The classifications have not only changed below family levels, but there have been major changes in higher level ranks, particularly in the creation of new ones. The last 40 years have seen the greatest changes, such the erection of the Supercohort Clupeocephala to contain all teleosts above the Cohorts Elopomorpha and Osteoglossomorpha and the removal of Ostariophysi (containing the Gonorynchiformes) from Euteleostei and its placement as sister aroup of the Clupeomorpha in the large clade currently ranked as Cohort Otomorpha (= Otocephala or Ostarioclupeomorpha) at the base of the Clupeocephala. Independent studies based on morphological and molecular data sets support the phylogenetic hypothesis of a sister-group relationship between clupeomorphs and ostariophysans. The first publications reporting this sister-group relationship were based on molecular studies published between 1993 and 1996. Morphological evidence-from fossils and extant clupeomorphs and ostariophysans-reaching similar results was published almost simultaneously. In contrast, the most recent publications based on mitogenome sequences and other molecular and genomic data have reached a different result, with the alepocephalids as a third member of the Otomorpha. However, the topologies of these non-morphological hypotheses do not agree. In one, the three major groups stand in a trichotomy, whereas in another the clupeomorphs are the sister to alepocephalids plus ostariophysans. Although both the ostariophysans and alepocephalids have been interpreted as primitive euteleosts in the past, their phylogenetic positions within Euteleostei were uncertain. The aims of this on-going research project are to test the two contrasting hypotheses concerning the content of the Otomorpha, searching for new morphological synapomorphies and testing previous ones. An examination of numerous fossil and extant taxa belonging to clupeomorphs, ostariophysans and alepocephalids is in progress. The studied material includes numerous ontogenetic series up to adult stages of clupeomorphs and ostariophysans, as well as larval stages and different subadult to adult sizes of ten alepocephalid genera. Two main analyses are conducted in parallel, one that includes fossil and extant species and another restricted to extant species, matching those in the molecular and genomic investigation. Preliminary results of over 200 characters (mainly osteological) of fossil and recent species or only recent ones have not found support for the inclusion of alepocephalids within the group. The investigation of a diverse set of soft anatomical structures is the next goal of this study.

Key words: Teleostei; Systematics; High level classification; Phylogenetic relationships; Morphology; Developmental morphology.



### The limitations of naming laterosensory-system pores in comparative morphological studies: examples from Neotropical fishes

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The laterosensory system is one of the most frequently used sources of characters for phylogenetic investigation of fishes. The general arrangement of these canals on head and trunk, including their association with surrounding bones, is significantly conservative, allowing tracing homologies even among phylogenetically widely separated groups with accentuated morphological disparity. In contrast, there is much variation at lower taxonomic levels, such that these differences are often used to diagnose species. In an attempt to allow comparisons between distinct taxa and to facilitate the incorporation of characters of this morphological complex into systematic studies, several authors have proposed different terminologies based on the naming of the main branches and their respective pores that open on the surface of the skin. However, these terminologies are usually incompatible with each other, since they have been established for specific taxonomic groups, hindering their use in analysis with broader taxonomic sampling. We provide examples from Neotropical fishes demonstrating that naming laterosensory system pores may be misleading even among closely related taxa. The main flaw of many of these terminologies is the failure to recognize that the pores in the skin of adults may arise from distinct ontogenetic paths. Some pores, especially those located at the terminal point of a given canal, are formed by the tube of a single neuromast. In other cases, however, the pore of the adult is formed by the confluence of tubes originated by two or more neuromasts. If at least one of the neuromasts involved in the confluence is present, the pore will still be present, even though in this case it cannot be considered homologous to the pore formed when two or more neuromast tubes are present in the confluence. Another flaw lies in the impossibility of homologizing pores of different taxa when one neuromast of a sequential series is lacking. In such cases, addressing the identity of the correspondent pores is impracticable due to the impossibility of establishing the homology of sequential neuromasts. These examples highlight the difficulties of applying terminologies to individual lateral-line pores, expressing the need of a deeper understanding of the laterosensory system prior to its incorporation in systematic studies of fishes.

Key words: Lateral line; Taxonomy; Systematics; Financial support: PPR: FAPESP (2014/10849-6; 2016/06677-0); MNLP: CAPES



#### Morphology, osteology, and systematics of fossil and living bonytongue fishes, Osteoglossomorpha

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Osteoglossomorpha, the bony-tongue fishes, have attracted the attention of systematic ichthyologists throughout history and were among the earliest groups of fishes to be analyzed in a cladistic framework. Over the last 50 years Osteoglossomoprha has become one of the most intensively studied groups of basal teleostean fishes, particularly considering the relatively low species-level diversity of the group among the extant global fish fauna (taxonomically, though, osteoglossomorphs are more numerous in the fossil record). Of the currently recognized valid extant species, approximately 90% are in a single family, Mormyridae (sub-Saharan Africa). Much of the attention that has been given to this group is because of its phylogenetic position at the base of extant teleostean fishes (it was long considered to be the sister group of all other Teleostei, a position now frequently recovered as being held by Elopomorpha). Osteoglossomorpha is morphologically diverse, with this heterogeneity being manifested by highly derived aspects of both the cranial and post-cranial skeleton: interestingly some taxa show relatively derived cranial skeletons and largely plesiomorphic post-cranial skeletons (e.g., Hiodon), and vice versa. This presentation will provide a review of the morphology and systematics of fossil and living Osteoglossomorpha, highlighting recent advances in the understanding the diversity and relationships among these fishes. For example, the recognized species-level diversity of the group has grown in recent years (e.g., the previously monotypic genus Arapaima has recently undergone substantial revision). Further, problematic issues related to the systematics of the group that require future work will be identified and discussed. The phylogenetic affinity of the monotypic African family Pantodontidae (sometimes included in the family Osteoglossidae, which includes the species of the South American genus Osteoglossum and Australasian genus Scleropages) is perhaps a key outstanding issue to be resolved. Continued analysis of the fossil record is also important for understanding the biogeographic and ecological evolution of the osteoglossomorphs.

Key words: Systematics; Paleontology; Gondwana, Teleostei



#### Review of the phylogeny and classification of the Callichthyidae armored catfishes (Ostariophysi: Siluriformes)

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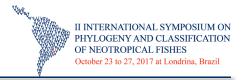
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Callichthyidae is a widespread family of Neotropical Ioricarioid catfishes composed of about 220 species. This group was formally recognized since the first classificatory schemes in the middle of the 19<sup>th</sup> Century on early ichthyological monographs of Charles Bonaparte and Pieter Bleeker. It reached an important taxonomic growth to the generic level on early and middle 1900's thanks to the works of Carl Eigenmann, Charles Regan, and William Gosline. However, the application of strict phylogenetic methods began on late 20<sup>th</sup> Century, and thereafter important taxonomic changes were revealed based on new research. The aim of this paper is to review the phylogeny and classification of the family, including all currently recognized hierarchical taxa, in regard to the analysis of published data and information generated in our ongoing work that includes a wide array of characters, comprising external morphology, osteology, and the exploration of new sources of data such as molecules. gross brain morphology, and digestive tract anatomy. Published literature revealed a long history of taxonomic names and classification changes of the established taxa which is here summarized and commented. Synapomorphies and valid taxa are also presented and discussed for the published data. Work in progress using multiple sources of phenotypic data along with molecular data in a strict phylogenetic context corroborates the monophyly of the family Callichthyidae, the subfamilies Callichthyinae and Corydoradinae, and most of its genera, further on the synonymy of Brochis and the revalidation of Scleromystax. However, it also reveals interrelationships of genera somewhat different from previous hypotheses, and the instability of the taxonomy within Corydoradinae, especially Corydoras, prompting the need to split this taxon in at least three genera. These findings support the necessity of a new phylogenetic classification for the Callichthyidae and the need of revised diagnoses for all genera. Moreover, brain and digestive tract morphology strongly support the monophyly of the family and its two subfamilies. Also, exploration of new sources of data emerge as an interesting quarry for phylogeny as revealed by the derived states consisting of exclusive features when compared to other loricarioid or even siluriform lineages. We discuss additional questions about the study of Callichthyidae, such as fossil data, distribution patterns, and future research.



### Systematics of Clupeiformes and testing for ecological limits on clade growth in a trans-marine/freshwater clade

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Clupeiformes (herring, sardines, shad, anchovies and allies) are a globally distributed clade with over 380 marine, freshwater, and diadromous species. Although best known as filter feeding fishes that form large schools, this group occupies a diverse array of trophic guilds and habitats, and exhibits various life history strategies. Theory suggests that species richness in clades is modulated by ecological limits, which results in diversity-dependent clade growth, a pattern that most clades exhibit. As a trans-marine/freshwater clade that has undergone repeated transitions between marine and freshwaters, Clupeiformes are an excellent system for investigating the interplay between ecological diversity and macroevolutionary dynamics. In this study I review the systematics of Clupeiformes and identify both well-supported relationships and groups in need of taxonomic revision. To provide a temporal context for the evolution of Neotropical Clupeiformes, I evaluate divergence times by integrating fossils and molecular age estimates of Clupeiformes. Finally, I use comparative methods to test whether ecological limits regulate diversity in Clupeiformes. My results suggest that trans-marine/freshwater clades are able to circumvent ecological limits on clade growth and exhibit a pattern consistent with exponential growth. This study demonstrates that phylogenies are a critical link between ecology and macroevolutionary dynamics and suggests that habitat transitions can play a key role in shaping diversity patterns.

Key words: Clupeiformes; Systematics; Diversification; Ecological Limits



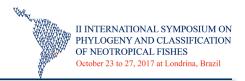
#### The biological explorations of the deep ocean in Brazil: what we know and what we don't know about the fishes

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The deep ocean is one of the most extreme environments on Earth. The pressure increases 1 atmosphere for every10 meters. The sunlight is absorbed exponentially making photosynthesis impossible starting from 200 m, and it is completely absent at the depth of 1,000 m. No matter how warm the surface layers are, the temperature falls to about 5° C between 300 and 1,000 m, and slowly decreases to 0-3°C near to the seafloor. The deep-ocean is also the largest environment on Earth, and corresponds to about 80% of the Brazilian Economic Exclusive Zone (EEZ). It is estimated that 10 to 15% of all fish species inhabit depths greater than 500 m, most of which having unique morphological and physiological adaptations. In Brazilian waters, the scientific biological explorations if the deep ocean started with stations made by the British Challenger Expedition (1872–1876) off the Northeastern coast and the São Pedro e São Paulo archipelago. For a long time, there was no specific program to explore the deep ocean, with the exceptions of a few fishery stations made by the German R/V Ernst Haeckel (1966) to Southern Brazil, and the American MIV Oregon (1957–1975) off the mouth of the Amazon river. Only in 1987, the French R/V Marion Dufresne made the first cruise between Rio de Janeiro and Espírito Santo States, including the Victoria-Trindade Chain. From 1994 to 2004, a few other expeditions were made as part of the Program REVIZEE on board of the French R/V Thalassa and the Brazilians N/Oc. Prof. W. Besnard, N/Pa Diadorim and N/Pa Soloncy Moura. Starting in 2000, the Brazilian oil and gas company PETROBRÁS has invested on studies focusing the deep ocean, mainly from the Campos and Potiguar Basins, in Rio de Janeiro and Rio Grande do Norte States, respectively. Besides, individual efforts have been made by the Projeto Tamar to obtain samples off Bahia using longline and traps. Based on the Catalog of the Brazilian Fauna, we evaluated records of 738 species of fishes known to occur in greater depths, including five species of Myxini, 80 of Chondrichthyes, and 653 of Osteichthyes. Most part of them are meso- or bathypelagic (40%) or bathydemersal (37%), followed by the demersal (19%) and pelagic-oceanic (6%) species which also occur in shallow waters. However, some of those records were based on few specimens, there are both latitudinal and depth gaps of sampling, and relatively few taxonomic revisions based on accurate comparisons available. As in other parts of the world, the Brazilian ichthyofauna of the deep ocean struggles under severe anthropogenic impacts caused by the destructive commercial fishing using bottom trawling, the extraction of oil & gas, and the mining for ferromanganese and sulfide deposits. Because the deep ocean is a delicate environment and the recovery of deep-sea species is considerably slower than for an equivalent in shallow water, it is possible that some part of the diversity is disappearing without our knowledge.

Key words: Taxonomy; western South Atlantic, Programa REVIZEE Financial support: CNPq Universal #433050/2016-0 (MRSM)



### The ambiguous correspondence of cichlid species challenges taxonomy but informs the evolutionary origin of Neotropical biodiversity

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The goal of systematic taxonomy, following the New Synthesis, is to discover evolutionary lineages, traditionally interpreted as documenting and diagnosing "species" units and inferring the patterns of descent among those units. These species are real if they are the products of biological evolution, the process producing phenotypic and genetic diversity, and they have value if they inform our understanding of those processes or our conservation of them. However, as we will demonstrate with several examples from Neotropical cichlid fishes, the products of taxonomy (species) are regularly not the same as the units of diversification (lineages). In some cases, poor correspondence between dichotomous units produced under a traditional taxonomic paradigm and well-quantified patterns of discontinuity (or lack thereof) reflects an obfuscation of evolutionary insight, implying a need for improved methods and sampling strategies for Neotropical fishes. However, discordance between taxonomic and evolutionary inferences also occurs in large part because the products of evolution are inherently amorphous and frequently can only correspond to diagnosable units with considerable ambiguity. In these cases, even well-quantified patterns of diversity defy all common representations for species as taxa or lineages, but this lack of correspondence provides greater insight into the diversification process than any subjective rendering of species could. There is now ample empirical evidence that nature exhibits nested endemism and reticulate lineages that are real products of evolution but cannot be adequately communicated as species under most taxonomies. Thus, the paradigm of "species" may act as a 'red herring', serving to distract rather than to inform. More importantly, the expanded paradigm for taxonomy, one that recognizes lineages as quantifiable but not necessarily dichotomous or unambiguously diagnosable, is fundamental to the ultimate goal of understanding the processes governing the origin and interaction of biological diversity. We demonstrate this point by relating ongoing work combining ecological and genetic analyses of the role of evolutionary constraints on osmoregulation in promoting diversification across the well-known blackwater-whitewater ecological gradient in the Neotropics. This work depends critically on a thorough and multifaceted view of Neotropical cichlid meta-population lineages.

Key words: species delimitation, hybridization, phylogeography, adaptation, gene flow



### Amazon basin drainage evolution: center stage for Neotropical fish diversification

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The past 20 years have seen substantial advances in our understanding of the geological processes underlying the formation of the exceptional Amazon basin, and the geomorphological history of Amazonian drainage networks. Modern research has largely confirmed the hypothesis first advanced by Hoorn and colleagues (1995) that the modern west-to-east-flowing transcontinental Amazon river system was assembled during the Late Miocene, by means of tectonic and erosional processes. During this time, the Solimões Basin (Western Amazon) became separated from the Llanos Basin (Central Orinoco) across the Vaupes Arch, and connected to the Amazonas Basin (Eastern Amazon) via breaching of the Purús Arch. Understanding the timing and sequence of these geomorphological events, cumulating in the largest documented mega-river capture event on Earth, is important for the study of Neotropical fish systematics and evolution. River capture is a perennial geomorphological process that moves the location of watershed divides between adjacent basins, merging and separating portions of drainage networks and their resident biotas. Importantly, river capture affects rates of both speciation and extinction, by subdividing species geographic ranges, and also by facilitating dispersal (geographic range expansion). Different age estimates for the origin of the transcontinental Amazon have important consequences for interpreting the landscapes on which Amazonian taxa diversified, and for understanding the role of rivers and river capture in the processes of biotic diversification. Here we review recent geological evidence supporting published dates for the timing of this seminal event, ranging from the Late Miocene (11 – 9 Ma) to Late Pleistocene (<1.0 Ma). We also review recent phylogenetic evidence supporting hypothesis advanced by Lundberg and colleagues at the 1997 ISPCNF in Porto Alegre, regarding the role of mega-wetland systems (e.g. Pebas and Acre systems, modern várzeas and pantanals) in the evolution of Amazonian fish diversity. Pertinent data are reviewed from geochronological (e.g. fission track, radiometric) dating methods, sedimentology, and biostratigraphy (e.g. palynology, macro-fossils), from localities in Llanos, Solimões and Amazonas Basins, and from offshore marine localities in Amazon Fan and Ceará Rise, and from species-dense time calibrated phylogenies of Neotropical fishes. The current literature consensus is that the modern transcontinental river system first formed in the Late Miocene, in association with major orogenic activity in the Northern Andes, with date estimates in the range 8.1 – 10.1 Ma. Further, there was substantial increase in Andean sediments reaching the mouth of the Amazon river in the Early Pliocene, about 4.5 Ma. These data indicate the onset of transcontinentalization was the result of multiple river-capture events over a time-interval of about 3.5 – 5.5 million years. Proposed Pleistocene dates for transcontinentalization are invalid as they are based on a method (i.e. quartz optically stimulated luminescence) that is only able to estimate dates up to a maximum of ~0.35 Ma. We conclude that the unique geological history of river capture events in the Sub-Andean foreland basin contributed substantially to the origin, diversification, and maintenance of species-richness in many clades large riverine fishes, including marine derived clades (e.g. Clupeidae, Potamotrygonidae) and primary freshwater clades (e.g. Apteronotidae, Pimelodidae).

Key words: Biostratigraphy, Geochronology, River capture, Sub-Andean foreland, Transcontinental Amazon Financial support: United States National Science Foundation awards 0741450 and 1354511 to JSA



#### Diversity and systematics of Gymnotiformes: A 20-year progress report

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The past 20 years have seen steady advances in our understanding of gymnotiform diversity and systematics. From 1997 to 2017 the number of valid gymnotiform species increased by 143 species (from 102 to 245 species, or a 140% increase) at an average rate of about 7.1 new species per year, but closely matching an exponential growth curve (R<sup>2</sup>=0.98). These new species represent 25 of the 34 (74%) currently recognized gymnotiform genera and all five families, concentrated in the following genera: Gymnotus (28), Sternarchorhynchus (28), Brachyhypopomus (22), Apteronotus (12), and Eigenmannia (12). During this time interval, five new genera in three families were also described: the apteronotids Tembeassu Triques (1998), Compsaraia Albert (2001), Parapteronotus Albert (2001), and Pariosternarchus Albert & Crampton (2007); the hypopomids Akawaio Maldonado-Ocampo et al. (2014) and Procerusternarchus Cox-Fernandes et al. (2014); and the fossil sternopygid *†Humboldtichthys* Gayet & Meunier (2000). This rapid growth in taxonomic knowledge was fueled in part by renewed commitment to field collections, and the application of new technologies for discovering and recognizing new taxa. Systematic use of portable electronic amplifiers, GPS, and digital photography is now routine. Electric signals are also increasingly used in recognizing and describing new gymnotiform species. Precision (or reproducibility) in species delimitation has been facilitated by the widespread use of molecular sequence (genetic) data, and increasingly rigorous and standardized techniques for comparative morphology; e.g. geometric morphometrics, whole-mount (cleared-and-stained) histology, scanning electron microscopy (SEM), and computed tomography (CT). Accuracy (or approximation to true values) in species delimitation has been improved by increasing attention to ontogenetic and geographic sources of variation in both molecular and morphological datasets. These new tools have greatly advanced the study of ecological and evolutionary processes governing gymnotiform diversification. New molecular tools are now revolutionizing higher-level gymnotiform systematics. All phylogenetic studies of Gymnotiformes since 1997 included molecular data, with datamatrices for ingroup taxa ranging over four orders of magnitude, from ~5.7 kbp (Tan et al. 2017) to ~15.8 mbp (Alda et al. 2017), while only two of these studies included morphological data (Albert, 2001; Tagliacollo et al., 2017). The most robust genomic analysis to date includes 966 ultraconserved element (UCE) loci for 42 gymnotiform species (Alda et al. 2017). There is high consensus among all these studies in inter-generic relationships, but little consensus regarding inter-family interrelationships. The current best-estimate indicates a rapid radiation at the base of crown-group Gymnotiformes, with interfamily divergences too fast to have left a strong phylogenetic signal; i.e. the tree does not strongly predict interfamily-level trait values. Further, a species tree (cloudogram) for a subset of 50 UCE loci shows support for two different patterns of inter-family relationships. Such gene-tree incongruences may arise from real biological processes (e.g. incomplete linage sorting, horizontal gene transfer), or systematic gene tree errors (e.g. incorrect models, low phylogenetic informativeness of short loci). These phylogenomic results add to a growing literature cautioning that more data do not guarantee the correct tree, and that judicious use of genomic data is more reliable than simply using all the available data.

Key words: Neotropical fishes, Phylogenomics, Species delimitation, Taxonomy, Ultra-conserved Elements (UCEs) Financial support: United States National Science Foundation awards 0741450 and 1354511 to JSA, and 1354149 to FA



### Taxonomy, systematics, and diversification of the New World Silversides (Atherinopsidae)

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The New World Silversides (Atherinopsidae) represent a widespread and diverse group of freshwater, estuarine, and marine fishes. Freshwater atherinopsids occur throughout the eastern United States and Canada, southward through Mexico and Central America. Marine and estuarine atherinopsids are distributed from Baja, California to Peru in the Pacific basin and from southern Canada to Northern Brazil in the Atlantic basin. Silversides are surface dwelling fishes that often occur in large schools. Some species are of ecological importance, serving as forage for larger predatory species, and others are economically valuable and provide a significant source of protein for human consumption. The objectives of this study are three fold. First, we will provide an overview of the taxonomic diversity of Silversides throughout their distribution, highlighting areas of diversity and significance. Second, we will provide an overview of the evolutionary history of the group and summarize morphological and molecular based hypotheses from previous studies, including several of our own. Finally, our previous work indicated higher rates of lineage diversification for freshwater species than marine species. We will expand on this study by incorporating morphological data to test whether freshwater lineages are morphologically more diverse and have faster rates of phenotypic evolution than marine Silversides. It is expected that rates of species diversification will be positively correlated with morphological diversification in freshwater species due to the high rate of isolation and reduction of gene flow in freshwater habitats compared to marine habitats.

Key words: Atherinopsidae, diversity, diversification rates



#### A Phylogenetic hypothesis for the New World clingfish genus Gobiesox based on morphological characters (Teleostei: Gobiesocidae)

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The New World clinafish genus Gobiesox contains 30 species, including 23 marine species and seven that are known only from freshwater but are tentatively considered to be amphidromous. A recent multi-locus molecular phylogenetic investigation of Gobiesox based on 12 species (four freshwater and nine marine) and multiple outgroup taxa obtained Gobiesox as monophyletic and showed that the freshwater clingfishes render the marine species paraphyletic. Despite this recent advance in our understanding of Gobiesox, the phylogenetic relationships of the majority of the species (including both marine and freshwater taxa) remain unknown and are unlikely to be clarified through molecular phylogenetic investigation because tissues are rare or unavailable. Examination of museum specimens representing 23 species of Gobiesox (six freshwater, 17 marine) and a number of suitable outgroup taxa has resulted in a morphological character matrix comprising 48 characters. Phylogenetic analysis of this data set using parsimony criteria results in cladograms that include a monophyletic Gobiesox but offer an alternative hypothesis on the relationships of the freshwater clade, which is the sister taxon to a clade comprising all marine species included for investigation. Derived morphological characters supporting the monophyly of Gobiesox and subgroupings of freshwater and marine taxa will be presented.



#### Comprehensive phylogeny of ray-finned fishes (Actinopterygii) enhanced by transcriptomic and genomic data

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Our understanding of phylogenetic relationships among bony fishes and their classification has been transformed in recent years with the influx of molecular data. Many novel aroups have been defined on evidence from a handful of genes, and a new fully phylogenetic classification has been proposed. However, some critical nodes of the fish phylogeny still remain controversial. Phylogenomics is a powerful yet challenging resource to improve this knowledge based on genome-wide evidence. Here we leverage 132 newly sequenced transcriptomes combined with genomic databases (5 new genomes) to investigate the evolution of fishes with an unparalleled scale of data: >500,000 bases of exon sequences from each of 305 species, representing all major bony fish lineages (66 out of 72 orders). Selection of genetic loci from genomic and transcriptomic data was based on stringent criteria to address potential systematic errors arising from whole genome duplications and base composition biases, establishing a novel set of 1,105 exon markers for general application in fish phylogenomics. Maximum likelihood analyses supported a well-resolved phylogeny that mostly agrees with previous hypotheses and gene genealogy interrogation resolved some long-standing uncertainties. The set of 1,105 molecular markers tested in this study, applied via hybrid enrichment and high-throughput sequencing, is a promising resource to increase taxonomic sampling to resolve remaining uncertainties in percomorph evolution and to consolidate the tree of life for fishes.



### Flatfishes (Teleostei: Pleuronectiformes): a contemporary view of species diversity

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Flatfishes (Teleostei: Pleuronectiformes) undergo an extreme form of ontogenetic development where one eye migrates to the opposite side of the head. Due to this morphological asymmetry, flatfishes are a distinctive group of fishes whose adults are easily recognized. Flatfishes occur in all of the world's oceans spanning a global distribution from north Polar seas to the waters off Antarctica. The majority of flatfish species occur in marine and estuarine waters, with relatively few species inhabiting freshwater ecosystems. Current estimates of the diversity of flatfishes indicate that approximately 800+ species in about 123 genera should be recognized as valid taxa. The most diverse flatfish families (with ca. 100+ species/family) include the Soleidae, Bothidae, Cynoglossidae, and Paralichthyidae. Families representing medium levels of diversity (ca. 27-64 species/family) include the Pleuronectidae, Achiridae, Samaridae, Rhombosoleidae and Poecilopsettidae. Other families, such as the Psettodidae, Citharidae, Scophthalmidae, and Achiropsettidae contain the lowest levels of species diversity (ca. 3-10 species/family) among pleuronectiform families. Geographically, the highest diversity of flatfish species occurs in the tropical Indo-Pacific region. Ecologically, the highest diversity of species is represented by flatfishes that inhabit the continental shelf. Relatively few flatfish species from a variety of families and genera occur in the deep sea (> 1,000 m). Current rates of species discovery indicate that ichthyologists are continuing to find new species at a pace similar to that when scientific nomenclature began in 1758 indicating that we are still not close to knowing the total diversity of flatfishes. A number of factors, including both those related to the fishes themselves and those related to activities of systematic ichthyologists, influence the probability that a flatfish species will be discovered. Several factors related to systematic activities have changed over time and the impact of these changes will be discussed relative to species discovery rates for the Pleuronectiformes, with specific examples highlighted from recent and on-going systematic studies of Indo-West Pacific and New Zealand flatfishes. Finally, some thoughts on several topics relevant to the progress and future direction of species discovery of flatfishes will be presented.



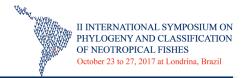
### Ontogeny and homology of the tripus (Teleostei: Otophysi)

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With more than 10.000 species, members of the Otophysi dominate the non-marine waters of our planet and include almost two thirds of all known freshwater fishes. Part of their evolutionary success has been hypothesized to be related to the evolution of a sound perception and transmission system, termed the Weberian apparatus. Consisting of anatomical modifications of the swim bladder, the first four vertebrae, and the inner ear, the Weberian apparatus increases the spectrum of hearing, its sensitivity and its accuracy when compared to most fishes without this apparatus. The swim bladder is connected to the inner ear via a chain of four tiny, highly moveable bones, the scaphium, claustrum, intercalarium, and tripus, together termed the Weberian ossicles. While there is a consensus that the Weberian ossicles are derived from the first three vertebrae, their precise homology has remained an area of long-standing and ongoing discussion and dispute. Applying the ontogenetic approach to resolving homologies, we here investigate the development of the tripus in representatives of all four otophysan orders, the cypriniforms, characiforms, siluriforms, and gymnotiforms. We discuss 13 different hypotheses of homology of the tripus previously formulated and compare these to our findings. We conclude that the tripus is a modification of the rib of the third vertebra in cypriniforms. In characiforms, siluriforms, and gymnotiforms the lateral process of the third vertebra forms at the base of the third basiventral before the third rib ossifies and subsequently fuses with it to form the tripus of the adult stage. This complex tripus is a synapomorphy of Characiphysi. The extent and size of the rib component of the complex tripus varies especially among siluriforms and formation of the tripus in this group may provide interesting and phylogenetically relevant information.

Key Words: Taxonomy; Systematics; Weberian Apparatus; Morphology



#### Pleistocene effects on Atlantic marine biogeographical barriers

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Two major marine biogeographic barriers in Brazil, the Amazon River outflow and the open water distance between the Brazilian coast and Trindade-Martim Vaz Insular Complex, are areatly affected by sea level fluctuations. The Amazon freshwater and sediment outflow, which started to influence the Atlantic about 10ma ago, is a strong barrier to shallow water reef fish and other organisms separating Brazil and the Caribbean, and it is probably responsible for most of the endemism found in Brazilian coastal habitats. During present high sea level conditions, many deep-water species can colonize reefs under the Amazon outflow and their populations provide a connection between Brazil and the Caribbean. In periods of low sealevel, the Amazon's sediment outflow is deposited directly onto deeper areas, closing this deep reef connection. The barrier between the Brazilian coast and Trindade-Martim Vaz Insular Complex is the 1170km separating these areas. However, several seamounts, some reaching depths as shallow as 15-20m depth, are present between the islands and the mainland. In current se-level, these sea mounts act as stepping stones to allow colonization of the islands by deep reef species. During low sea-level periods, many sea mounts become islands and serve as a corridor for both deep and shallow species. Therefore, these two barriers respond in opposite ways to sea level fluctuations: the Amazon barrier becomes stronger during low sea-levels, whereas the opposite happens to the distance barrier between the coast and Trindade. Here we will discuss how these barriers operate and how they influence endemism along the Brazilian coast and Trindade-Martim Vaz.

Key words: Phylogeography, Ichthyology, Sea-level Fluctuations, Biogeography Financial support: California Academy of Sciences, National Science Foundation



#### Systematics of the Gymnotiformes: advances and future prospects

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The knowledge on the species diversity, nomenclature, and relationships at species level greatly advanced in the last two decades. In opposition, the number of studies published on the relationships above species level, and in fields as comparative anatomy, molecular systematics and phylogeography remain almost unchanged. Currently, 240+ valid species are known to Gymnotiformes and several new species are in process of description. The substantial raise in the species numbers is consequence of a series of factors, as such: an active generation of ichthyologists working on the group, the utilization of previously poorly used or new tools to identify species (e.g., DNA barcooding, Next generation Sequencing, electric organ discharges and 3D CT scan), expeditions to inadequately explored or unexplored areas, and comprehensives taxonomic revisions as the first step to recognize additional diversity into a clade. Similarly, Ferraris et al. scrutinized and summarized the nomenclature of the order in a comprehensive catalog only just published. In the same way the phylogenies based on morphology increased considerable. Those phylogenetic studies added new characters, proposed new hypotheses of relationships, reveled patterns and process in the evolution of several taxa of the Gymnotiformes. On the other hand, in despite of the phylogenetic reconstructions, there is still a clear deficit on the knowledge of the anatomical diversity of the group. As consequence, several poorly explored or unexplored anatomical systems remain to be investigated. The recent discovery of the caudal skeleton in the electric eel, the largest member of the order, exemplifies the topic. Comprehensives studies published on molecular systematics and phylogeography are still uncommon in the order. In the last 20 years only a hand full of papers dealing with the phylogeography and molecular phylogeny were published. Those numbers greatly contrast with the large amount of papers published on remain orders of Otophysi. On going studies on osteology, musculature and neuroanatomy will supply the deficiency on the anatomy and offer new insights on phylogenetic relationships of the Gymnotiformes. Likewise, expeditions to Gymnotiformes undersampled regions associated to an integrated taxonomic approach, and genome-wide phylogeographic and phylogenetic studies will advance the knowledge on species diversity, patterns and process of diversification in the order.

Key words: Neotropical electric fishes; Diversity; Comparative Anatomy; Molecules Financial support: Smithsonian Institution

## **General Session**



#### A new threatened species of *Hypomasticus* Borodin, 1929 (Characiformes: Anostomidae) from rio de Contas, southern Bahia

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Characiformes and Siluriformes are the richest and most abundant groups of freshwater fishes in South America. Anostomidae is the second richest family of Characiformes with more than 150 valid species. That diversity is yet underestimated, as species are being discovered in field expeditions of unexplored areas and described in taxonomic revisions every year. Here, a new species of Hypomasticus is described and illustrated using morphological data, and compared to all other species of the family. Specimens were illustrated using digital photograph, measured using digital calipers, and had teeth, scales and fin rays counted under a stereomicroscope. The morphological data, partially summarized in tables, were compared to original data obtained from specimens of other species or to data available in the literature. The new species is distinguished from all other Anostomidae, except Hypomasticus julii, H. pachycheilus, H. mormyrops, H. thayeri, and Leporinus melanopleurodes, by having inferior mouth, with a mouth cleft longitudinally aligned with or slightly ventral to ventral margin of opercle, and four premaxillary and dentary teeth with blunt cutting edge and arranged side by side (vs. mouth subterminal, terminal or superior with teeth incisiform and arranged in a stair-like pattern in most anostomids). The new species is distinguished from H. julii, H. pachycheilus and Leporinus melanopleurodes by having three dark midlateral blotches on body (vs. body with numerous blotches or with a dark longitudinal stripe); and from H. mormyrops and H. thayeri by having three scale rows between dorsal-fin origin and lateral line and three scale rows between lateral line and pelvic-fin origin (vs. four). The new species is allocated in Hypomasticus for sharing numerous features with H. mormyrops, the genus type species, including inferior mouth with four premaxillary and dentary teeth with blunt cutting edge and arranged side by side, and three midlateral blotches on body. The new Hypomasticus species is only known from two specimens, and it is possibly endemic to rio de Contas in southern Bahia, where it occurs with Megaleporinus brinco, Megaleporinus sp., and Leporinus bahiensis. The rio de Contas basin is severely impacted due to water pollution from urbanization, and highly fragmented by small reservoirs used for maintaining water in a dry savanna. This is a new threatened species according to IUCN criteria.

Key words: Systematics; Taxonomy; Anostomoidea Financial support: Fundação Araucária (177/2014)



### A new species of *Microschemobrycon* (Characiformes: Characidae) from the rio Xingu, Brazil

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Microschemobrycon is a group of small size characids inhabiting freshwater bodies in the Amazon region of South America, including the Amazonas, Casiguiare, Essequibo, Orinoco and Paru do Oeste river basins. According to Géry (1977), Microschemobrycon can be diagnosed from the remaining Aphyoditeinae by the presence of a terminal mouth (except M. melanotus); a single row of numerous feeble tricuspid or conic teeth in jaws (including maxilla); premaxilla with ascending process; "postorbitals" present; lateral line series complete or incomplete and caudal fin scaleless (except M. casiguiare). Composed by seven valid species, the genus is the largest unit in the Aphyoditeinae, a subfamily branded by the high frequency of monotypic genera (e.g. Aphyodite, Leptobrycon, Parecbasis, Oxybrycon, Thrissobrycon). Microschemobrycon sp n. is known only from the middle rio Curuá, a black water tributary of the rio Xingu, Pará, Brazil. The new species can be easily distinguished from all congeners by the presence of a unique conspicuous, dark and round caudal-peduncle spot. Additionally, Microschemobrycon sp.n. can be distinguished from all congeners, except M. elongatus, by the presence of a longitudinal dark stripe along the lower jaw. The new species differs from M. elongatus by having lateral line scale series completely pored (vs. lateral line incomplete, with 15-19 pored scales) and by having tricuspid teeth (vs. conical teeth). Additionally, other aspects of its coloration differ the new species from all congeners, including M. elongatus: the presence of a small, but always present, dark spot in the dorsal-fin oriain and a dark and wide lateral band on the caudal region of the body. The species is also characterized by a conspicuous pseudotympanum, and caudal fin scaleless. Males of the new species are sexually dimorphic, presenting bony hooks on pelvic- and anal-fin rays.

Key-words: fishes, Aphyoditeinae, body transparency, Neotropical region Financial support (FCJ): CNPq (process 453850/2014)



### Searching for characters in the skin of Pseudopimelodidae (Actinopterygii: Siluriformes) for systematics purposes

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Pseudopimelodidae is a family of catfishes with seven genera and 48 species. The systematics of Pseudopimelodidae has been investigated for the last 20 years, but the relationships of several species remain unresolved. It is difficult to include all species in a phylogenetic analysis because several of them are rare in collections, which difficult the use of traditional invasive techniques like osteology or myology. The loss of integrity, or specimen destruction, is the main obstacles, stimulating the search for new sources of characters. Pseudopimelodidae has a rough skin, with several tubercles, probably related to the rheophilic habits. In the search for characters with low level of destruction and useful to the systematics of Pseudopimelodidae, the skin on the side of head of one species from six genera was histological examined, using the traditional technique of hematoxylin and eosin, and scan electron microscopy. A small piece of skin (c. 0.5x0.5cm) was removed from the lateral of head, nearly cheeks, and properly prepared according to each type of analyses. In all the species a stratified epithelium is observed, with presence of tubercles with different morphologies, and large club cells. In the tubercles, the number of layers of cells was clearly larger than in other regions of skin. In Microglanis garavelloi and Batrochoglanis villosus the tubercles have pointed unculi, but in Pseudopimelodus bufonius and Lophiosilurus alexandri they are rounded; in Cephalosilurus apurensis was not observed the presence of unculi in the tubercles because of the deep layer of keratin. In Rhyacoglanis, the unculi has several rounded projections on surface. Therefore, the architecture of tubercles is very promising as source of information. Also, a layer of melanin in the dermis is easily observed in some species, but absent in others. In light of morphological variations, there is a good potential in skin histology as a source of informative characters for the Pseudopimelodidae systematics, allowing the inclusion of rare species.

Key words: Catfishes; Histology; Scan Electron Microscopy Financial support: PIBIC-CNPq/UEL; CNPq (proc. 304868/2015-9)



#### Tackling a recent freshwater radiation of silversides (Atherinopsidae: *Odontesthes*) in southeastern South America

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Odontesthes encompasses 19 species inhabiting marine, estuarine and freshwater environments in South Brazil, Uruguay, Argentina and Chile. A large monophyletic subgroup of the genus, distributed in freshwater of southeastern South America, is a radiation containing 10 putative species. This subgroup is found in lower Paraná and Uruguay rivers and Laguna dos Patos and Tramandaí systems. Within this region, some species appear to be restricted to riverine environments (e.g. Odontesthes perugiae, and O. yucuman), some inhabit lakes and lower portions of rivers (e.g. O. bonariensis, O. humensis, O. retropinnis, O. mirinensis, and O. sp. "cha") and others are restricted to lacustrine environments (e.g. O. bicudo, O. ledae, and O. piquava). The three large-sized species (O. bonariensis, O. humensis and O. retropinnis) are distinguished among themselves through gill rakers counts, and all small-sized species in the O. perugiae species group (remaining seven species) differ mostly on snout and mouth shape, features often correlated with diet. Preliminary investigation of relationships using mitochondrial (COI and D-Loop) and nuclear (SH3PX3) shown none to little phylogenetic informativeness among the O. perugiae species group, suggesting a recent radiation during the Pleistocene (<2Mya). Given the mismatch between morphological and genetic data we evaluate the extent to which feeding behavior may be responsible for high speciation rates in this recent clade. For that we reconstruct phylogenetic relationships using next generation sequencing and describe morphological variation using geometric morphometrics. Genomic data was generated for 124 individuals representing several populations of these 10 species of freshwater Odontesthes and O. argentinensis. Two double digest reduced representation libraries (ddRAD) were sequenced, which after assembled represented 6,696 SNP's with 25% missing data. Phylogenetic results show large-sized species (O. retropinnis, O. bonariensis and O. humensis) form a grade where clade supports are stronger, in which O. bonariensis is the sister group of the smaller body size species of the O. perugiae species group. Within this group a monophyletic riverine group represented by two allopatricly distributed species in the upper Uruguay and lower Paraná and Uruguay rivers (O. yucuman and O. perugiae, respectively). Sister to this group are species endemic to lacustrine environments (Patos: O. mirinensis, O. sp. n. "cha"; Tramandaí: O. ledae, O. bicudo, O. piquava), which are part of a recent radiation with low genetic structure observed between species. Analyses using geometric morphometrics and principal component (PCA) on head and body shape, indicates that most variation between species (PC1) observed within this group corresponds to snout and body elongation/shortening. Changes in this axis are observed in at least three instances within the O. perugiae species group indicating similar and recent phenotypic evolution disregarding region or habitat (lacustrine and riverine) and instances of sympatry. The secondary axis of variation (PC2) represents changes on head/body depth and prognathy/hypognathy associated mostly with the older radiation of the large-sized species. Data on feeding ecology from Odontesthes species suggests that differences in diet may correlate snout elongation/shortening and prognathy/ hypognathy to changes from piscivory (long snouts) to invertivory (short snouts) and to benthivory (hypognathy) to omnivory (prognathy).

Key words: Next-gen Sequencing, Incomplete lineage sorting, Geometric morphometrics Financial support: CNPq (#Universal), CAPES (#PNPD)



# Morphological differentiation of the Species Complex Hoplias malabaricus (Bloch, 1794) (Teleostei: Characiformes: Erythrinidae) in the Neotropical region

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Hoplias malabaricus (Bloch, 1794) is a species complex (=paraspecies) that shows the widest distribution in the Neotropical region, from northern Argentina to Panama. A species complex is composed of populations that have not responded morphologically to vicariant and/or geodispersal processes. Since 1980, cytogenetic and molecular biology data unveiled the hidden diversity within this paraspecies. This group requires a taxonomic revision, however, that is a difficult iob due to the broad distribution of the species. Geometric morphometrics has been considered an effective tool to search for species boundaries. Our aim was to explore the morphological variation from the cephalic skeleton of H. malabaricus using samples from the Neotropical region. The cephalic regions are not affected by formalin fixation. We photographed 1200 samples from several ichthyological collections. We selected 13 landmarks, using tpsDIG 2.29 software. We used MorphoJ 1.06 to performed Procrustes fit, Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA), for this analysis our priors groups were Basins and Fresh Water Ecoregions. Analysis including all samples from the Neotropical region does not allow the detection of morphological differences among basins. On the other hand, a separate CVA within the rio La Plata basin successfully separated Paraná, Paraguay, and Uruguay river basins. CVA shape changes showed differences in the supra-pre-opercular bone and eye width among these three populations. Those characters could diagnose different species within the H. malabaricus complex. Future perspectives include comparing geometric morphometrics with molecular and cytogenetic characters and extending morphological variation to the whole geographical distribution range of this paraspecies.

Key words: Systematics; Taxonomy; Freshwater; Neotropical



### Phylogeny of Pterygoplichthys Gill, 1858 (Siluriformes: Loricariidae) sensu lato

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The genus Pterygoplichthys belongs to the widely distributed Loricariidae, the largest family of Neotropical Siluriformes, and it was described to accommodate Hypostomus duodecimalis and H. multiradiatus, loricariids with more than 10 branched dorsal fin rays and fully plated abdomen. Posteriorly, Liposarcus was proposed to allocate H. multiradiatus, H. pardalis and a new species, Liposarcus altipinnis. Liposarcus was diagnosed by the lack of hypertrophied odontodes on the cheek plates. In 1991, in a review of the loricariids with extranumerary branched dorsal fin rays, it was proposed a new genus, Glyptoperichthys, diagnosed by an elevated supraoccipital process and hypertrophied odontodes on cheek plates (vs. supraoccipital not elevated, lack of odontodes and three plates limiting the supraoccipital process in Liposarcus; and presence of odontodes, supraoccipital not elevated and supraoccipital limited by one single plate in Pterygoplichthys). In 2004, after a cladistic analysis of Hypostominae, Liposarcus and Glyptoperichthys were synonymized in Pterygoplichthys, and a new tribe was proposed, Pterygoplichthyini. Currently, Pterygoplichthys comprises 15 species, distributed in Amazon/ Orinoco, La Plata, São Francisco, Magdalena, and Maracaibo basins. The present work aimed to realize a thorough phylogenetic analysis of the species of Pterygoplichthys and to test the validity of Liposarcus and Glyptoperichthys. We used 203 osteologic and morphologic characters, already known and newly proposed, of 109 terminal taxa. The tree was rooted in Delturus brevis and the ingroup comprised 13 species of Pterygoplichthys. The parsimony analysis was conducted using TNT© 1.1, heuristic search, 10,000 replicates and saving 10 trees per replicate. To reduce the influence of homoplastic characters in the analysis, we performed an implied weighting analysis with a constant of 3 (k=3). The analysis resulted in three most parsimonious trees, with 1509 steps; fit= 94.78; adjusted homoplasy of 97.22; and 20,786,671 total-rearrangements examined. The strict consensus tree agrees with the latest hypothesis and corroborated the tribe Pterygoplichthyini, although with a relation of Pterygoplicthyini+(Hypostomini+Ancistrini). Pteriaoplichthyini is diagnosed by one unique characteristic, large number of vertebrae until last bifid neural spine (20-23), and ten other non-unique synapomorhies. Two monophyletic groups were recovered, Liposarcus and Pterygoplichthys. Liposarcus presented the following synapomorphies: presence of seven infraorbitals and lateral walls of anterolateral process of the basipterygium with same length. Pterygoplichthys presents hypertrophied odontodes on cheeks and a fully evertible cheek plate. The proposition to consider just one single genus was not corroborated here. On the other hand, to keep Glyptoperichthys valid, a new genus would have to accommodate P. punctatus, P. weberi and P. zuliaensis. As two monophyletic groups were found, we propose to keep Liposarcus with L. ambrosettii, L. disjunctivus, L. pardalis and L. multiradiatus and Pterygoplichthys with P. etentaculatus, P. gibbiceps, P. joselimaianus, P. lituratus, P. parnaibae, P. punctatus, P. scrophus, P. undecimalis, P. weberi, P. xinguensis and P. zuliaensis.

Keywords: Hypostominae; Pterygoplichthyini; Liposarcus; Glyptoperichthys Financial support: AGB benefited from a DCR/AM fellowship from FAPEAM/CNPq (process 062.01066/2015)



#### Phylogenetic relationships of the species within *Metynnis* Cope, 1870 (Characiformes: Serrasalmidae)

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The parsimony analysis of phylogenetic relationships among species of Metynnis was conducted with 162 morphological characters observed across 46 terminal taxa, including all genera of Serrasalmidae, inclusive the extinct Megapiranha paranensis, and the 14 Metynnis species recognized as valid herein. Other authors had already proposed most of the characters examined in this study, from which only 11 are unpublished, and mainly related to color pattern. The analysis was performed in PAUP\*4.0a152 using heuristic searches with 10.000 random sequence additions used to obtain starting trees via stepwise addition and tree bisection reconnection branch swapping method. All characters were unordered and weighted equally, and optimized based on accelerated transformation (ACCTRAN). The final hypothesis resulted in a strict consensus tree generated from the resulting eight equally parsimonious trees, with 794 steps, consistency index (CI) 0.307, and retention index (RI) 0.727. As expected and already proposed by previous authors, Metynnis is recovered as the sister group of the remaining genera popularly known as "piranhas" (Catoprion, Megapiranha, Pygocentrus, Pristobrycon, Pygopristis, and Serrasalmus). The other genera of the family are organized as follows: a basal clade composed of Mylossoma, Colossoma and Piaractus, sister group of a more inclusive clade, with Acnodon in the basis as sister group of Myloplus (including Utiaritichthys, proposed here as a junior synonym of the former genus), closely related to a clade composed of Ossubtus, Myleus, Mylesinus, and Tometes, and finally this group as sister to the clade composed of Metynnis and "piranhas". The monophyly of Metynnis was supported by 20 synapomorphies, from which three are exclusive as already proposed by other authors: adipose-fin base length eaual or superior to distance between dorsal-fin end and adipose-fin origin; descending arm of maxillary expanded and broad, forming almost a straight angle with ascending arm; and lateral surface of vertical canal of preopercle uncovered and situated posteriorly to musculature and infraorbitals. Within Metynnis, the species are divided into two most inclusive monophyletic clades: the first composed of M. mola, M. cuiaba, M. otuquensis, M. altidorsalis, M. maculatus, M. anisurus and M. lippincottianus; and the second of M. guaporensis, M. luna, M. fasciatus, M. hypsauchen, M. longipinnis, M. melanogrammus, and an undescribed species. The first clade is supported by nine synapomorphies, including presence of dark maculae on flank; infraorbital 1 dorsally overlapping the premaxillary dentigerous process; distal portion of supraoccipital spine straight; and epiotic with a single forame. In turn, the latter clade is supported by 13 synapomorphies, comprising the presence of a concavity on anterior lobe of males anal-fin; three dorsal-fin rays articulated with first dorsal pterygiophore; opercle shape, with ventral half approximately twice as large as dorsal half; and lack of fusion of hypurals 1 and 2. This study represents the first hypothesis of relationships among all Metynnis species, involving a great number of characters investigated throughout all genera within Serrasalmidae.



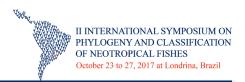
#### Hyphessobrycon panamensis Durbin, 1908 as senior synonym of Hyphessobrycon condotensis Regan, 1913 and Hyphessobrycon sebastiani García-Alzate, Román-Valencia & Taphorn, 2010 (Characiformes: Characidae)

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The alpha-taxonomy of Hyphessobry con panamensis is very complex due to its brief characterization in the original description, be widely distributed and present population variations, besides that other similar species have been synonymized and then revalidated along time. Hyphessobrycon panamensis was described from the río Boquerón, Panamá, and has been recorded in drainages from Belize to Colombia. In Colombia, its distribution includes the río San Juan, where it is syntopic with H. condotensis, and H. sebastiani, two morphologically similar species. It is noteworthy that H. condotensis has already been suggested as junior synonym of H. panamensis by some authors, and was described from the río Condoto, tributary to the río San Juan, which is the same typelocality of H. sebastiani. In a recent work, H. panamensis was restricted to drainages in Costa Rica and Panamá, whereas H. sebastiani was the valid species occurring in Colombia. Thus, we aimed to analyze these species, covering their entire geographic distribution, investigating the main diagnostic characters, and other characters that could distinguish one from another. Counts and measurements followed the traditional methodology for Characidae. We analyzed the syntypes of H. condotensis and H. panamensis, the holotype of H. sebastiani, topotypes of the three species, and specimens from other drainages, and we verified the overlapping of several characters, including in the same drainage. Therefore, as no evidence for distinction of these species was found, not even with combination of characters, we purpose here H. condotensis and H. sebastiani as junior synonyms of H. panamensis, highlighting some variations of populations. In Panamá, the variations included a larger standard length (48.6 mm SL vs. up to 40.0 mm SL), completely pored lateral line (33 vs. 6-17) and less branched anal-fin rays (19-21 vs. 24-27), although these features totally overlap considering its whole distribution. The number of anal-fin rays of the syntypes of H. condotensis and H. panamensis is discreet (25-27 and 21-23, respectively), however, in other specimens from the río San Juan and drainages in Panamá, this count is overlapped. In Costa Rica, the variations included a distal margin of the anal fin very darkened (vs. little darkened), and up to 23 total gill rakers (vs. 17-20, rarely 21 or 22, in Panamá). Thus, Hyphessobrycon panamensis can be diagnosed from its congeners by having two dark humeral blotches, first darker than second; no dark blotch on caudal peduncle and dorsal fin; no chevron marks along flank; a pair of large bony hooks from first to fifth anal-fin rays of mature males; outer row of premaxilla with conical to tricuspid teeth; inner row of premaxilla with up to pentacuspid teeth; maxilla with one to three teeth; body depth 34.7-46.5% SL; and 18-23, rarely 17, total gill rakers on first arch. Finally, we widened the distribution of H. panamensis to the ríos Atrato, Baudó and San Juan basins in Colombia, ríos Bejuco, Boquerón, Calovébora, Chagres and Llano Succio basins in Panamá, ríos Sixaola and Tortuguero basins in Costa Rica and drainages in Belize.



#### Is Hemisorubim (Siluriformes: Pimelodidae) a monotypic genus?

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Currently, Hemisorubim Bleeker, 1862 is represented by one species, Hemisorubim platyrhynchos (Valenciennes, 1840), wide distributed in Amazon, Maroni, Orinoco, Araguaia-Tocantins, Paraná-Paraguay River basins, and coastal rivers of Maranhão State. Multivariate morphometrics analysis of samples from Amazonas, Turiaçu, Upper Paraná and Paraguay River basins, and molecular analysis of samples from Amazonas, Itapecuru, and Paraguay River basins were employed to test the monotypy of genus. The Canonical Variate Analysis clearly discriminate the populations in the two first axis (Wilk's lambda = 0.006456; F = 4.116, p = 6.198E-10). In the first axis, which accounted for 70.05% of total variance, Amazonas and Turiacu samples were discriminated of Paraguay and Paraná samples. In the second axis, which accounted for 19.71% of total variance, Amazonas was discriminated of Turiaçu, and Paraguay of Paraná samples. The discriminant characters were: distance between dorsal fin to adipose fin, maxillary barbel length, caudal peduncle length, body depth, adipose-fin base length, eye diameter, anal-fin base length, interorbital width, eye diameter at vertical, inter-nostrils distance, eye to posterior nostril distance and mouth width. The Neighbor Joining analysis of COI, also clearly discriminate the populations, corroborating the results observed with morphometric analysis. The distance between Paraguay and Amazon is 0.03±0.007, Paraguay and Itapecuru is 0.048±0.009, Itapecuru and Amazon is 0.045±0.009. Two populations were distinguished in the Amazon River basin, in the upper and in the middle Amazon, with a distance of 0.015±0.005. In the light of those results, it is evident that more than one species are included in the genus, allowing to refuse the hypothesis of Hemisorubim as a monotypic genus.

Key words: Catfish; Amazon River; Paraguay River; Turiaçu River; Itapecuru river Financial support: PIBIC CNPq/UEL



### On the type-locality of *Hemisorubim platyrhynchos* (Valenciennes, 1840) (Siluriformes: Pimelodidae)

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The genus Hemisorubim Bleeker, 1862, is monotypic, represented by H. platyrhynchos (Valenciennes, 1840). The type-locality of this species has been imprecisely considered as Brazil. The species was described based on one specimen deposited in Museum d'Histoire Naturelle, Paris, collected by Alexandre Rodrigues Ferreira. According to morphometric and molecular analysis, this species is not monotypic with, at least, three new species. However, it is difficult to identify the species without the precise type-locality. Ferreira's expedition was carried out in the period of 1783-1793, traveling 39.372 km through the Amazon River in the States of Pará and Amazonas (until Tefé), Negro and Branco Rivers in the States of Amazonas and Roraima (to the northern region of the state), Madeira and Guaporé Rivers, in the States of Amazonas and Rondônia, and Upper Paraguay River, in the States of Mato Grosso and Mato Grosso do Sul (from Cuiabá to Corumbá region). Therefore, two main hydrographic basins are candidate to be elected as H. platyrhynchos type-locality: Amazon or Upper Paraguay River basins. The period and effort of work of Ferreira were bigger in the Amazon basin, which increase the possibility of Amazon basin to be the type-locality of this species. In the original description of species, the number of black blotches is six to seven (maybe counted in both sides of body). Interestingly, the Hemisorubim platyrhynchos specimen depicted by Ferreira's Expedition illustrators (Joaquim José Codina or José Joaquim Freire) has four rounded black blotches, three on the flanks and one on the end of caudal peduncle, and several light brown rounded blotches on dorsal region of flanks, indicating that the illustrated specimen is not the preserved specimen. Although the color pattern is quite variable in Hemisorubim, the number of black blotches of examined specimens from Amazon River basin is significant higher (n = 14, mean = 8±3.9, median = 8, 25% and 75% percentils of 5.8 and 9.5, respectively) than that of specimens from Paraguay River basin (n=15, median =  $3.8\pm1.6$ , median = 4, 25% and 75%percentils of 3 and 4, respectively) (t = -3.78, p < 0.05). Therefore, the original description of black blotches on H. platyrhynchos closely relates the species to the Amazon River basin, the probably type-locality.

Key words: Catfish; Amazon River; Paraguay River; color pattern Financial support: PIBIC CNPq/UEL



### Hidden diversity in species complex Heptapterus mustelinus (Siluriformes: Heptapteridae)

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Heptapterus mustelinus is a species of the family Heptapteridae, originally described as Pimelodus mustelinus from Río La Plata, widespread throughout Paraná River basin, Uruguay river basin and coastal streams of Uruguay and southern Brazil. Several populations of this species were examined and compared along its distribution area. Morphometric (44) and meristic (34) data were taken from 190 specimens with caliper and stereomicroscope. Counts and descriptions of osteological features were made from cleared and stained specimens and X-ray images. DNA was extracted and sequenced from 25 specimens, using two mitochondrial (COI and CytB) markers. Analyses were conducted using both Maximum Likelihood and Bayesian Inference methods. Morphological and genetic data reciprocally support the recognition of two new species, similar to H. mustelinus in general body shape. Heptapterus mustelinus group (H. mbya, H. gengo, H. mustelinus, Heptapterus sp.n. A and Heptapterus sp.n. B) can be distinguishing from H. bleekeri and H. tapanahoniensis by the position of the adipose-fin origin, located anterior to anal-fin origin (vs. adipose-fin origin, posterior to analfin origin); additionally from H. panamensis and H. tapanahoniensis by the confluence of the adipose and caudal fins (vs. adipose and caudal fins separated); additionally differ from Heptapterus fissipinnis and H. panamensis by having the caudal-fin truncate (vs. caudal-fin bifurcate); from H. multiradiatus, H. stewarti and H. sympterygium by having 15-23 anal-fin rays (vs. 36, 30, 22-29 respectively); additionally differs from H. sympterygium by having the anal-fin not continuous with the caudal-fin (vs. anal-fin continuous with the caudal-fin). The two new species are distinguished from their congeners by the number of vertebrae, width of posterior cranial fontanel, head width, number of lateral line pores, unbranched anal-fin rays, and caudal-fin rays on the ventral lobe. The new species have a restricted distribution, suggesting that both are endemic to the sub-drainages they are found. Heptapterus sp.n. A is distributed in upper portion of río Uruguay (rio Pelotas) and Heptapterus sp.n. B endemic from the upper portion of rio Ibicuí. Species delimitation analyses with GMYC method obtained in Heptapterus mustelinus populations support the presence of the new species.

Key words: Taxonomy; morphology, catfish, molecular analyses Financial support: CAPES



#### Molecular phylogeny of the neotropical freshwater stingrays (Chondrichthyes: Myliobatiformes: Potamotrygonidae), with biogeographical inferences

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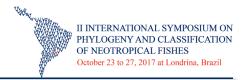
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Neotropical freshwater stingrays of the family Potamotrygonidae, which includes over 40 described species in 4 genera within the subfamily Potamotrygoninae, form a diverse and geographically widespread group, found in almost all major river basins in South America. This freshwater clade is sister to the recently created marine subfamily Styracurinae, composed of two amphi-American species of Styracura – S. pacifica and S. schmardae. The diversity and biogeography of freshwater stingrays are still poorly understood. Here, we address both population- and species-level relationships among potamotrygonid lineages using four molecular markers, both nuclear and mitochondrial, using over 350 terminal taxa. Our dataset includes almost all presently described species of this family and an unprecedented biogeographical representation. We time-calibrated our tree using well-documented vicariant events and fossil priors, informed by the paleogeography of South America. The phylogeography of each genus and species were evaluated, to test whether observed patterns are congruent with other Neotropical freshwater fishes. The phylogeny corroborates the monophyly of the family and its two subfamilies. Within Potamotrygoninae, we provide molecular evidence for the paraphyletic status of Potamotrygon, which includes members of Plesiotrygon. This clade is sister to the clade formed by Heliotrygon and Paratrygon. We present hypotheses of relationships among lineages of Paratrygon, as well as lineages of Potamotrygon, taking in consideration their distribution in different areas of South America. Concordance of time estimates and paleogeographical events suggests that the evolution of potamotrygonids is correlated with geological processes such as marine incursions, river captures, and changes in river drainage patterns. We consider the age of species and lineages of Potamotrygon, and propose that lower Amazon lineages may have originated as the modern channelization of the Amazon occurred. We hypothesize that endemic species may have originated from widespread species after invasions of the lower Amazon and the Paraná-Paraguay regions. Finally, we discuss the diversity of the freshwater lineages and possible evidence for hybridization and introgression, which might explain the lack of reciprocal monophyly for many nominal species of Potamotrygon.

Key words: Systematics, Hybridization, Distribution, Phylogeography

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#### Does water color matter? Phylogeny and phylogeography of the genus Steatogenys (Boulenger, 1898) (Gymnotiformes: Hypopomidae)

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The rivers of the Amazon can be divided into high-conductivity systems (comprising sedimentrich white waters) flowing out of Andean erosion zones, and low-conductivity systems, comprising black waters (with high humic acid and low sediment concentrations) draining lowland Cenozoic formations and clear waters (with intermediate sediment composition) draining Paleozoic formations. Waters with different conductivities are likely to affect the electric communication and electrolocation systems of weakly electric gymnotiform fishes. To investigate the evolutionary influences of water-type on electric fishes, we use the genus Steatogenys (Boulenger, 1898), with three valid species (S. elegans, S. duidae and S. ocellatus), as a model system. The species S. elegans and S. duidae are widely distributed across the Amazon but have different habitat distributions: S. elegans occurs in all three main water types while S. duidae occurs mainly in low conductivity waters. S. ocellatus occurs primarily in central and upper Amazon black water rivers. We generated a phylogeny for Steatogenys using both mitochondrial and nuclear genes and evaluated the phylogeography of S. duidae and S. elegans. Our analyses confirm reciprocal monophyly for each recognized species of Steatogenys, and indicate two distinct lineages within S. elegans, one mainly associated to lower conductivity environments and one to high conductivity environments. We also produced a haplotype network for S. elegans, which corroborates the recovered phylogenetic lineages, and supports the effect of habitat and geography on haplotype distributions. Using a multivariate analysis correlating molecular distances, geographical distances and environmental variables, we tested for the influence of different environments over the phylogeny. The results of this novel approach indicate that the undescribed lineages within S. elegans are sympatric over broad spatial scales, but one lineage is associated with black and clear water, while the other is associated with white water habitats. This suggests that conductivity, as well as other environmental variables such as pH, temperature, dissolved oxygen, may play a role in lineage diversification of electric fishes.

Keywords: Conductivity, ATPase, Haplotype, Isolation by Environment, Isolation by Distance Funding: CNPq (Process: 207384/2014-2), NSERC, NSF (Process: DEB-1146374)



## One hundred years later: monophyly, phylogenetic position and taxonomic revision of the Neotropical catfish genus *Scleronema* Eigenmann, 1917 (Ostariophysi: Siluriformes: Trichomycteridae)

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Trichomycteridae is a monophyletic group of Neotropical freshwater fishes comprising eight subfamilies: Copionodontinae and Trichogeninae, closely related and sister group to the rest of the family; Trichomycterinae, a non-monophyletic assemblage as presently conceived; Glanapteryginae, Sarcoglanidinae, Stegophilinae, Tridentinae, and Vandelliinae, which form the monophyletic clade socalled TSVSG. Scleronema and the genera Bullockia, Eremophilus, Hatcheria, Itualanis, Rhizosomichthys, Silvinichthys, and Trichomycterus belong to Trichomycterinae, the largest and most complex group within the family. Scleronema comprises three psammophylous species, S. angustirostre, S. minutum and S. operculatum, which are restricted to low altitudes in the subtropical region of the La Plata basin (formed by the Paraná, Paraguay, and Uruguay rivers) and coastal drainages in the South Brazil and Uruguay. Since its proposition in 1917 and later by most subsequent authors interested in the systematic of Trichomycteridae, Scleronema has been recognized mainly by two derived characters: the presence of a skin fold in the fleshy base of the maxillary barbel as well as in the posterior margin of the opercle. However, its monophyly has never been tested in an analysis including its three species and members of each subfamily of Trichomycteridae. Additionally, contradictory hypotheses about the phylogenetic position of Scleronema into the family have been found along these years. Recently, phylogenies based on musculature and molecular data recovered the monophyly of Trichomycterinae with Scleronema included. Other studies, however, proposed the genus as more closely related to the TSVSG clade than to trichomycterines. Other questions lie about its diversity and the accurate delimitation of its species. Are all species of the genus valid? And how about the undescribed species mentioned in previous checklists? Based on considerations above, the present work focused in Scleronema has the following major objectives: to make a taxonomic review, to test its monophyly, and to investigate its position inside Trichomycteridae. The ingroup includes the three species of Scleronema and 24 taxa as outgroups, representing all subfamilies of Trichomycteridae, with the root in Nematogenys inermis. The search for characters was done in published papers, unpublished dissertations or via personal observation. The matrix was constructed with 192 characters, 24 of them treated as continuous without discretization, and the phylogenetic analysis was performed under parsimony. A unique most parsimonious tree was found recovering the monophyly of Scleronema based on four exclusive synapomorphies: skin fold in the base of the maxillary barbel and in posterior margin of the opercle, autopalatine with an archshaped process dorsally and articulating with vomer in its ventral surface. Additionally, Scleronema is defined by seven synapomorphies homoplastically found in other taxa and two synapomorphies related to continuous characters. The affinity of Scleronema with members of the TSVSG clade was strongly supported by 19 characters. Two information sources were used for the resolution of the taxonomic questions: morphological (including the analysis of the type material) and molecular data (the mitochondrial genes COI and Cytb). As a result, S. angustirostre is proposed as junior synonym of S. minutum. Six new species on genus are recognized and all species are diagnosed and delimited geographically.

Key words: Systematics; Taxonomy; Trichomycterinae Financial support: CNPq (processes 142010/2012-0 and 152354/2016-6)



#### Integrative taxonomy of the genus *Trichomycterus* Valenciennes, 1832 (Siluriformes: Trichomycteridae) in coastal drainages of South and Southeast Brazil

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Trichomycteridae is the second most diverse family of Siluriformes comprising about 300 valid species. Members of the family are distributed throughout continental freshwaters from Costa Rica to Patagonia. The most problematic genus in trichomycterid systematics is Trichomycterus, the largest genus of the family with more than 170 species. Despite the description of more than 70 new species in the last two decades, Trichomycterus still includes a large number of undescribed taxa. This taxonomic study focuses on coastal drainages from Tramandaí River system (South) to the Ribeira do Iguape River basin (North) in South and Southeast Brazil. Nine species where previously reported in those drainages: Trichomycterus balios, T. cubataonis, T. davisi, T. guaraguessaba, T. iheringi, T. jacupiranga, T. nigricans, T. tupinamba and T. zonatus. The aim of this study is to provide an integrative taxonomic revision of the genus based on morphological and molecular data in this region. We took 25 measurements and examined fin-ray counts and laterosensory canal system and associated pores. Based on a preliminary analyses, T. alternatus, T. balios, T. zonatus, T. cubataonis, T. davisi and four additional morphotypes were confirmed as valid in the study being diagnosed by a combination of characters such as pectoral-fin rays, extension of filament on the first pectoral-fin ray and color pattern. Trichomycterus alternatus and T. zonatus occur in the Ribeira do Iguape basin and show similar color pattern composed of vertical black bars in the body. These species are distinguished by the number of pectoral-fin rays (i+7 vs. i+6, respectively) and the presence of the pores i1 and i3 of the laterosensory canal system (vs. absence in the latter species). Trichomycterus alternatus is a new record to Ribeira do lauape basin. Trichomycterus davisi is diagnosed by 1+6 pectoral-fin rays, first pectoral-fin rays without prolongation and mottled color pattern. Trichomycterus jacupiranga, T. tupinamba, T. guaraguessaba are currently known only to the type-locality. Trichomycterus cubataonis, described from the Cubatão River (Santa Catarina State), has a wider distribution than previously reported and is found in the Biguacu, Tijucas, Itapocú and Cubatão Norte rivers. Trichomycterus balios is distributed in the Mampituba, Araranguá and Itajaí rivers. Trichomycterus nigricans, as redescribed by Arratia, was not confirmed in the area. Four additional morphotypes were registered in the study area and are likely new species endemic to coastal basins: Trichomycterus sp. 1 is distributed to Tramandaí, Mampituba and Araranguá rivers; Trichomycterus sp. 2 is endemic to the Tubarão River; Trichomycterus sp. 3 is distributed to Cubatão Sul, Biguacu and Tijucas River; Trichomycterus sp. 4 is restricted to the Florianópolis Island. Within the described species, six are endemic to the coastal basins (T. cubataonis, T. guaraquessaba, T. jacupiranga, T. nigricans, T. tupinamba and T. zonatus). In contrast, T. balios, T. iheringi e T. davisi also occur in continental drainages, suggesting that stream capture was an important event promoting biotic exchange. In the next stages of the study, for a better resolution on the identification and species delimitation, we are sequencing mitochondrial markers to include in the integrative analysis.

Key words: Neotropical; Taxonomy; Trichomycterinae; Species delimitation Financial support: Capes (process 1681448), Capes (PNPD), CNPq (152354/2016-6)



### Phylogeny of the genus Oligosarcus (Characiformes: Characidae): an analysis of total evidence

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Oligosarcus is distributed in the southeastern portion of South America and most of its species have an allopatric distribution. A recent, parsimony-based phylogeny, using morphological characters demonstrates that the group is monophyletic, supported by two synapomorphies in which O. pintoi is the sister species to remaining Oligosarcus. Putative Oligosarcus, O. itau, O. amome, O. platensis, and O. varii were not included, the latter because its description occurred after that study of the genus. Molecular phylogenies of Characidae included few species of Oligosarcus, and in-depth study on relationships of Oligosarcus using molecular data is warranted. Therefore, we performed a preliminary total evidence examination using a concatenated dataset with morphological and molecular characters. Nineteen species of Oligosarcus were included, with 34 morphological characters and molecular data from COI (669pb). The most parsimonious set of relationships among the species was reconstructed in TNT v1.1, using traditional search with 10.000 replicates holding 10 trees in each replicate. Both Bootstrap (1000 replicates) and Bremer support values were used for examining node support. A Bayesian Inference of phylogeny was completed using MrBayes 3.2.6 within CIPRES website. Forty million generations of the Markov Chain Monte Carlo (MCMC) using four chains sampling every 1000 generations were completed, using four different models. One for morphology (MarkovK model with a gamma rate) and three for each codon position of COI (were selected using PartitionFinder). The species Bryconops melanurus, Tetragonopterus argenteus and Astyanax altiparanae were included as outgroups and the root was the first species. Both Maximum Parsimony and Bayesian analyses recovered the monophyly of the genus Oligosarcus, presenting A. altiparanae as sister species. The Parsimony resulted in four trees with 476 steps. The consensus topology is moderately resolved with 12 nodes connecting ingroup taxa. Substantial uncertainties remain, with particularly large polytomies occurring in the basal taxa O. planaltinae, O. pintoi, O. paranensis, O. macrolepis and O. brevioris. Both Bremer's support and Bootstrap values were low, indicating uncertainties in these branches. Bayesian analyses recovered a topology with more polytomies, resulting in only 6 monophyletic resolved groups and with low posterior probability values. Some groups were similar in both analyzes: (O. schindleri + O. bolivianus), (O. solitarius + O. jacuiensis) and (O. robustus + (O. hepsetus + O. oligolepis), supported by 6, 2 and 3 sinapomorphies respectively. These low branch supports may be due to missing data, as already indicated by other authors, in which the Bayesian analysis suffers greater interference in its results. A larger number of characters may help to better understand the relationships between such species. For further steps, it is intended to include other molecular markers as well as to raise more morphological characters.

Key words: Systematics; Maximum Parsimony; Bayesian Inference; Freshwater; Neotropical Financial support: CAPES



#### Phylogeny of the genus Ageneiosus and Tympanopleura (Siluriformes: Auchenipteridae) estimating node time using molecular data

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The genera Ageneiosus and Tympanopleura (Auchenipteridae) were recently revised taxonomically, with the revalidation of the latter. The major morphological features distinguishing both genera are the presence of a large non-encapsulated swim bladder and of a pseudotympanum in Tympanopleura vs. a swim bladder strongly reduced and ossified in adults and reduced pseudotympanic area, with different levels of epaxial muscles covering the tympanic region in Ageneiosus (except A. pardalis, where the swim bladder is large). Thus, the aim of the present work is to unravel the phylogenetic relationships of both genera under a molecular approach. We sampled representative species of both genera Ageneiosus and Tympanopleura, and we appointed the auchenipterids Auchenipterichthys longimanus and Auchenipterichthys puctatus as outgroups based on former cladistic hypotheses. We used mitochondrial DNA genes cytochrome c oxidase subunit I (COI) and ATP synthase 8 and 6 (ATPase 8/6) and, also the nuclear marker recombination-activating genes (RAG-1) for reconstructing phylogenetic trees. We used PartitionFinder to identify the best models for each gene that fit the data. We employed the maximum-likelihood (ML) analysis using RAxML program and Bayesian Inference (BI) phylogeny employed MrBayes program. The divergence time among the species of the genera was estimated using \*BEAST, under Yule speciation process, an uncorrelated lognormal relaxed clock and a rate of evolution of each gene per million years. We ran MCMC with 10,000,000 generations, discarding 10% as burn-in. The convergence was conferred in Tracer. The evolution models used were the GTR+I+G for the mitochondrial genes and K80+I for the nuclear. The topology of the generated trees was incongruent among each other under ML and BI analyses and among the markers as well. However, some consistencies were observed: Ageneiosus inermis was always depicted as sister-group of A. polystictus, and A militaris was always closely related to A. magoi; six species were undistinguishable genetically; Tympanopleura came always inserted within Ageneiosus and non-monophyletic. The time of divergence for Ageneiosus was estimated in approx. 16.5 mya (Miocene). Isolation of A. pardalis may have occurred around 12.5 mya provoked by periods of the Andean uplift. Diversification of the "Tympanopleura" lineage might be related with the alteration of the Amazon River flux from west to east, discharging into the Atlantic, dated around 7 mya according to some hypotheses. The group of six species genetically similar was shown by ML and bayesian analyses to be extremely recent, having differentiated around 1.5 mya from now. And finally, the molecular analyses showed that Tympanopleura is not monophyletic but rather a parafiletic branch inserted within Ageneiosus, despite the contrasting morphology between their representatives.



### Convergent evolution and classification: the Characiformes dilemma (Teleostei: Ostariophysi)

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The traditional definition of classification is the grouping of objects into classes owing to their shared possession of attributes. For the sake of communication, such classes have to be distinguished and named. Traditional classification relies on some basic principles related to homogeneity, shared attributes, difference degrees, and hierarchical arrangements of the classes. Phylogenetic systematics brought an objective methodology for the biological classification, although with some undesirable collateral effects, as too many groups and ranking difficulties. Phylogenetic, or cladistic, classification is expected to be free of subjectivity and arbitrariness, but objections to this has been often pointed out. One of these objections is that any constructed cladogram is only an inference on the true phylogeny and then, classifications may be extremely variable, since other cladograms are also legitimate alternatives. Modern phylogenetic methodologies based on comprehensive molecular database have been rewriting the understanding of several fish groups and, sometimes, shading light on obscure relationships, sometimes bringing doubt to well-established ones. We performed a comprehensive phylogenetic analysis based on molecular ultraconserved elements (UCES) for the order Characiformes. This analysis recovered well-established groups but also unexpected relationships, mainly related to the subfamilies and genera of the Characidae. Our results would require a huge amount of taxonomical adequacy with profound modifications on subfamilies and genera composition and diagnosis. Well supported groups on putative synapomorphies (e.g. Jupiaba, Curimatella, Poptella, etc.) are now understood as paraphyletic along with several other characid genera, probably evidencing the major rule of adaptive convergence on the evolution of this group of fishes. Such comprehensive analyses, based on modern and refined methodologies and algorithms, give us the opportunity to better understand convergent evolution and the putative and unsuspected dependency of traits modifications. Our results showed an unsuspected connection between the presences of a pelvic spine with specific tooth morphology in putatively unrelated species, currently recognized in the genus Jupiaba. Unrelated species of the genera Moenkhausia, Astyanax, and Ctenobrycon showed complexes transformation on the tooth and jaw morphology converging to a specific kind of mouth that seems to be related to a grassy seed diet. Such observations can be associated to the exhaustively discussed convergent traits in Characidae such as perforation degree of the lateral line, caudal-fin squamation, number of tooth series in premaxilla, etc., all important features extensively employed in diagnoses of Characid genera. Our results demonstrate that many of these characters should be reevaluated and, differentiation and definition of some evidenced groups should be addressed in an unprecedented dimension of morphological, molecular, and ecological features that may end on non-operational but scientifically supported diagnostic system.

Key words: Characidae, Phylogeny, UCE, Dependent characters Financial support: Support: FAPESP 2014/26508-3, 2016/11313-8, CNPq



#### Inference of population structure in the endemic southern Brazilian genus *Eurycheilichthys* (Siluriformes: Loricariidae) using ddRADseq data

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Eurycheilichthys is a Neotropical genus of small loricariids endemic to two river basins in southern Brazil and better known by two of its species: E. pantherinus, from the upper Uruguay River basin (in Rio Grande do Sul and Santa Catarina states) and E. limulus, from the upper reaches of the Jacuí River basin, which is part of the Laguna dos Patos drainage (in Rio Grande do Sul State). In early 2017, however, seven additional new species of Eurycheilichthys were described, showing not only that the genus is also present in the Taguari-Antas River, a tributary to the Jacuí River, but mainly the unexpectedly high diversity of the group in that microbasin. Thus, eight out of nine species of the genus occur in the headwaters of the Laguna dos Patos drainage, being restrictedly distributed throughout the high plateaus of Rio Grande do Sul State. The present work aims to better understand the evolution of those lineages through time, and investigate and assess levels of population genetic structuring within and among populations and species of the genus. Data from ddRADSeg libraries for 65 individuals of all species of Eurycheilichthys were used in a Bayesian clustering analysis of multilocus genotype data, performed with fastStructure, an algorithm for approximate inference underlying the software Structure, to assess genetic partitioning. As a result, the analysis suggested four main lineage clusters: one from the upper Uruguay River, representing E. pantherinus, one from the Jacuí River, which corresponds to E. limulus, and another two lineage clusters from the Taguari-Antas, detecting low differentiation among those seven recently described species. Further fastStructure analysis of the Taguari-Antas data recognized some of the morphological species. Yet, the analysis still show, on one hand, the clustering of allopatric species with very contrasting morphological features and, on the other, the clustering of two quite similar morphological species, but with very strong heterogeneity signatures, and the presence of foreign components. The low number of lineages recognized indicates a very recent diversification of the group, especially of those morphospecies from the Taquari-Antas microbasin. Additionally, these results suggest the possibility of phenotypic plasticity due to habitat heterogeneity, the presence of cryptic species, and/or hybridization. Next steps in this research include further analyses to test those hypotheses.

Key words: Neotropical; Freshwater; endemism; next-generation sequencing Financial support: CAPES, CNPq, Lakeside Foundation (USA)



### Morphological overlapping in two species of *Microglanis* (Siluriformes: Pseudopimelodidae) from Southern Brazil

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Identifications and descriptions of species based only on morphological characters may present limitations when the characters used are masked by the effect of phenotypic plasticity and/ or genetic variability. This situation would bring instability to species recognition and producing biased reports of species distribution and diversity. Studies analyzing only the morphology have been identifying some phenotypes from Uruguay River as Microglanis cottoides, a species described from Laguna dos Patos basin. The aim of this study was to investigate if M. cottoides really occur in Uruguay River using single-locus DNA sequences of the COI gene and morphological information. For molecular analysis, a total of 19 specimens were analyzed: Five M. cottoides from Camaquã basin (type locality; Laguna dos Patos tributary), 10 M. malabarbai from two different localities: Arroio Albino (type locality, n= 5) and Ijuí River tributary (n= 5), and four M. aff. cottoides from Arroio Guarita. The genetic distances among and within species were calculated with Kimura-2-Parameter (K2P) distance model. A neighbor-joining (NJ) dendrogram of K2P distances was created using MEGA v 6.0 software to provide a graphic representation of divergence patterning among species. The species delimitation was initially carried out using 2% divergence as a cutoff value, as employed in others barcoding surveys. The genetic distance analysis revealed that M. aff. cottoides did not differs from M. malabarbai, and this species differs from M. cottoides from Camaquã basin by more than 6% of the K2P distance, and both species showing low intraspecific genetic variation, indicating a clear "barcode gap" between them. For morphological analysis, a total of 65 specimens were analyzed: 25 M. cottoides from Camaquã basin, and 40 M. malabarbai from Arroio Guarita (previously identified as M. aff. cottoides, n = 4), Arroio Albino (n = 15) and liuí River tributary (n = 21). No secure morphometric discrimination was observed through principal components analysis (PCA), with superposition of specimens of both species along the second and third principal component. The PC2 retained 3.7% and PC3 retained 1.8% of the total variance. The population of M. malabarbai from Arroio Albino, the type-locality, and Ijuí River tributary, had great morphological variation, showing superposition with M. cottoides from Camaguã basin. The population of M. malabarbai from Arroio Guarita, with color pattern similar to M. cottoides, differed slightly from M. cottoides by higher values of posterior internarial distance, anterior to posterior nostrils distance, maxillary barbel length, and smaller values of body depth at anal fin origin, head depth, and dorsal-fin base length. The counts showed that there is superposition of lateral line pores number between the species. Therefore, M. malabarbai and M. cottoides have a wide intraspecific morphological variation, which cause the superposition and making difficult a correct identification. However, this is ensured by the great genetic distance between species.

Key words: Catfishes, DNA barcoding, Uruguay River basin; Morphological convergence Financial support: CNPq, Fundação Araucária



### Genetic diversity of *Gymnogeophagus* setequedas (Cichlidae: Geophagini) from the Lower Iguaçu River

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Gymnogeophagus seteguedas is a rare and rheophilic species of Geophagini tribe, considered threatened by all Brazilian red lists. Its known geographic range of distribution was the Paraná river basin, in Paraguay, and tributaries of the Itaipu Reservoir in Brazil. However, the recent records of G. setequedas in the low Iguacu River, in a region considered highly endemic for the ichthyofauna, extended the known geographical distribution of the species. The aim of this study was to estimate the genetic diversity and population structure of G. setequedas from this restricted area of occurrence, using microsatellites markers and mitochondrial haplotypes (D-loop). Muscular tissue samples of 87 specimens were obtained from eight locations in the Lower Iguacu River, between upstream of the Iguacu Falls and downstream of the Salto Caxias Reservoir. Seven microsatellite loci and 449 bp of D-Loop mitochondrial region were examined. Both markers revealed high levels of genetic diversity (e.g. A= 122, NA= 17.429, HE= 0.675), absence of inbreeding, non-significant substructuring ( $F_{st}$ = 0.0204), and lack of HW disequilibrium, indicating the occurrence of a single G. setequedas population distributed along a stretch of approximately 190 km long. Examining the D-Loop region, 12 haplotypes were revealed, and low  $\pi$  values (0.00729) and high h values (0.750), suggesting an ancient bottleneck followed by rapid population growth and accumulation of mutations. In addition, the pattern of mismatch distribution was multimodal, which is usually ascribed to populations on demographic equilibrium. The genetic information obtained in this study suggests satisfactory levels of genetic diversity in the population of G. setequedas, in spite of the small area of occurrence. Since species description, there have been a number of unsuccessful attempts to collect new specimens from the known geographical range of the species. Thus, the population of G. seteguedas of the Lower Iguacu River may be one of the last remnants of this species, for this reason it is necessary to maintain the Iguacu River tributaries and the downstream area from the Baixo Iguaçu Reservoir free of additional dams, to guarantee the survival of this species.

#### Key words: Conservation; Freshwater; Neotropical

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### Genetic diversity of Andinoacara latifrons (Steindachner, 1878) as conservation strategy in Colombian basins

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Genetic differentiation in the genus Andinoacara have showed a congruence between geological history and species diversification, guiding predominantly vicariance processes in the Neotropical region, especially in the Northwest region of South America. In this sense, Andinoacara latifrons as a trans-Andean endemic specie of Colombia have showed similar patterns that Andinoacara genus, but information about populations genetic and phylogeography have to be more explored. Our aim is to analyze the genetic diversity of Caribbean and Andean individuals in order to establish a relationship in evolutionary and phylogeographic terms. We amplified the mitochondrial gene Cytochrome C oxidase 1 (COI) and a phylogenetic tree was constructed in Geneious v7.1.3 using the best evolutionary model to fit our data, following the Bayesian information criterion (BIC) in the JModeltest v2.1 software, also a haplotype network was constructed using the software SplitsTree4 v4.14.5. We are amplifying cytochrome b and the nuclear gene Recombination Activating Protein 1 (rag1) for a more consistent results. A haplotype differentiation between Caribbean and Andean individuals was confirmed through the mitochondrial gene cytochrome oxidase 1 and a marked differentiation ratify the separation of Cauca, Maracaibo, and Caribbean basins in a population level, but that haplotype inference also show a shared diversity that could indicate an ancient history explained by its geological structure, ratifying a vicariance process. Also, results of mitochondrial and nuclear genes need to be analyzed to give a comprehensive approach. Caribbean basins show a substantial differentiation as Musilová described in previous works, explained by a higher species potential caused directly because Andean orogeny. We highlight Colombian basins as regions with a great environment and richness specie principally in the trans-Andean zone, allowing the establishment of a considerable number of endemic species, by other hand, the haplotype approximation of the individuals of different basins could show as first elucidation about the role of hydrographic basins as barriers in the possible genetic fluxes. Finally, a more complex analysis is ongoing to a robust discussion.

Key words: trans-Andean; Phylogeography; Neotropical Financial support: CAPES, UEPG



# Phylogenetic supertree for Siluriformes (Teleostei: Ostariophysi) constructed with a molecular supermatrix of seven concatenated genes available in databases

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Siluriformes represents the sixth major order in vertebrates, and is one the most diverse in Neotropical fishes. The order has a history of controversies in the phylogenetic relationships, monophyly recognition of some groups and even about the number of recognized families. The number of families varies in literature, but in majority of publications it is recognized 36 families, with 478 genera and over 3000 species. The monophyly of Siluriformes is well established, through molecular and morphological studies, although these different sources of data are not consensual when the issue is the investigation of phylogenetic uncertainties in the higher level of phylogenetic relationships between the main groups inside Siluriformes. One interesting approach to integrate different sources of data is grouping the information available in supertrees that could be more informative for phylogenetic and evolutionary inferences than analysis of a limited data. There are several methods to construct supertrees, including the supermatrix and MRP approaches that are used in this work. The aim of the work was construct a phylogenetic supertree for Siluriformes using a molecular supermatrix built with the concatenation of seven gene regions used in the most relevant papers regarding the higher-level phylogeny for Siluriformes, and compare the results with a MRP supertree constructed previously using both molecular and morphological data (data not published), and highlight the main differences between these two approaches. For this purpose, were data mined the molecular information available in databases to constructed a supermatrix resulted from a concatenation of seven genes founded in the main higher-level phylogenies for Siluriformes, the regions included: Cytochrome Oxidase C Oxidase Subunit I (COI or COX1), Cytochrome B (cyt-b), NADH dehydrogenase subunit 4 (ND4), tRNA-His, tRNA-Ser, Recombination Activating Protein 1 (rag1) exons 1, 2 and 3 and Recombination Activating Protein 2 (rag2). The sequences were aligned separated by gene region in ClustalW and then concatenated in Geneious 7.1. The final concatenated alignment contains about 5450 base pairs. The individual alignments for each gene portion were analyzed in JModeltest 2.0, using the Akaike Information Criterion correction (AICc) to search for the best evolutionary model to fit to each partition. A maximum likelihood analysis in PhyML and a Bayesian analysis in MrBayes were run using the best model fit to each partition. Bootstrap and posterior probabilities analyses were used to estimate the branches support. The final topologies contain more than 250 species and constitute one of the broad analysis of Siluriformes. The supertrees shows some relationships that are constantly found in molecular analysis, like the poliphily of Bagridae and the basal position of Loricarioidea as sister group of the remain Siluroidei, in contrast with morphological approaches that places Loricarioidea as sister group of Sisoroidea. In a higher-level the supertree built with a concatenated supermatrix shows the following arrangement: (Gymnotiformes, (Loricarioidea, Sisoroidea, (Siluroidea, Doradoidea, Bagroidea))). This sort of analysis constitutes a tool that is available for future considerations regarding Siluriformes and represents an additive approach, once the resolution of the trees could be improved with the addition of more data that will became available soon.

Key words: Catfishes, Systematics; Taxonomy; Freshwater; Neotropical Financial support: CAPES, CNPq, UEPG, UFPR, Fundação Araucária



### A hundred-year-old new species of Geophagus Heckel, 1840 (Cichliformes: Cichlidae) from the rio Teles Pires, rio Tapajós Basin, State of Mato Grosso

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Geophagus (sensu Kullander) includes 20 species widely distributed in northern South America (Amazon and Orinoco river basins and coastal rivers from Venezuela to the Brazilian State of Maranhão), sharing the presence of swim bladder prolongations into the caudal region, which are sided by caudal ribs. Among Geophagus species, only G. argyrostictus, G. gottwaldi, G. arammepareius, G. harreri and G. taeniopareius present a complete dark suborbital stripe at least in part of the life cycle. We hereafter refer to those species as the "G. harreri speciesgroup". Recent field work in the upper rio Tapajós basin in the State of Mato Grosso, yielded an additional and very distinctive undescribed species that fits into the G. harreri-group. Subsequently, an additional specimen collected in 1915 was located among type specimens in the MNRJ fish collection, which Alípio de Miranda Ribeiro already had recognized as a new species. However, he never formally described this specimen. The new species is distinguished from all other congeners by having two intensely pigmented flank bars, almost as dark as the midlateral spot (the one containing the midlateral spot and the one anterior to it; vs. flank bars weakly pigmented, much lighter than midlateral spot). Also distinguished from all other congeners by having a black spot (as dark as midlateral spot) immediately ventral to dorsal-fin base and anterodorsal to midlateral spot, never connected to any flank bar (vs. absence of a similar spot). Additionally, the new species can be distinguished from all other congeners by the paedomorphic retention of an area of highly concentrated pigmentation in the portion of each flank bar that covers E1 and E2 scale series and on the portion that is in contact with dorsal-fin base (vs. flank bars uniformly pigmented in specimens larger than about 30 mm SL; adult G. mirabilis present several spots along E1 and E2 series, but they are distinct from the spots seen in young specimens). Also distinguished from all other congeners by the paedomorphic retention of a small dark spot on the base of middle caudal-fin rays and the absence of well-defined light spots and stripes on caudal fin (vs. absence of such spot and presence of light spots or stripes or both). Distinguished from congeners, except those in the "G. harreri species-group", by the presence of a conspicuous, complete dark suborbital stripe.

Key words: Neotropical region, Fishes, Geophagini, Taxonomy

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#### The genus Characidium Reinhardt, 1867 (Characiformes: Crenuchidae) in the Chapada Diamantina, Bahia, with description of a new species

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The genus Characidium Reinhardt, 1867 currently includes 68 valid species of small, Neotropical fishes commonly known as the south-american darters (EN), peixes-mocinha, mariposa or charutinho (PT). They are typical inhabitants of streams of first and second orders and are particularly highly diversified in the coastal drainages of the eastern South American basins. The Chapada Diamantina is an oasis in the middle of the hot and dry Semiárido Baiano, with a mild weather and several water bodies which are part of the headwaters of the coastal drainage rio Paraguacu, offering a variety of aquatic ecosystems for the freshwater fishes. Four species of Characidium occur in the Chapada Dimantina: C. bahiense Almeida, 1971; C. clistenesi Melo & Espíndola, 2016; C. deludens Zanata & Camelier, 2015; and an undescribed species. Characidium deludens is distributed in the tributaries of rio Preto in the west and the rio Jacuípe in the north, but it also occurs elsewhere in the São Francisco basin. In the Chapada Diamantina, C. bahiense is restricted to a small area known as the Pantanal do Marimbus, a flooding plain of the rio Santo Antônio interconnected by several lagoons; outside of the Chapada Diamantina, it is known from the rio São Francisco basin and other coastal drainages in Northeastern Brazil. Characidium clistenesi and Characidium sp. n. are sympatric species restricted to the tributaries of rio Paraguacu in the southeastern side of the Chapada Diamantina. Most species of Characidium, such as C. deludens, C. clistenesi and Characidium sp. n., are bottom dwellers and inhabit creeks with fast-flowing waters; they have the posterior chamber of the swim bladder reduced and negative buoyancy, and the expanded pectoral and pelvic fins works as flaps to help them to stay near to the ground, swim against the currents, and even to escalate small waterfalls. However, a few other species, such as C. bahiense, use a different kind of habitat, preferring water bodies with low or no currents, so they can lay on the submerged vegetation next to the margins; these species have a well-developed swim bladder, deeper body, and the fins are short and more fragile. The recent discoveries of two new species of Characidium restricted to the headwaters of rio Paraguaçu, allied to a better understanding of the local patterns of distribution, which molted by both the complexity of habitats and historical isolation of the Chapada Diamantina rivers, highlights high the level of endemism of the ichthyofauna of the region and the necessity for conservational policies.

Key words: Taxonomy; Caatinga Biome, Freshwater; Neotropical Finantial Support: CNPq Universal #433050/2016-0 (MRSM), PPBIO do Semiárido - MCTI/CNPq (ACAS)



### The Trichomycterus (Siluriformes: Trichomycteridae) from rio Doce, Southeastern Brazil: integrative taxonomy sheds light on species diversity and distribution patterns

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The identification of Trichomycterus species at rio Doce is a difficult task as local species diversity and limits are less understood, deserving further investigation. Morphological diagnosis of the three species recorded from rio Doce and distribution limits are herein revised. We recognized the following morphologically distinct Trichomycterus from rio Doce: Trichomycterus alternatus, from lower portions of rio Doce at Espírito Santo and eastern Minas Gerais; T. pradensis, from coastal tablelands at northern Espírito Santo and southern Bahia, with limits on western portions of middle rio Doce basin, and T. argos, from mountain portions along southern portion of rio Doce; the latter recognized as a member of Trichomycterus brasiliensis species group. Additionally, an unnamed Trichomycterus species was found on upper rio Piracicaba sub-basin differing from congeners at rio Doce by asymmetric or unpaired supraorbital sensory pore S6 (vs. symmetric, paired S6); 6-7 branched pectoral fin rays (vs. 8 in T. pradensis); large eyes, 15-17% in Head Length (vs. small, 8-10% in T. argos); first pectoral fin ray in a long filament, 20-23% in pectoral fin length (vs. short, less than 15%); high caudal peduncle depth, 11-14% in Standard Length (vs. small, less than 11%) and 38 vertebrae (vs. 37 or less). The difficulties regarding Trichomycterus taxonomy are the wide variety of coloration patterns and overlap on some external morphological features among populations. In order to check species identity and their limits, an integrative taxonomic study was taken. Barcoding DNA mitochondrial gene Cytochrome C Oxidase Subunit I (COI) nucleotide sequences were generated for identification of T. alternatus (3) haplotypes), T. argos (3), T. pradensis (6), and also from populations on headwaters along the Serra do Espinhaço at upper rio Piracicaba (3), rio Santo Antônio (2) and rio Corrente Grande (2) river basins. The generated sequences presented 669 base pairs (bp), resulting in a final matrix composed by 32 haplotypes. The phylogenetic tree with maximum-likelihood (ML) recovered the monophyly of all Trichomycterus species with high support and minimum genetic distance of 4%, identifying the seven haplotypes of gene COI from lineages along Espinhaco formation, confirming the presence of a single potentially new Trichomycterus along upper rio Doce. The distribution patterns of such species is congruent with vicariant events promoted by isolation of river stretches between Dom Silvério group and the Espinhaço escarpments. The tectonic history of Araçuaí belt, at southern Serra do Espinhaço, molded the hydrographic basin by abrupt inflection of upper rio Doce channel, isolating a population at upper rio Piracicaba and rio Santo Antônio sub-basins. Integrative taxonomic studies, together with geological evidence, contribute to clear the difficulties in recognition of taxonomic complex species, representing an important tool for solving the problems of species identification at rio Doce basin.

Key words: Taxonomy; Systematic; Freshwater; Northeastern Mata Atlântica; cambeva Financial support: CNPq- PCI DA grant (process 302248/2016-1). Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)



### Taxonomic status of *Imparfinis borodini* Mees & Cala, 1989 (Siluriformes: Heptapteridae)

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The Neotropical genus Imparfinis Eigenmann & Norris, 1900 was erected based solely on I. piperatus. In 1974, the genus Imparfinis was re-evaluated by Mees, and rearranged into 13 species. Nowadays, Imparfinis corresponds to the largest assemblage in Nemuroglanis clade, with 21 valid species of controversial taxonomy because of weak diagnostic features. The inclusion of Imparfinis borodini is one of the unsolved problems in the genus. Mees and Cala proposed the name I. borodini in face of a homonymy case, in substitution of I. longicauda Borodin, 1927, preoccupied by its senior homonym I. longicauda Boulenger, 1887. The inclusion of I. borodini Mees & Cala, 1989 in Imparfinis was based on the orbital margin free, although the authors consider this species as quite different from congeners. An attempt to provide a phylogenetic diagnosis in Imparfinis was carried out by Bockmann by combining four apomorphic features, and within the apomorphic features pointed out in that study only the caudal processes of last vertebrae is shared with I. borodini. Nonetheless, Imparfinis is still considered a poorly diagnosed genus, with generic recognition based on an array of osteological features, and by the presence of a dark band along lateral line. Morphological examination of Imparfinis borodini specimens from the rio São Francisco (rio Ondas) and coastal basins (rio Contas) in Bahia, Northeastern Brazil, suggests that this species does not fit on Imparfinis generic diagnosis sensu Bockmann, as evidenced by comparisons with I. piperatus, type species of Imparfinis as: plain mesial surface of ascendent portion of Meckel's cartilage in suspensorium (vs. extremely concave in I. piperatus); the posterior margin of the first epibranchial segment with no process covering epibranchial 2 on brachial arch (vs. process present); absence of dark stripe along lateral line (vs. presence); and long adipose fin representing 28.13% in relation to standard length, SL (vs. comparatively short adipose fin, 20.9-21.6% in SL). The uncertainties regarding I. borodini generic assignment are more evident when comparing its dorsal positioned eyes (vs. lateral eyes in all Imparfinis); falcate caudal fin (vs. bifurcated); and variable adipose fin, separated or almost contiguous to caudal fin (vs. separated from caudal). Although well distributed throughout the Neotropical region, little is known about the phylogenetic and systematic position of this catfish group. The monophyly of Imparfinis was tested, revealing issues in the current systematic arrangement of this genus that might hinder proper inferences about species interrelationships.

Key words: Systematics; Freshwater; Neotropical; Bahia

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### How many species of *Brevoortia* (Clupeidae: Clupeiformes) in the south-Western Atlantic Ocean? An integrative molecular-morphological approach

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Brevoortia is a genus of the Clupeidae that comprises moderately sized plankton-filtering marine coastal fishes distributed in the north and south Western Atlantic Oceans. Six species are currently recognized. Four of them are reported as sympatric or partially sympatric in the north Atlantic and the Gulf of Mexico: B. tyranus (Latrobe 1802), B. gunteri Hildebrand 1948, B. patronus Goode 1878, and B. smithi Hildebrand 1941. Brevoortia aurea (Spix & Agassiz 1829) and Brevoortia pectinata (Jenyns 1842), in turn, are reported from the south-Western Atlantic. However, the number of species of Brevoortia has been a matter of controversy in recent literature, especially in the south Atlantic, where some geographically restricted molecular and morphological studies suggest that a single species of the genus might be actually present. Brevoortia aurea is reported from off Bahia state (Brazil; the type locality), south to Rio Grande do Sul state (also Brazil). Records of Brevoortia pectinata are geographically more restricted in the literature and fish collections, ranging from Rio Grande do Sul state to southern Buenos Ayres (Argentina; type locality: Bahia Blanca). Even if the two species are valid, there is still some controversy as their actual geographic range, given that the characters reported in the literature as diagnostic for both are extremely similar. In order to address the question of the validity of the two species of Brevoortia reported for the south-Western Atlantic and their possible geographic distributions we used an integrative morphological and molecular approach. A total of 27 morphometric and 13 meristic characters were measured and counted in 176 specimens of Brevoortia from Rio de Janeiro, São Paulo, Rio Grande do Sul and Bahia Blanca, Argentina, based on specimens collected in this study and in specimens deposited in Museum collections (MNRJ, MZUSP, NPM, CIUFES). A multivariate discriminant and Canonical Variate analyses was employed in order to test morphological divergence among geographic samples. Molecular phylogenetic analyses were based on three markers, two mitochondrial (16s and COI) and one nuclear (RAG2). Gene genealogies were assessed by Parsimony, Maximum-Likelihood and Bayesian analyses, based on specimens collected in Rio de Janeiro, Rio Grande do Sul and Bahia Blanca that were also included in the morphological study. Morphometric analyses revealed significant differences between specimens from Rio Grande do Sul and Rio de Janeiro. However, meristic characters showed a less pronounced segregation between those samples, with a relatively large number of intermediate specimens. Despite some degree of morphological variation between samples, molecular-based phylogenetic analyses did not evidence species-specific clades on gene trees or haplotype networks, and genetic divergence between samples was not larger than that within samples, indicating that a single species of Brevoortia (B. aurea, the senior synonym) should be recognized in the south Atlantic. If that hypothesis is correct, it is likely that the morphometric separation between populations of Rio de Janeiro vs. Rio Grande do Sul results from the fact that only a few specimens from intermediate regions were included in the morphological analysis, a question that will be addressed in future stages of this study.



### A 100-year conundrum solved: Hyperoglyphe macrophthalma (Miranda Ribeiro 1915) is a junior synonym of Schedophilus velaini (Sauvage 1879) (Centrolophidae)

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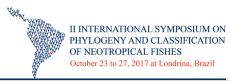
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Originally named as Toledia macrophthalma Miranda Ribeiro 1915, the Brazilian barrelfish (locally known as 'Lírio') was described based on a single specimen, 68 cm SL, bought in the fish market of the city of Macaé, and deposited in the fish collection of the Museu Nacional, Universidade Federal do Rio de Janeiro. In 1967, the species was reallocated in Hyperoglyphe, with a remark that it might be a junior synonym of H. moselii (Cunningham 1910). However, neither the holotype of T. macrophthalma nor any other specimen attributed to the species were checked at that moment. Hyperoglyphe moselii, in turn, was synonymized with Schedophilus velaini in 1995. In spite of this confusing taxonomic situation, T. macrophthalma has been considered as valid under Hyperoalyphe since 1967 and up to this day. The species is frequently caught on the shelf break, between 160 and 250 m, by bottom hook-and-line fishing off northern Rio de Janeiro State, where it attains a high price in local markets. However, since its description and until recently other specimens of *H. macrophthalma* were not known in fish collections. Recently, six specimens (72-86 cm TL) of H. macrophthalma were collected from the commercial landings at Cabo Frio and Macaé (the type locality) and are now deposited in the Fish Collection of the Núcleo em Ecologia e Desenvolvimento Socioambiental de Macaé (NPM). Based on these specimens, phylogenetic analyses were conducted using three genetic markers (two mitochondrial sequences, COI and 16S and one nuclear, RAG2). Gene genealogies were assessed by Parsimony, Maximum-Likelihood and Bayesian analyses. Sequences from six genera of the Centrolophidae were used in the analyses, all of them obtained in Genbank and referenced in published literature. The number of specimens and species varies depending on the marker, but the results are congruent among them. All specimens of H. macrophthalma sequenced were clustered with specimens of Schedophilus velaini and "H. moselii", independent of the genetic marker or methodological approach employed. Three haplotypes are shared between H. macrophthalma and S. velaini in the haplotype network. Genetic divergences between these two species were not larger than that within each group of species, indicating that all specimens most likely represent a single species. Meristic and morphometric data of specimens attributed to H. macrophthalma, including the holotype, compared with published information on S. velaini is also congruent with the conclusion that both species are synonyms. Therefore, the Brazilian barrelfish or "Lírio" is actually S. velaini.



## Systematic review of *Pyrrhulina* Valenciennes (Characiformes: Lebiasinidae) with comments on *Holotaxis* Cope

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The genus Pyrrhulina comprises 19 nominal species endemic of cis-andean South America. The group includes many taxonomic challenges and species lacking well preserved types and its monophyly is supported by the presence of two series of premaxillary teeth, the opercular membrane partially united to the isthmus, the absence of postcleithrum 3, and the variably shortened dorsalmost paired longitudinal series of scales on the body. Herein, the first comprehensive taxonomic review is proposed with the recognition of six new species. Additionally, five nominal species are considered or confirmed as synonyms. The previously proposed synonymies of P. macrolepis and P. rachoviana with P. australis are confirmed. On the other hand, the controversial synonymy of P. semifasciata with P. laeta is refuted, with P. eleanore being proposed as the synonym of P. laeta, instead. Pyrrhulina maxima is considered a synonym of P. semifasciata, given the comparison between both species seem to have been based in misidentified specimens. Pyrrhulina obermulleri, originally described as a subspecies of P. lugubris, is suggested as a synonym of that species. Finally, Holotaxis melanostomus, is removed from Pyrrhulina and is suggested herein as the senior synonym of Copeina autatta. A potential synonymy between both species based in the shared type locality of both species and also in the alleged single row of premaxillary teeth was recently proposed, but not formally, likely because the loss of the holotype of H. melanostomus, but also because the shared type locality per se would not be indicative of any synonymy. A feature missed so far allowing unequivocal evidence for the removal of the species from Pyrrhulina is the diagnostic presence of seven longitudinal rows of scales on the flanks, which contrasts with the presence of five longitudinal rows present in the holotype of H. laetus, and also in all Copella and Pyrrhulina species, agreeing, instead, with the number of rows observed in all Copeina specimens examined herein.

Key words: Pyrrhulininae; Taxonomy; Ostariophysi; Neotropical Financial support: FAPESP (process 2010/19088-7)



# Preliminary data on the morphology of Anchoviella cayennensis (Puyo, 1946), a possible junior synonym of Anchoviella perfaciata (Poey, 1860) (Clupeomorpha: Engraulidae)

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Anchoviella is a genus of the Engraulidae composed by seventeen species of the fishes commonly known as herrings or "manjubas" in Brazilian Portuguese. Herrings are relevant in artisanal fisheries worldwide and, in some cases, also in industrial fisheries. They are also relevant to the maintenance of coastal ecosystems, since they compose a substantial portion of the primary consumers among coastal fishes. Anchoviella cayennensis (Puyo, 1946) was described based on specimens collected in the Cayenne river, in French Guiana. Records of the species are known in the south-Western Atlantic between the French Guiana and Espírito Santo, Brazil. The morphologically similar Anchoviella perfasciata (Poey, 1860), in turn, was described based on specimens collected in Cuba. Its geographic distribution is reported as being restricted to certain parts of the Central and north-Western Atlantic, such as North Carolina and Florida in the US, Panama, Central America, the Antilles, Cuba and Trinidad. Literature on engraulid taxonomy is scarce, but some studies pointed out that the two species are highly similar in terms of morphology. The diagnostic characters proposed so far for those two species partially overlap, therefore identification of both is problematic even if they are valid. As a consequence, the geographic distributions currently reported for A. cayennensis and A. perfasciata are also questionable. The final goal of the present study is to solve this taxonomic uncertainty, using 23 morphometric and 7 meristic characters measured and counted in specimens of Anchoviella attributed to A. cayennensis and A. perfasciata deposited in fish collections and collected for the study. Cleared and stained specimens are also being analyzed in order to present an osteological description of A. cayennensis if the species is valid. A total of 44 specimens attributed to A. cayennensis were analyzed so far. Eighteen of them were collected in the Paraiba do Sul river in Rio de Janeiro State, Brazil, and are deposited at NPM. Twenty-three further specimens attributed to the same species were also collected in the Rio Doce basin in Espírito Santo, Brazil, whereas three specimens were collected in the Rio São Francisco basin in Sergipe, Brazil, all of them deposited in CIUFES. Results indicate that counts and measurements taken so far are within the range proposed in the literature for A. cayennensis. However, the number of gillrakers in the lower branch of the first branchial arch of 14 specimens (28(2); 29(2); 30(10)) also falls within the range reported for A. perfasciata in the literature (24-30). In addition, size of the upper jaw of 33 among the 44 specimens studied (63.6% - 65.5% of head length) also overlap with values reported for A. perfasciata. In the next stages of this study more specimens attributed to A. cayennensis will be examined, with the addition of specimens identified as A. perfasciata deposited in fish collections, especially those that include collections from the north Atlantic.



### A new species of Hyphessobrycon Durbin (Characiformes: Characidae) from the Rio Jequitinhonha basin, eastern Brazil, with comments on the Astyanax scabripinnis species complex

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Hyphessobrycon is the most species-rich genus of Characidae distributed from southern Mexico to Río de La Plata in Argentina. The paraphyletism of the genus is consensual. Despite that, primarily for empirical reasons many species are still being described within consensually non monophyletic genera whithin Characidae, after the classification proposed by Eigenmann. In this study, a new species that fits in the current definition of Hyphessobrycon with a distinct shape of humeral blotch is described from the upper portion of the Rio Jequitinhonha basin. It is distinguished from most congeners by the presence of a humeral blotch and a caudal peduncle blotch. Among these, Hyphessobrycon sp. n. distinguishes from all species with the aforementioned colour pattern, except H. anisitsi, H. balbus, H. boulengeri, H. duragenys, H. hamatus, H. isiri, H. langeanii, H. rutiliflavidus, H. santae, H. uaiso and H. vinaceus by possessing the greatest body depth on the vertical through somewhere anteriorly to dorsal-fin origin (vs. greatest body depth on the vertical through dorsal-fin origin). Hyphessobrycon sp. n. distinguishes from these species by the presence of a single well-delimited round to vertically oval conspicuous humeral spot dorsally to the lateral line scale row (vs. humeral blotch, when present and single, with a widest vertically or horizontally elongate dorsal portion, and a narrower ventral extension from the lateral line). Hyphessobrycon sp. n. shares with the aforementioned species the following characters: 1) humeral blotch with the general position and shape as described above; 2) greatest body depth on the vertical trough anteriorly to the dorsal-fin origin; 3) body depth smaller than 41%, varying from 30% to 33%; 4) low number of anal-fin rays (12-23); 5) shape and distribution of bony processes on the fins of males; 6) one to two maxillary teeth; 7) anal-fin distal margin of males with a very discrete anterior lobe, slightly convex in some species; 8) a variable concentration of chromatophores on the distal margin of the scales, forming a reticulated pattern; 9) presence of a longitudinal stripe from humeral blotch to the caudal peduncle blotch, or to the distal tip of the medial caudal-fin rays. Among characids this combination of characters are also found in Hasemania kalunga, H. piatan and H. crenuchoides, and the species of the Astyanax scabripinnis species complex. Following the current paradigm Hyphessobrycon differs from Astyanax by the presence of incomplete lateral line (vs. complete) and from Hasemania by the presence of adipose fin (vs. absence). However, some authors, based on the sharing of supposed exclusive characters, described species in genera different from those classically delimited, and even proposed putative monophyletic groups within these genera without cladistic analyses. In spite of the sharing of all, apparently exclusive, characters mentioned above by all the species of Hyphessobrycon and Hasemania aforementioned, and the species of A. scabripinnis species complex, we simply hypothesize a close relationship among these species without a generic rearrangement, which obviously require a broad phylogenetic analyses.

Key words: Neotropical; Freshwater; Coastal drainage; Taxonomy; Sexual dimorphism Financial support: CAPES (ISP, SAS), CNPq (GMD 300066/2016-3)



### A new sexually dimorphic species of Aphyocharacidium Géry (Characidae: Aphyocharacinae) from the Marañón river, Peru

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Presence of hooks on fins has been proposed as a synapomorphy of a group of Characiformes, including Characidae. Some characid taxa, however, are known to lack fin hooks as such is the case of the species of the genus Aphyocharacidium. This genus is easily recognized among characids by the presence of two tooth series in the dentary. Currently there are two valid species from this genus, Aphyocharacidium bolivianum Géry, 1973 and Aphyocharacidium melandetum (Eigenmann, 1912), both lacking hooks on fin rays. We herein describe a new species from the Marañón river, at Morona and Andoas districts from Peru. The new species can be assigned to Aphyocharacidium by the presence of two tooth series in the dentary with tricuspid teeth becoming conical in the posteriormost region of the bone, also sharing with the other species of the genus a single series of numerous maxillary teeth, pseudotympanum, lateral line complete, and falciform anal fin. It can be diagnosed from other species of the genus for bearing well developed bony hooks on the anal and pelvic fin rays. Anal fin bony hooks are observed from the last unbranched ray and from 1st to 7th branched rays of adult males, limited to the midlength region of the rays, inserted along the posterolateral border of the rays, and absent on the secondary branches. One to four hooks (usually two) are found per segment of the lepidotrichia, showing a robust base (approximately twice the width of the distal half hook) gradually sharpening to a retrorse tip. Anal fin rays bearing bony hooks are expanded in the sagittal plane. Pelvic fin bony hooks present on the unbranched ray and from 1st to 6th branched rays of adult males, along midlength portion and ventromedial margin, usually present on the secondary branches of the rays. One to three hooks (usually two) per segment of the lepidotrichia, with a robust base gradually sharpening to a retrorse tip. The new species is further diagnosed by other characters related to body measurements and lateral line scales count. The presence of hooks on fins of this new species, so far unknown among Aphyocharacidium species that are deeply inserted in the phylogeny of Characidae, further demonstrates the loss of this secondary sexual character is secondary and may have occurred among the members of this family.

Key words: Sexual dimorphism; Hooks; Characiformes; Secondary sexual characters Financial support: CAPES, CNPq



### Cryptic diversity in the Cis-Andean *Rhamdia quelen* species complex (Siluriformes: Heptapteridae)

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Rhamdia is a genus distributed throughout Neotropical's major hydrographic basins. Despite its commercial importance, the taxonomic status of the genus is still controversial and complex. Currently, three valid species occur in the Cis-Andean region: Rhamdia voulezi, R. branneri and R. quelen. These two former species occur only on the Iguaçu River Basin, while R. quelen is widely distributed throughout South America basins. In the latest morphological taxonomic review carried out for Rhamdia, 46 nominal species were synonymized as R. quelen. However, it is possible that there are more divergent lineages than the species currently recognized. In order to recognize potentially undescribed taxa, our aiming was to reconstruct the evolutionary relationship among these three Cis-Andean species using DNA sequences of cytochrome C Oxidase I. We sampled 33 specimens of Rhamdia, representing all Cis-Andean species. Specimens were collected from the Iguaçu, Uruguay, Benedito, and Itapocu rivers, and Peri Lagoon. After aligned and edited all recent COI sequences, we combined them with COI reference sequences of Rhamdia available in the BOLD system. The final dataset, including outgroup (Rhamdia guatemalensis and Pimelodella sp.), was composed of 60 sequences. To characterize the evolutionary relationship among samples, we used the COI data set to generate phylogenetic tree under a Bayesian coalescent framework. We also used GMYC analysis to assign individuals to putative independently evolving lineage, identifying seven well-supported lineages; only two of those monophyletic lineages corresponded to currently recognized species: R. branneri and R. voulezi. Under the name R. guelen, five distinct lineages were identified, evidencing a non-monophyletic group. Divergence times were estimated under an uncorrelated lognormal relaxed clock model and birth-death model was used as species tree prior. We calibrated the phylogeny using a mutation rate defined for Characiformes, once this information is unknown for Siluriformes. The COIbased tree recovered two main geographic groups: Northeast and South/Southeast. Within this latter group, two clades geographic well-defined are visualized: inland and coastal groups, that split at 5.88 Mya. This biogeographic pattern is probable resulted of sea level fluctuations that occurred during the Miocene. The marine transgression and regression has been indicated as an important diversification process in several Neotropical fishes. The ancestral lineage of South/Southeast group could be present in the Brazilian inlands, and after a marine regression event part of this ancestral reached the coastal through headwater captures events. However, we do not reject that vicariance and geodispersal have played a key role in the currently complex distribution pattern of Rhamdia. It is probable that the interaction among these events shaped the diversity of lineages in this genus. Although all Cis-Andean basins were not sampled, the results corroborate that R. quelen is a species complex, and is composed of at least five potential species.

Key words: Jundiá; Marine transgression/regression; Neotropical freshwater fishes; Paraphyly; Phylogeography Financial support: PNPD Capes



### Secondary sexual characters in Characidae (Teleostei: Characiformes): morphological variation and evolutionary patterns

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Among Characiforms families, Characidae, with 1276 valid species is, undoubtedly, the most problematic. This is due to the great diversity and morphological variation among its members. Although some recent studies, based on both morphological and molecular data, were performed, its delimitation, and intra and inter-familial relations are still controversial. These studies however, corroborate the monophyly of a group characterized by the absence of supra-orbital bone, a seemingly reductive character. As summarized by many authors, the lack of information on the color in life and sexual dimorphism of its members is one of the main obstacles preventing further comprehensive phylogenetic analyses on Characidae. Although, there has been a great advance in the knowledge of the sexual dimorphic characters in the family since Eigenmann, with works proving its phylogenetic importance in the resolution of well-corroborated groups (e.g. Glandulocaudinae sensu Weitzman & Menezes), it is clear that a great part of this studies are restricted to taxa with more evident and specialized secondary sexual characters (e.g. caudal organ complex), and in less inclusive groups, often composed by less than five species. Along the years, the authors observed a great unveiled diversification in some sexual characters, which description is restricted to the simple presence or absence (e.g. bony process). These observations and the proved phylogenetic informativeness of the secondary sexual characters led us to perform a detailed broad comparative anatomical analyses of sexual characters such as bony process, gill gland and fin shape and length in Characidae, with the purpose of providing information which, when analyzed together with characters from other anatomical complexes, could help in the resolution of aroups whose relations remain controversial in the literature (i.e. incertae sedis), as well as of finding evolutionary patterns. Therefore, 73 species of Characidae, representing all suprageneric groups (sensu Mirande), plus 3 species of Crenuchidae, 2 species of Gasteropelecidae, and 1 species of Serrasalmidae, were analyzed. As a result, an incredible morphological variation in these characters was unveiled, which might be potentially informative in resolving relations in less inclusive groups in Characidae. Regarding the bony processes, it was possible to delimitate different shapes, patterns of distribution of such processes on the fins and on a particular fin ray, and dependence or independence of the presence of bony processes among the fins in a species. Regarding the shape and length of fins and gill gland, it was possible to delimitate different discrete conditions along Characidae. The comparative anatomic analyses of such characters in a great number of species provided us the opportunity to observe possible evolutionary patterns by plotting the characters on the different available phylogenetic hypothesis on the family. Also, derived anatomical features are identified as new putative synapomorphies for some less inclusive groups. Further, our results indicate that the presence of a certain condition of an individual secondary sexual character, and the combination of conditions of different secondary sexual characters in species or suprageneric groups along the evolution of Characidae might be related to the evolution of the reproductive strategy in the family.

Key words: Secondary sexual characters; Taxonomy; Sexual dimorphism, Freshwater; Neotropical Financial support: FAPESP (process 2012/01263-2)



### Seasonality of some secondary sexual characters of Hysteronotus megalostomus Eigenmann (Characiformes: Characidae: Stevardiinae)

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Secondary sexual characters are those that differentiate males from females, indirectly related to sexual activity, and selected through sexual selection. It is expected that they would increase the reproductive success rather than survival. Secondary sexual characters have supposedly a pre-copulatory role in the reproductive activity, thus it might emerge before reproductive period. Characidae has a huge morphological diversity of secondary sexual characters. These characters are related to body size, bony processes, caudal organ, gill gland, fin shape and length, nuptial tubercles and color pattern. Secondary sexual characters have been well studied in Characidae, both taxonomically and phylogenetically, or on their development in relation to the reproduction period. This last approach has enriched the discussion regarding a big question: are the bony processes of Characidae species seasonal or permanent? Many authors have concluded that bony processes once developed show no sign of regression after reproduction period, while others concluded the opposite. The matter is therefore still controversial. While re-describing Hysteronotus megalostomus, we found some evidence that would rather change this question, and create others. We analyzed a great number of specimens, with different sizes, which permitted us to determine the dimorphic characters and its natural most developed state. Sexual characters of H. megalostomus are related to caudal organ, gill gland, bony processes, color pattern, and body and caudal-peduncle depth. Among the analyzed specimens however, there were some large males that had only the caudal organ, and the dimorphism on body- and caudalpeduncle depth. Macroscopic analyses of the gonads of both the males with highly developed sexual characters and the males without bony processes and gill glands revealed gonad in an advanced development stage vs. gonad in a rest or initial development stage, respectively. Also, through macroscopic analyzes of gonads of both mature males and females we concluded that the species has a seasonal reproductive period. The gonadal information, plus the fact that the analyzed males have the same size, and that the males without bony processes and gill glands has a fully developed caudal organ complex, which indicates a previous sexual maturity, clearly indicate the seasonality of the bony processes and the gill gland in this species. Additionally, images through scanning electron microscopy revealed bony scars where the bony processes should be, which also indicate that they break and are discarded rather than regress in this species. Furthermore, these results show that there could be seasonal and permanent sexual characters, even in the same species, and that the seasonality of the bony processes might be related to the very seasonality of the reproductive period. Published studies discussing this matter analyzed different species, with probably different reproductive strategies. Therefore, questions should rather be: In which species those characters are seasonal, and in which they are permanent? Which characters are seasonal or permanent in each species? Is the seasonality or permanency related to the complexity of the character? Is the seasonality of these characters related to seasonality of reproductive period?



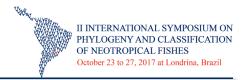
#### The color pattern and osteological description of Corydoras undulatus Regan, 1912 (Siluriformes: Callichthyidae)

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The present study was conducted to provide a description of coloration and osteology of Corydoras undulatus Regan, 1912 from the La Plata and Laguna dos Patos basins. The original description is scarce in terms of information on morphological characteristics and, in particular, color pattern and osteological characteristics, and was based on only three specimens. Additional information in the scientific literature on this species is also scarce. Specimens from the known area of distribution of the species were analyzed; Paraná and Paraguay River basins in Argentina, Uruguay River basin in Argentina, Brazil and Uruguay, and the Laguna dos Patos system in Brazil. The morpho-anatomic analysis was performed through the observation of bone structures, using stereomicroscope, in cleared and stained specimens (c&s). The osteological terminology was based on a bibliography already established. A description of the color pattern and osteological features of Corydoras undulatus was provided. Based on the results, Corydoras undulatus can be distinguished from all congeners by the body's color pattern, its flanks thickly covered by irregular black spots predominating throughout the lateral series of the dorsal plates, and at the top of the ventral plates; Inferior part of the lateral series of the ventral plates without pigmentation. Some specimens may present one to three irregular black longitudinal stripes on the flank. Dorsal, Pectoral and caudal fin usually with irregular diffuse dark blotches; Flap adipose with some dark spots; Anal fin usually hyaline. Corydoras undulatus can be distinguished from Corydoras elegans by the absence of ventral contact between the scapulocoracoids and the cleithrum laterally, and by the scapulacoracoids covered by skin (vs. cleithrum and scapulocoracoid in contact and exposed laterally). The present work adds to the literature important data about coloration and osteology of this species, and may also contribute to an improved understanding of the phylogenetic relationships among all congeners.

Key words: Freshwater, Laguna dos Patos, La Plata, rio Uruguai



## Identity and type-locality of Astyanax taeniatus (Jenyns, 1842): a tetra collected by Charles Darwing during the Beagle's Voyage

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In 1832, Charles Darwin spent three months travelling in Rio de Janeiro State, Brazil, between April 4<sup>th</sup> and July 5<sup>th</sup>, in a break from his journey in the HMS Beagle. During his wanderings throughout the interior of the State, Darwin visited the "Fazenda Socêgo", a farm located north to the Cabo Frio region. About "Socêgo", Darwin wrote in his diaries that "... it is not possible to give an adequate idea of the higher feelings of wonder, astonishment, and devotion, which fill and elevate the mind". In a running brook of this farm, he collected two small specimens of a fish that was described in 1842 by Jenyns as Tetragonopterus taeniatus, which was later transferred to Astyanax. The identity of the species has remained somewhat elusive since then, due mostly to the brevity of the features presented as diagnostic. Astyanax taeniatus was redescribed in 2001, but the type specimens of Tetragonopterus taeniatus were not examined in this study. The geographic region covered in this revision was also relatively restricted, therefore doubts on the type locality of the species and its precise identity still remain. In this study, we intended to clarify the accurate identity and the type-locality of A. taeniatus associating molecular tools to the usual morphological data. The two syntypes of Tetragonopterus taeniatus were examined and a tissue sample was extracted from one of them. Genetic comparisons were made between the type-specimen and those of Astyanax collected recently in the "Fazenda Socêgo", city of Conceição de Macabú, Rio de Janeiro State. Based on information from Darwin's journal and the "Caminhos de Darwin" project, we recognized the "Fazenda Socêgo" as the type-locality of A. taeniatus. Cytochrome Oxidase Subunit I (COI) sequences of the syntype and of specimens of Astyanax collected in the "Fazenda Socêgo" were included in a matrix with 207 specimens of the Characidae from coastal rivers of Brazil. The smallest values of p-distance (0.03) were obtained between the sequence of the syntype of Tetragonopterus taeniatus and sequences of specimens of Astyanax collected in rio Aduelas, Rio Macaé basin, in "Fazenda Socêgo". The anatomical and meristic information of the modern specimens collected in rio Aduelas are also in accordance with data from the syntypes. With the use of ancient DNA techniques, we therefore succeeded to determinate the type-locality of A. taeniatus in Conceição de Macabu, RJ, Brazil, with the collection of modern topotypes of the species.

Key words: Ancient DNA; Darwin; Characidae; Freshwater; Neotropical Financial support: CNPq scholarships and grants to PCS (Proc. 205678/2014-9; 141407/2013-1) and LRM (307890/2016-3)



### Revision of genus *Hypostomus* Lacépède, 1803 (Siluriformes: Loricariidae) from rio Paranapanema, Southeastern Brazil, with description of a new species

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The species of Hypostomus Lacépède from the rio Paranapanema basin, left bank tributary of the upper rio Paraná, are reviewed and morphologically distinguished. One probably new species of the genus is identified and described. Morphological and morphometric analyses were used to study and characterize the species. Comparative material of Hypostomus from type localities in the upper rio Paraná basin assisted the species identification. Fourteen species of the genus were recognized in the rio Paranapanema basin, which can be separated in two groups based on the color pattern. One constituted by light-brown ground color body with dark spots or bars, and a second group with darker ground color body and pale spots or vermiculated. The new species belongs to the first group and is promptly diagnosed relative to the pale spotted species group that includes Hypostomus albopunctatus (Regan, 1908), Hypostomus margaritifer (Regan, 1908), Hypostomus microstomus Weber, 1987, Hypostomus multidens Jerep, Shibatta e Zawadzki, 2007, Hypostomus paulinus (Ihering, 1905), Hypostomus regani (Ihering, 1905) and Hypostomus strigaticeps (Regan, 1908). Hypostomus n. sp. can be distinguished from the remaining dark blotched species of the basin by presenting three plates bordering the supraoccipital [vs. one plate in Hypostomus ancistroides (Ihering, 1911), Hypostomus brevis (Nichols, 1919), Hypostomus hermanni (Ihering, 1905), Hypostomus iheringii (Regan, 1908), Hypostomus nigromaculatus Schubart, 1964 and Hypostomus topavae Godoy, 1969]. The new species is also characterized by having small body size (maximum 134,3 mm SL); presence of hypertrophied odontodes in the pectoral fins spine; body covered by spiny plates; plates of the anterior region of body forming conspicuous keels; and abdomen almost naked, except by small plates anteriorly to the anus and between pectoral fins. Hypostomus n. sp. has background color of head and trunk lightbrown and with dark spots (smaller and more densely in the head, greater and sparser on the trunk and caudal peduncle). Some specimens present three dark dorsolateral bands, two below the dorsal fin and one below the adipose. Abdominal region and ventral region of the caudal peduncle lightbrown and in some specimens with dark spots. Fins light-brown with dark spots similar to those of the body; spots of the interradial membranes and rays frequently aligned, forming transversal bands in all fins. The new species resembles and may be confounded with Hypostomus nigromaculatus and Hypostomus paulinus in the Paranapanema basin. From the former is promptly diagnosed by having shorter snout (snout length 59.6 to 61.3% in head length vs. 63.7 to 71.7% in H. nigromaculatus) and greater eyes (eye diameter 17.2 to 18.9% in head length vs 13.3 to 15.9%). Relative to the latter it can be mainly distinguished by the number of teeth (37 to 56 teeth vs. 58 to 83 in H. paulinus) and by the color pattern (H. paulinus has body predominantly grayish background color with pale vermiculation, more conspicuous on the head). An identification key, descriptions and illustrations for all the species recognized from the rio Paranapanema basin are provided.



## Morphological study of species of *Characidium* Reinhardt in the upper Rio Todos os Santos, Minas Gerais (Teleostei: Characiformes: Crenuchidae)

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The genus Characidium belongs to the Characiformes, a group of Neotropical fishes with a wide diversity of species, body shapes and behavior, comprising 18 families, about 270 genera and over 1700 species. The genus is speciose, including relatively small fishes that do not exceed 100 mm in standard length and is particularly diverse in streams of the Atlantic Forest. Characidium is a monophyletic aroup characterized by the black spot on the base of the caudal fin rays, the only synapomorphy of the genus, which may be secondarily lost in some species. Recently, many specimens of Characidium were collected in Rio Todos os Santos, an affluent of Rio Mucuri in Minas Gerais State. Some of these specimens were initially identified as Characidium timbuiense, but others seemed morphologically distinct and did not fit the description of other species of Characidium from the region. Our initial goal was to perform a morphological study to determine if both forms were indeed distinct, and to possibly describe a new species of Characidium. The study was based on the analysis of 25 morphometric and 12 meristic data from 130 specimens of Characidium sp and 30 specimens of "Characidium" timbuiense", both collected in many stretches of Rio Todos os Santos, municipality of Teófilo Otoni, MG in 2010. In addition, counts of vertebrae, branchiostegal rays, teeth, supraneural bones and others osteological observations were performed in cleared and stained specimens. Observations on color pattern of the two forms, general body shape, sexual dimorphism and pseudotympanum configuration were also performed to complement the diagnosis. After a careful examination of specimens of both forms, and in addition to confirming that they represent two different species, we also hypothesize that specimens previously assigned to "C. timbuiense" differ from representatives of this species as traditionally recognized based on the adipose fin with little or no pigmentation, larger number of teeth in the premaxilla, absence of the inner row of dentary teeth, larger number of teeth in the ectopterygoid and orbital diameter not conspicuously shorter than the length of the snout. Some of the main diagnostic features between the two species studied herein include number of vertical bars, size and number of teeth in the premaxilla, ectopterygoid and dentary. Both forms in the upper Rio Todos os Santos, hence, are considered as new species of Neotropical fishes and are presented herein.

Keywords: Taxonomy, Neotropical, new species



### Ontogeny and Neotropical Fishes: perspectives from a still poorly explored source of information

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Ontogeny studies of organisms have provided important information to understand the development of form since earlier times. Such studies are nowadays useful to better understand similarities and differences among lineages and relate them with the phylogeny and taxonomy of organisms. In the case of fish, there are examples of ontogenetic studies that brought new interpretations about anatomical structures, sometimes impacting hypotheses of relationships among groups. There are also cases of features that appear during ontogeny and serve as diagnostic for different species, aiding in the identification of eggs and larvae. It is evident, therefore, the importance of ontogenetic studies to elucidate issues regarding the phylogeny and classification of fish. On the other hand, and most probably due to the inherent difficulty in obtaining adequate developmental series, ontogenetic studies including Neotropical fish are still insipient in spite of the incredible diversity of species in South and Central America. To surpass this obstacle, there have been recent efforts to compile collections of developmental series of different Neotropical fish groups, including freshwater, marine and estuarine species. Studies resulting from these collections approach different taxonomic or phylogenetic questions according to each group, some of them detailed herein. Among the Characiformes, the recent complete skeletogenesis of Salminus brasiliensis have been used as a model to understand reductions and ontogenetic truncations in miniature characids such as Priocharax. A similar approach has been used with Lebiasina bimaculata serving as model to tackle miniaturization in the Lebiasinidae. In addition, the comparison of the development of the maxilla in Salminus and Brycon has revealed a common pattern, until now unique among characiforms, suggesting an ontogenetic synapomorphy of the clade comprising these two genera. More recently, the ontogeny of the skull of Hoplias intermedius is being addressed to better understand synapomorphies of the Erythrinidae related to the infraorbital series, in addition to the development of their dentition and formation of the dilatator groove, a structure present in most Erythrinoidea. Among marine fishes, different ontogenetic changes in color pattern of distinct species of Trachinotus (Carangiformes) are useful in the diagnosis of juveniles of distinct species, aiding in the identification of small pompanos abundant in sandy beaches that could not be properly told apart in the field. Among Albuliformes, the skeletogenesis of Albula vulpes revealed an interesting pattern in which ossification of the skull is delayed in leptocephali larvae until metamorphosis, when development of most bones clearly increases. At last, the development of the skull and eye of Anableps (Cyprinodontiformes) showed an interesting pattern in which the overgrowth of the frontal bone in the orbital region is temporally related to the rapid growth of the eye, functional horizontal divide of the pupil and differential expression of opsins in the dorsal and ventral region of the retina, an adaptation to life in the water surface. Developmental Biology of Neotropical fish is a vast and still poorly explored universe that will help further advances if the phylogeny and classifications of these organisms.



### A new genus in Crenuchidae, with inferences on phylogenetic positioning and impacts on Characidiinae phylogeny

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Crenuchidae is composed by 95 species of small fish (maximum 10 cm of standard length) divided into two subfamilies (Crenuchinae and Characidiinae). The family is widely distributed in the tropical and subtropical regions of the New World. However, the highest taxonomic and morphological diversity of Crenuchidae is found in drainage areas in the north of South America, including Guianas and Western Amazon. A new taxon recently discovered in Upper Paraná River drainage is currently being investigated as a possible new genus, attributed to Characidiinae for its morphological similarities shared with the group. Here we present preliminary results on the diagnosis and phylogenetic positioning of the new taxon in the Characidiinae phylogeny. The new taxon was included in an array of morphological data with 40 taxa and 64 characters, with representatives of most of the genera of Crenuchidae, with the exception of Poecilocharax, Geryichthys and Skiotocharax. A new matrix was analyzed with the software TNT version 1.5, through parsimony analysis. The most parsimonious trees retrieval was performed by heuristic search with 1.000 replicates, hold 50, initial seed at 0. The Branch Swapping algorithm used was TBR (Tree bisection and reconnection). The polarization of the states of characters were done through comparison with external groups, constituted by Crenuchus and rooted in an outgroup composed by all-zeros, based on information obtained from other Characiformes, summarizing for a comparison, the assumed polarity. The Bremer index, retention index (RI) and consistency index (CI) were also performed with TNT. The RI and CI indexes were obtained through the statsall.run script. A phylogenetic analysis resulted in 44 equally more parsimonious trees (139 steps; RI = 0.8; CI = 0.5) summarized in a strict consensus tree. The new taxon is recovered as the sister group of the others Characidiinae being diagnosed (phylogenetic sense) by 7 autapomorphies. In addition, the new taxon can also be differentiated from most Characidiinae for the combination of characters: reduction of the principal caudal rays, presence of 2 pleural centers in the caudal complex, anterior post-temporal margin fully in contact with the parietal, epiocciptal bridge present, together with various morphometric and color characteristics. The inclusion of the new taxon reduced the previously recognized synapomorphies for Characidiinae from thirteen to eight. The genus Characidium is recovered as paraphyletic in an unresolved clade along with other characidiines. The initial hypothesis of the new taxon representing a new genus can be accepted. However, new morphological conditions were observed, some seemingly exclusive and others shared with other clades. In the light of these new evidences, an inclusion of additional taxa and the revaluation of some characters and their conditions are necessary to corroborate the new hypothesis.

Key words: Crenuchinae; Cladistic; Characiformes; Freshwater Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES



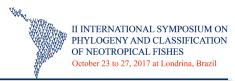
#### Phylogeny and phylogeography of Characidium (Characiformes: Crenuchidae) in southeastern Brazil

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Phylogenetic evolution of Characidium remains largely unknown due to poor morphological differentiation, continental scale distribution and large number of species. With 80 nominal species distributed through most of the Neotropical Region, testing hypotheses of species delimitation and phylogeny is a major challenge. In this study, we present a phylogenetic study of DNA sequences of species of Characidium from coastal streams of southeastern Brasil located between northeastern Rio Grande do Sul and northern Espírito Santo. In this region, mountain ridges separating small rivers draining the coastal slopes of the Serra do Mar, the Serra Geral, and the mountains situated eastward of the Serra do Caparaó isolate species and populations of Characidium. Our study included a phylogenetic analysis of next generation (Illumina) genomic data, a phylogeographic analysis of nuclear and mitochondrial genes, and species delimitation analyses based on traditional DNA barcoding methods. To recover deep phylogenetic relationships, DNA readings of the entire mitochondrial genome of nine species of Characidium were obtained, and the complete gene coding region, encompassing 37 genes (13 protein coding genes, 22 tRNA genes, and 2 rRNA genes) and the light strand replication origin loop, was assembled into a data matrix containing 15,802 nucleotide positions (after Muscle alignment). Phylogeographic relationships among populations from 70 localities were recovered based on fragments of two mitochondrial and one nuclear gene from 500 specimens. Maximum likelihood analysis of the sequences revealed the presence of up to four sympatric lineages in most river drainages. Analysis of genetic distances resulted in excellent species delimitation based on molecular data from single river drainages, even when morphologically cryptic species were involved. Comparisons of closely related populations among various coastal drainages, however, revealed varying degrees of molecular differentiation spanning the 2% threshold limit usually accepted as a barcode gap among sympatric species. These varying degrees of phylogeographic differentiation among allopatric populations are correlated with a hierarchical pattern of isolation among coastal drainages. The combination of phylogenomic and phylogeographic methods allowed us to recognize lineages that correspond to various speciescomplexes within the clade of species with naked isthmus and variegated color patterns: the Characium alipioi group with at least four species, a group of undescribed species closely related to the C. alipioi group, and a large assemblage of species associated with C grajahuensis. Within the latter, we recognize the C. interruptum group, the C. pterostictum group, the C. timbuiense group, an unnamed species from coastal streams of Rio de Janeiro, and the C. grajahuense complex senso stricto. The C. lauroi complex is included in this large assemblage, but its monophyly is poorly supported. However, this result is tentative, because our sampling of the complex was incomplete. Within each of the main clades, the various lineages are largely allopatric and associated to a hierarchical pattern of Pleistocene isolation caused by sea level changes. However, the geographic distribution of these clades is largely overlapping indicating that old speciation events were followed by subsequent dispersal along coastal plains.

Key words: Phylogenomics; Mitogenome; Phylogeography; Systematics; Neotropical Financial support: CAPES, CNPq (proc. 476822/2012-2, 440566/2015-0), FAPERJ



### Phylogenetic analysis of the genus *Brachyhypopomus* (Gymnotiformes: Hypopomidae) based on characters of sperm morphology

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The genus Brachyhypopomus (Gymnotiformes, Hypopomidae) has been recently reviewed with the total number of valid species increasing from 13 to 28 species. A species-level phylogenetic reconstruction of the genus has also been recently published, based on morphological and molecular data. The characters of sperm morphology have the potential to elevate the number of characters used in phylogenetic analysis, enhancing its power of resolution and assumptions concerning ecology and evolution of the species. In the present study, 18 of the 28 valid species of Brachyhypopomus (B. alberti, B. arrayae, B. batesi, B. beebei, B. benjamini, B. bennetti, B. brevirostris, B. bullocki, B. diazi, B. flavipomus, B. hamiltoni, B. hendersoni, B. occidentalis, B. pinnicaudatus, B. regani, B. sullivani, B. verdii, B. walteri) had testis samples analyzed through transmission electron microscopy aiming to describe the ultrastructure of the sperm cell, and three species had data compiled from bibliography (B. bombilla, B. draco, B. gauderio). Additionally, data from representatives of different families of gymnotiforms (Ramphichthys hahni, Sternopygus sp., Apteronotus albiforns, Eigenmannia virescens, E. trilineata) were also gathered from bibliography. Among the observed characteristics of spermatozoa ultrastructure of the different species, 12 characters were considered informative. In phylogenetic analysis using only characters of sperm cell morphology, six characters has supported the monophyly of the genus Brachyhypopomus. Within Brachyhypopomus, three groups were observed: B. regani and B. arrayae, supported by four characters; B. alberti, B. sullivani, and B. bombilla, supported by three characters; one aroup including all the remaining species. A group including R. hahni, Sternopygus sp., E. virescens, and E. trilineata was observed supported by the presence of axonemal fins, and A. albiforns was positioned alone according to the presence of long midpiece, long citoplasmic canal, and elongated vesicles in the midpiece. The insertion of characters of spermatozoa ultrastructure in the total evidence matrix comprising the combined data from morphology, cytb, and rag2 presented in the phylogenetic reconstruction of Brachyhypopomus resulted in a slightly different tree topology concerning a few Brachyhypopomus species groups.

Key words: Systematic, electric fishes, ultrastructure, testis

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#### Morphology-based phylogeny of the small catfishes Hypoptopomatinae (Teleostei: Siluriformes: Loricariidae)

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Twenty years ago, in the I International Symposium on Phylogeny and Classification on Neotropical Fishes, S. A. Schaefer provided a phylogenetic analysis for the Hypoptopomatinae, based on the considerable increase of new species of these small catfishes commonly found associated with the marginal vegetation. From the about 70 species known in that time comprised in 12 genera, the author included 30 valid species (36 with the new taxa), merged in 17 terminal taxa of the subfamily. Since this approach, the number of new species of Hypoptopomatinge increased exponentially, reaching today 169 valid species organized in 23 genera, an average of five new species described each year. This unthinkable number of species has encouraged many morphological and molecular studies in these last two decades, however, the still crescent number of new descriptions results in many uncertain relationships involving the members of Hypoptopomatinae, and also of them with species of the Neoplecostominae, sister group of the subfamily. Considering this scenario, we present here a phylogenetic hypothesis for the Hypoptopomatinae sensu Martins et al., 2014. The analysis was performed based on 123 morphological characters, including osteological and external features. These characters were observed in 104 terminal taxa comprising Astroblepidae, and member of all the six Loricariidae subfamilies, Delturinae, Lithogeninae, Loricariinae, Hypoptopomatinae, Hypostominae e Neoplecostominae. We analyzed 86 species of the Hypoptopomatinae, 82 of them valid species from all of the valid genera of the subfamily, the greatest phylogenetic analysis for the subfamily until now. The analysis was performed using TNT, with all characters considered as non-additive and receiving equal weights. To find the most parsimonious tree, we performed a heuristic search using new technology: ratched (200 iterations) and drift (50 iterations), with random addition sequences, 1,000 replicates. From the equally parsimonious trees we constructed a strict consensus tree. Bremer support was performed using suboptimal trees with up to 10 steps more than the fundamental trees. We obtained 16 equally parsimonious trees (overall length 1,503 steps; consistency index=0.136; retention index=0.577), which were used to constructed the strict consensus tree. Hypoptopomating was recovered as a monophyletic group supported by 12 unambiguous synapomorphies. On the other hand, Hisonotus, Microlepidogaster, Otothyropsis, and Parotocinclus were recovered as non-monophyletic groups. As these genera comprise 45% of all valid hypoptopomatine species, there are many taxonomic problems to be solved. Many aspects of the evolution of the Hypoptopomatinae can be explained by their association with marginal vegetation. Many changes involving their morphological features resulted in an increase of their capacity of fixation on marginal vegetation, such as the reduction of body size (with many pedomorphic features related), exposition of pectoral girdle, enlargement of odontodes on snout, direction of odontodes of pelvic fin, abdominal coverage of plates, and expansion of swimbblader capsule. The Brazilian crystalline shield probably is the cradle of the subfamily, sheltering its most basal members. Thus, the close association of the hypoptopomatines to the marginal vegetation made them able to colonize and disperse to other habitats on lowlands, certainly a success factor to the group.

Key words: Biodiversity; Cascudinhos; Freshwater fish; Neotropical; Systematics

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### Skeletal development and ossification sequence of Astyanax lacustris (Lütken, 1875) (Characiformes: Characidae)

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An ontogenetic study regarding the skeleton development of the characid Astyanax lacustris is currently being carried out to propose an ossification sequence for this species and to describe and illustrate the development of its dermal and endoskeletal components. The ontogenetic series was obtained at an aquaculture station in the state of São Paulo from stocks of farmed Astyanax lacustris. The series consists of approximately 25 specimens, fixed in buffered formalin, for each day of the first 30 post-hatch days. Specimens were cleared and stained, measured and examined with the aid of a stereomicroscope. The smallest specimen examined so far is 3.3 mm of notochord length (NL) and already has eight partially or completely ossified elements: the anterior portion of the notochord, dentary, maxilla, premaxilla, opercle, tooth plate associated to pharyngobranchial 4, tooth plate associated to the ceratobranchial 5 and cleithrum. A similar condition with the same number of ossified elements occurs at 6.5 mm NL in Salminus brasiliensis. The largest examined specimen is 17.4 mm in standard length (SL) and already has 141 of the 145 ossified elements expected in an adult specimen. A similar condition with the same number of ossified elements occurs at 20.3 mm SL in Salminus brasiliensis. Thirty-three specimens were examined so far. The ossification of four elements was not observed yet, those being the interhyal and the three pelvic-fin radials. Among the skeletal complexes only the elements of the anal and dorsal fins have an unambiguous ossification sequence to this moment. It is possible to identify the size range between 3.3 mm NL and 8.1 mm SL as critical for the skeletal development of this species. Specimens up to 8.1 mm SL already show ossification signs or complete ossification for 90 of the 145 expected skeletal elements. To date, the only complete ossification sequence for a characiform is the one proposed for Salminus brasiliensis. Partial sequences were proposed for Hepsetus odoe, Prochilodus argenteus and for the characids Brycon moorei, and Moekhausia sanctafilomenae. The increase of ontogenetic studies for more characiforms could provide a promising source of information to investigate variation in ontogenetic timing, the nature of phylogenetic characters, to help in the proposition of homology hypothesis and to understand heterochronic events in the evolution of the order.

Keywords: Skeletogenesis, ontogeny, Neotropical, Freshwater fishes Financial support: CNPq (process number: 142195/2015-4)



### Headwaters capture evidenced by paleo-rivers' reconstruction and genetic structure of the armoured catfish (*Pareiorhaphis garbei*) in the Serra do Mar mountain range, South-eastern Brazil

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Paleo-drainages' connections (PDC) and headwaters stream capture (HSC) are the two main historical processes shaping the distribution of strictly freshwater fishes. Recently, GIS-based methods to reconstruct paleo-drainages have enabled new possibilities of investigation on how both PDC and HSC have shaped the genetic structure of strictly freshwater fishes. In this context, the present study used PDC's reconstructions and single-locus cluster delimitation analyses to examine the genetic structure and population dynamics of Pareiorhaphis garbei, a 'near threatened' catfish from the Fluminense ecoregion, South-eastern Brazil. Sequences of mitochondrial cytochrome c oxidase subunit 1 (COI) and cytochrome b (cytb) gene were acquired from five sampling sites belonging to four coastal drainages: Macaé - KAE, São João - SJO, Guapi-Macacu - GAC, and GMI, and Santo Aleixo - SAL (only cytb). Ten haplotypes were found, none shared between localities. Coalescent-based delineation methods (sGMYC, mGMYC, bPTP, mPTP), as well distance based methods (ABGD), revealed five genetic clusters corresponding to each sampling site. Paleo-drainages' reconstructions showed two putative paleorivers: an eastern, connecting KAE and SJO, and a western one, merging in the Guanabara bay (GAC, GMI, and SAL). There are, at least, two disagreements between past riverine connections and current genetic structure. The first regards SJO being more closely related to populations from Guanabara paleo-river than to KAE population, which belongs to the same paleo-river system. The other concerns GMI, which besides belonging to the same drainage as GAC is indeed more closely related to SAL, an adjacent basin. These discordances between PDC and phylogenetic structure may indicate ancient HSC events in headwaters of Serra do Mar region. Furthermore, all analyses, including p-distance, showed high divergence of KAE from other linages (5.1-5.3% for COI and 7.5-8.0% for cytb), suggesting that specimens from other basins may represent a different species. If this is confirmed, P. garbei would be restricted only to Macaé river basin, since it is its type-locality. In this region, impacts such as invasive species (rainbow trout) and habitat loss have been identified, which can be especially threatening for species with restricted distribution. The p-distance between SJO and Guanabara bay drainages were also high (2.3% for COI and 3.6-4.4% for cytb), suggesting an ancient split and also possibly another cryptic species. However, further morphological and molecular analyses are required to define if P. garbei indeed represents a species complex and, if so, which historical processes regulated the dynamics and distribution of these restricted distributed freshwater catfish species. Our results suggest that headwaters and coastal plains' freshwater fish species might have different biogeographical patterns in Serra do Mar mountain range.

Key words: Biogeography, Atlantic Forest, phylogeography, conservation genetics, molecular systematics Financial support: SMQL was awarded a Science without Borders (Ciência sem Fronteiras) post-doctoral scholarship from CNPq (PDE 203476/2014-0)



### Phylogenetic and taxonomic approach of the genus Trichomycterus (Siluriformes: Trichomycteridae) of the Magdalena-Cauca basin, Colombia

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The current state of knowledge about biological diversity implies two aspects: the large number of groups that differ from others and the presence or absence of unique characters in the organism. Studies point out that current diversity accounts for only 1% of all biodiversity in history, as millions of species have been extinct over billions of years of biological evolution. The phylogenetic systematics emerged to understand processes and present a general system of reference on the origin of biodiversity. The Neotropical Region is the most biologically diverse on Earth. The ichthyofaunal region has enormous potential for description of new species. Estimates indicate that about 100 species are described per decade. It currently has 9100 species described of fish, of which 5150 are freshwater. The freshwater fishes are distributed in 20 orders, 69 families and 739 genera. The Siluriformes are the order of greatest diversity and Neotropical distribution of the Ostariophysi, having 36 families, 478 genera and more than 3200 species. Characiformes and Siluriformes are the most representative orders. Trichomycteridae is among the largest catfish families of the Neotropical Region, after Loricariidae with approximately 300 species and 41 genera being Trichomycterus the most representative genus with 173 valid species. The Trichomycterus genus problematic is questioned in several studies mainly due to the absence of synapomorphies that make it difficult to study relationships. The Magdalena-Cauca basin is the fifth largest in South America covering an area of 357 000 km<sup>2</sup> having 36 families, 96 genera and 205 species of fish. The system was formed approximately ten million years ago from the elevation of the Eastern Cordillera causing the geographical isolation of many species. The aim of this work is to disseminate partial results of the PhD thesis on phylogenetic systematics of Trichomycterus from Colombia. To that end, we compiled morphometric, meristic and osteological data of the species Trichomycterus striatus, T. bogotensis, T. cachiarensis, T. banneaui, T. ruitoquensis, T. spilosoma, T. chapmani, T. caliensis and T. taenia Magdalena-Cauca basin, Colombia. This group is expected to be phylogenetically more closely related to geographic barriers. The results indicated the Trichomycterus genus as a non-monophyletic aroup corroborating with information from the literature. This fact may be related to the genus possessing a very great diversity and distribution, being able to lead to the geographic and genetic isolation of certain populations, evidencing non-monophyletic. According to phylogenetic analysis, the Trichomycterus species of the Magdalena-Cauca basin form sequential basal groups within the idea of transandine ancestry, corroborating the hypothesis of this study. Plesiomorphic characters such as morphology of thin and long mesethmoid rinds with triangular base wider than its end, orientation of the sphenotic spine directed laterally, dentary tightly joined together forming a symphysis, single process in the stem of the anteroventral portion of the operculum and developed metapteryaoid were evident in the species of the genus with distribution to the Magdalena-Cauca basin. Thus the phylogenetic and distribution patterns generate a strong relation with the biogeographic history of the continental margins of South America.

Key words: Catfishes; Systematic; Osteology, Neotropical Financial support: Partially funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico -CNPq (Process #302877/2015-0) 170



### Sexual dimorphism of Ancistrus brevipinnis (Regan, 1904) (Siluriformes: Loricariidae)

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The genus Ancistrus, which belongs to the family Loricariidae is the most numerous genus of the tribe Ancistrini, with 70 valid species widely distributed in the Neotropical region. The more conspicuous synapomorphies of the genus Ancistrus are the presence of cutaneous tentacles disposed over the bare margin of the snout and the presence of retractable dermal denticles in the opercular region. The group is complex taxonomically, making identification of specimens difficult. Even in the presence of some polymorphisms, species of that genus are generally identified based on sampling locality. The original description of Ancistrus brevipinnis (Regan, 1904) lacks detailed information and little data is available in the literature, thus a revision of that species is necessary. In any species revision process, it is essential that sexual dimorphism characters are identified since, for many species, some sexual dimorphic characters can be so striking to the point of confusing them with interspecific differences. Thus, the goal of this study was to identify the sexual dimorphism characters of A. brevipinnis, in order to help in subsequent taxonomic studies. All analyzed specimens are deposited in the Fish Collection of the Science Museum of PUC-RS and in the Reference Collection of the Ichthyology Laboratory of UNISINOS. With the use of a 0.1 mm precision digital caliper, 30 measurements of 44 specimens (19 males and 25 females) were obtained. All data was normalized and with the use of PAST program, a Major Component Analysis was carried out in order to identify the possible ordination pattern within the groups. Subsequently, 18 specimens (5 males and 13 females) were cleared and stained in order to enable the visualization of the bone structures. Afterwards, the analysis of the internal and external morphology was undertaken using a stereoscopic binocular microscope. Sexual dimorphism in characters of external morphology of the species, such as the width variation of the bare margin of the snout, the orientation of the hypertrophied dermal denticles, the lack of infraorbital first plate in males, the disposition of "Y" shaped cutaneous tentacles of the bare margin of the snout in males, the lack of dermal denticles in portions of the parieto-supraoccipital process in females, and larger dermal denticles of the pectoral and pelvic fin spine in males were identified, illustrated and described. No relevant morphometric variations among males and females of that species were found. The present study contribute to the taxonomic knowledge of A. brevipinnis, since the delimitation of sexual dimorphism characters is essential for the identification process and for description of new species.

Keywords: Fish; Taxonomy; Freshwater; Neotropical

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## Ontogenetic study of the large trahira Hoplias intermedius (Günther, 1864) (Characiformes: Erythrinidae)

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The large trahira, Hoplias intermedius, is a predatory species that occurs in the basins of rio São Francisco, upper rio Paraná and affluents of rio Doce in the state of Minas Gerais, Brazil. It belongs to the Erythrinidae, a family defined by four synapomorphies: presence of five branchiostegal rays, a lamellar suprapreopercle, first infraorbital bifurcated anteriorly and antorbital absent. This study aims to document the main morphological aspects in the initial development of Hoplias intermedius that will serve as a basis to access relevant systematic issues in the ontogenetic context. The study was based on 52 specimens of Hoplias intermedius obtained through fish farming. Of these 52 specimens, 28 were cleared, double-stained and preserved in alycerin 70%. The other 24 specimens were whole specimens preserved in alcohol 70%. Description and photographic documentation were realized with the aid of a Zeiss stereoscope, connected to an Axiocam 105 Color digital camera and a software with Z-stack option. For comparative effects, an adult specimens of Hoplias aff. malabaricus was skeletonized using dermestes larvae. The ontogenetic series includes pre-hatching stages (e.g., eggs) of 2.1 mm in diameter and larvae with notochord length (NL) of up to 8.9 mm. The opening of the mouth occurs at 5.0 mm NL. The first tooth in the dentary is present at 6.3 mm NL, in the anterior region of this bone. The first tooth in the premaxilla is present at 6.6 mm NL, also in the anterior region of the bone. At 7.5 mm NL, the specimens analyzed present approximately 11 teeth in the dentary and 8 teeth in the premaxilla. All teeth are exclusively conical and the emergence of additional teeth occurs antero-posteriorly along the ontogeny. The reduction of the yolk sac is more conspicuous from 8.2 mm NL and at 8.8 mm NL the sac is fully absorbed. Inflexion of the notochord begins at 8.2 mm NL, and at 8.9 mm NL the inflexion seems complete. The pectoral-fin bud is evident at 6.7 mm NL, with no fin rays. Differentiation of caudal-fin rays begins at 8.0 mm NL. The first anal-fin rays are observed at 8.2 mm NL, however, they do not develop considerably until 8.9 mm NL. The first rays of the dorsal fin arise at 8.9 mm NL. The temporal upper limit of the ontogenetic series studied herein (e.g., maximum length: 8.9 mm NL) has not allowed access to the development of pelvic-fin buds and pectoral-fin rays, something that should be addressed with better sampling of older specimens.

Key words: Larvae, Early Life History, Wolf-fish Financial support: CNPq (Process 120909/2017-0)



## A new species of *Leporellus* Lutken, 1875 (Characiformes: Anostomidae) from the Arapuanã river in Brazil

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Anostomidae is the most species rich family of Characiformes excluding Characidae, with more than 150 species currently listed as valid. As other groups of Neotropical fishes, the diversity of Anostomidae is yet far from fully understood. The family includes 15 genera, and is considered to be composed of two clades, one exclusive of Leporellus and another including all other anostomid. Leporellus possesses five nominal species, which are indistinguishable based on current knowledge: L. cartledgei, L. pictus, L. retropinnis, L. sexdentatus, and L. vittatus. These species are often treated as synonyms in the literature, as Leporellus vittatus being the older and preferred name. The present contribution aims to describe a new species of Leporellus using morphological data, and comparing it to congeners. Specimens were illustrated using digital photograph, measured using digital calipers, and had teeth, scales and fin rays counted under a stereomicroscope. The morphological data, partially summarized in tables, were compared to original data obtained from including types, topotypes and other specimens of all nominal congeners. A distribution map was elaborated using Quantum Gis. The new species is distinguished from congeners by having 12 scale rows around the caudal peduncle (vs. 16). Additional features that diagnose the new species among anostomid include: 42 or 43 scales in the lateral line, five scale rows between dorsal fin and lateral line and four between lateral line and pelvic fin, caudal-fin rays extensively covered by diminutive scales, caudal fin with five longitudinal dark stripes, dorsal with a median dark blotches, subterminal mouth with four truncate teeth on each jaw. The new species is apparently endemic to the upper portion of the Arapiauna river, above the Dardanelos falls, an area where several species are known to be restricted to. The importance of the number of scale rows around caudal peduncle as a diagnostic feature in Anostomidae is discussed.

Key words: Neotropical, Taxonomy, Systematics Financial support: CNPq, CAPES



### Comparative morphology and evolution of the brain in the family Cetopsidae, with phylogenetic implications (Teleostei: Ostariophisi: Siluriformes)

Vitor P. Abrahão, Mário C. C. de Pinna

The Neotropical Souht American catfish Cetopsidae is a family of Siluriformes that includes five genera and 42 recognized species, occurring over a large portion of northern and central regions of South America, to both sides of the Andean cordilleras. The monophyly of the family is supported by several previous studies, both morphological and molecular, however its internal relations require further clarification because there are some not monophyletic clades. Despite the scarcity of evolutionary studies on the nervous system of the neotropical catfish, this complex shows great variation potentially informative and promising to phylogenetic studies. To test the informative power of these characters, the gross morphology of brain subdivisions in the catfish family Cetopsidae is described, illustrated and interpreted. For this, the specimens should be only cleared and counterstained for bone and cartilage and the brains were removed from braincase. An ellipsoid model was used to determine the volume of each brain region. A comprehensive comparison based on shape, relative position, and volume of the main brain subdivisions is presented for representative species of all genera and most available species in the family. Comparisons with other phylogenetically related siluriform families provide a broader context for the understanding of the main evolutionary transformations which shaped the cetopsid brain. Profoundly distinct morphological patterns are identified for each of the two main cetopsid subdivisions, subfamilies Helogeninae and Cetopsinae. Few intraspecific variations on major subdivisions of the brain are presented among all species examined herein. The monophyly of all genera is supported by putative neuroanatomic characters that are in accordance with previously phylogenetic hypothesis. Additional morphological variation is hierarchically interpreted against previously proposed phylogenetic hypotheses. Phylogenetic mapping reveals some recurrent morphological patterns suggestive of an association with specific feeding specializations and other lifehistory traits. These results support the idea that such neuroanatomic characters are useful to phylogenetic analyzes and that morphologic complex is a promising universe to be explored.

Key-words: Comparative neuroanatomy, catfishes, systematics Acknowledge financial support: FAPESP proc. n. 2014/11397-1 and proc. n. 2015/26804-4



# New taxonomic status to *Bryconamericus* exodon Eigenmann and *Knodus moenkhausii* (Eigenmann & Kennedy) and the description of a new species of *Knodus* (Characiformes: Stevardiinae)

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Bryconamericus exodon Eigenmann, 1907 was described from Puerto Max and Assunción, rio Paraguay, in Paraguay, and designated as type species of the genus Bryconamericus. Its current distribution includes the rio Paraguay and the upper rio Paraná basins. Knodus moenkhausii (Eigenmann & Kennedy, 1903) was described as Poecilurichthys moenkhausii from a brook near arroyo Trementina, rio Paraguay basin, also in the Paraguay. The species has passed through taxonomic adjustments along the years, being recognized as Bryconamericus moenkhausii by Eigenmann (1910) and Bryconamericus (Knodus) moenkhausii by Géry (1964), being nowadays recognized as Knodus moenkhausii sensu Eigenmann (1918). After its original description, K. moenkhausii was not recorded anymore in rio Paraguay basin, however it was identified by Castro, R.M.C. in upper rio Paraná basin in 2005. The types P. moenkhausii are severely damaged, specially the caudal fin, making impossible to verify the presence of scales which were the main diagnostic character from Bryconamericus. We have done an exhaustive taxonomic and phylogenetic analysis in the types and topotypes of P. moenkhausii (= K. moenkhausii) and we verified that the specimens from the type series of Poecilurichthys moenkhausii has misaligned pentacuspid teeth (vs. aligned and heptacuspid teeth in the Knodus species from the upper rio Paraná). Furthermore, those tooth characters, measurements and counts, evidenced that the type series of P. moenkhausii are actually specimens of Bryconamericus exodon (the type species of genus). Therefore, we recognize Poecilurichthys moenkhausii as a member of Bryconamericus and senior synonym of Bryconamericus exodon. The species of Knodus from the upper rio Paraná, rio São Francisco, rio Doce and rio Jequitinhonha basins, so far identified as "Knodus moenkhausii", is actually a new species of Knodus. Those species are diagnosed by the following characters: "Knodus" moenkhausii, from the rio Paraguay basin, has dark and naked caudal-fin lobes, misaligned teeth in the inner row of the premaxilla, pentacuspid teeth on the maxilla, one vertically humeral spot, 20-24 anal-fin branched rays, and 34-40 pored scales in lateral line; Knodus sp. n. present scales over the base of the caudal fin, squamation more developed on lower caudal-fin lobe, five rows of scales between the lateral line and the dorsal-fin origin, 18-21 branched anal-fin rays, heptacuspid teeth in the inner row of the premaxilla and heptacuspid teeth on the maxilla.

Key words: New species, Systematics; Poecilurichthys; Freshwater; Neotropical

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### Systematics of Hyphessobrycon Durbin, 1908 (Teleostei: Characidae)

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Hyphessobrycon Durbin, 1908 is the second most species-rich genus in Characidge, currently with about 150 valid species. The original diagnosis of the genus, improved by Eigenmann almost one century ago, is still in use and is given by a combination of characters non-exclusive to the genus. The merophyletic nature of the genus has been discussed since Eigenmann. Weitzman & Palmer have proposed a possible monophyletic group for Hyphessobrycon referred as "rosy tetra clade", based on characters present in the type species, Hyphessobrycon compressus, and other 25 characid species. The monophyly of the "rosy tetra clade" was tested based on a more encompassing analysis than that of Weitzman & Palmer, adding new taxa and characters to the phylogeny of Characidae sensu Mirande. The hypothesis of phylogenetic relationships was accessed using equal weighting and implied weighting parsimony analyses, using 227 taxa (including 42 species of Hyphessobrycon) and 391 characters of external morphology, osteology, myology and spermatozog ultrastructure. Hyphessobrycon was found as a monophyletic aroup [now treated as Hyphessobrycon stricto sensu (s.s.)] in both equal and implied weighting parsimony analyses, and was limited to 25 species in Characidae, defined by the following non-ambiguous synapomorphies: anterior paired projections of parasphenoid absent; conspicuous dark spot on dorsal fin present; ventral portion of the third infraorbital canal contacting the laterossensorial preopercle, and longitudinal stripe on the flank absent. The Hyphessobrycon sister groups is composed by 'Hyphessobrycon' tortuguerae and Parapristella georgiae, respectively. The other Hyphessobrycon species will be treated as Hyphessobrycon lato sensu (s.s.) and will be phylogenetically restructured and realigned into a broad Characidae classification.

Key words: 'rosy tetra clade'; Classification; Pristellinae; Morphology Financial support: CNPq and FAPESP (process 2011/11422-8; 2012/03404-2)



# Revised taxonomic standards for the systematics of *Trichomycterus* (Siluriformes: Trichomycteridae), based on an evaluation of phenotypic traits and COI data in an exceptionally large sample

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Trichomycterus is one of the largest genera of neotropical fishes and new species continue to be described at a fast rate. However, the validity of many old and not-so-old names is doubtful due to various factors. One of them is a persistent tradition of heterogeneous (or vague) taxonomic and descriptive standards. That fact, plus normally incomplete assessments of intraspecific and ontogenetic variation, make an understanding of Trichomycterus systematics one of the most daunting tasks in neotropical ichthyology. This study proposes a reassessment of morphological descriptive standards for Trichomycterus taxonomy. As a baseline, we performed morphological and molecular analyzes on an exceptionally large sample (730 ex) of Trichomycterus maculosus Barbosa & Costa, 2010, deposited in MZUSP collections. External and internal morphological data (pigmentation, osteology, meristic and morphometric) were investigated for possible covariation and contrasted with Cytocrome Oxydase Sub-unity I (COI) data. Three well-defined color patterns were identified in the sample, but they do not match variation observed in other morphological traits. DNA barcoding analysis does not refute the hypothesis that the sample belongs to a single species and thus supports all variation observed as intraspecific variation. Availability of a large sample allows the identification of low-frequency specimens which are phenotypically intermediate among apparently welldefined clusters that might otherwise be mistaken for distinct species. The detailed mapping of such variation in a large sample allows a clear picture of the range and pattern of intraspecific variation minimally expected in Trichomycterus. The present case can serve as a model of expected potential variation in the genus. We combine our results on intraspecific variation with a new proposal of taxonomic standards for descriptive work on Trichomycterus taxonomy, which hopefully will remedy the chronic and long-standing descriptive confusion mentioned above. Ultimately, this will help to prevent descriptions of new species based on taxonomically unreliable features and thus avoid taxonomic inflation and resilient proliferation of synonyms in Trichomycterus.

Key words: Standardization, Catfish, Taxonomy, Freshwater, Neotropical Financial support: FAPESP: 2016/25467-7



### Anatomy and morphology of *Trichomycterus taenia* Kner, 1863 (Siluriformes: Trichomycteridae) from Colombia's Pacific Coast

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The genus Trichomycterus is the most representative within its family Trichomycteridae, having a wide geographical distribution, from Costa Rica to Patagonia. Currently, this genus contains 173 valid species. In 1863, Kner described Trichomycterus taenia based on collections on the western slope of the Andes in Ecuador. This species appears to be distributed along the Pacific coast from Colombia to Peru. The description was published with a limited amount of characters such as reduced eyes, a truncate caudal fin, and a dark lonaitudinal band extending from the head to the caudal fin. The holotype was deposited in the old collection of the Zoologische Staatssammlung München (The Bavarian State Collection of Zoology), in Germany, which was destroyed in World War II. Therefore, the objective of the present work is to describe morphologically and anatomically the species T. taenia, in views of obtaining additional diagnostic characters, in order to make possible a re-description of the species in the future. Morphometry and meristics were performed, as well as morphological and osteological characterization of 17 specimens of Trichomycterus taenia from the Patia River basin, in Cauca department, in the Colombian Pacific coast. The material examined belongs to the scientific collections of the Colombia. Trichomycterus taenia (59.44 - 116.98 mm SL) is easily distinguished from its congeners by having a dark horizontal band in the lateral region of the body that extends from the operculum to the caudal fin. Additionally, it has 116 conical teeth in the premaxillary, distributed in 4 rows of teeth and 104 teeth in the bands of teeth divided into 3 bands, 39 total vertebrae, 13 inferior and 18 superior procurrent rays. Insertion of the first ray of the dorsal fin in the vertebra 20 and insertion of the first ray of the anal fin in the vertebra 25. Pectoral-fin rays i,7; dorsal-fin rays ii,7; pelvic-fin rays i,4; anal-fin rays ii,5; caudal fin rays 14. 13 pairs of ribs. 30 opercular dermal denticles and 39 interopercular dermal denticles. It presents a unique process in the stem of the antero-ventral portion of the operculum, reduced or absent metapterigoid and 1-2 vertebrae between the first pterygiophore of the dorsal fin and the first pterygiophore of the anal fin. In addition to the meristic and osteological data presented, T. taenia has a maxillary barbell that extends to the tip of the pectoral fin, a nasal barbel that cannot reach the opercular spines, the length of the pelvic fin rays reaching the anus, head wider than long, and the length of the pectoral rays equal to the length of the eye and the post-orbital part of the head. However, an in-depth description of the species and its redescription, wherever necessary, is of utmost importance in order to contribute to the increase of information and diagnostic characters to better understand the biodiversity of the species and its geographical distribution and conservation.

Keywords: Taxonomy; Catfishes; Colombia; Anatomy

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### Phylogenetic relationships of fossil and living species of *Steindachneridion* (Siluriformes: Pimelodidae) of the Paraíba do Sul river

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Steindachneridion Eigenmann & Eigenmann, 1919 is a genus of large Neotropical catfishes with a basal phylogenetic position in the Pimelodidae family, and it has been placed as a sister group for all other Pimelodidae genera. Six species compose this genus, Steindachneridion amblyurum, described from the Jeguitinhonha River; Steindachneridion parahybae, from the Paraíba do Sul River; Steindachneridion doceanum, from the Doce River; Steindachneridion scriptum and Steindachneridion punctatum, from the Uruguay River; and Steindachneridion melanodermatum, from the lower Iguaçu River. Besides these living species, two more fossil species are also described for this aenus, Steindachneridion iherinai and Steindachneridion silvansantosi, both from Paraíba do Sul River basin, at the Tremembé formation, dated from the upper Oligocene. The first fossil species described was S. iheringi, originally included in the genus Arius, and then transferred to Steindachneridion, which has several characters distinguishing from the other congeneric species. In turn, S. iheringi is diagnosed by the presence of a very ossified dorsal spine and anterior and median pit-lines on skull roof. Aiming to test the hypothesis that S. parahybae is more closely related with one of the fossil species than to the living congeners, we present a phylogenetic analysis based on 12 discrete morphological characters for all known species belonging to the genus Steindachneridion from the Paraíba do Sul River basin, plus S. scriptum, and Zungaro zungaro as out group. The most parsimonious tree, with consistency index of 0.933 and retention index of 0.857, shows the following phylogenetic relationship: (S. silvasantosi (S. iheringi (S. scriptum, S. parahybae))). In the S. silvasantosi description, the author pointed different shapes for the vomerian plates of the Steindachneridion species here analyzed, but such hypothesis was not corroborated by the present study, since the shape of such structure in S. iheringi compared to S. parahybae is very similar. Regarding the characters used to distinguish S. *iheringi* from its congeners, the dorsal-fin spine in S. parahybae is so ossified as in S. iheringi, and the anterior and median pit-lines on skull roof were also observed in S. parahybae and S. scriptum, showing that the diagnosis for the fossil species is inconsistent and should be revised. Despite this, the present phylogenetic analysis corroborates that fossil species of Steindachneridion from Paraíba do Sul River basin, S. silvasantosi and S. iheringi, are basal in the group and S. parahybae belongs to a more recent lineage.



### Morphometric and meristic variation of the armored catfish Ancistrus brevipinnis (Siluriformes: Loricariidae) from the Patos Lagoon system, Rio Grande do Sul, Brazil

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Ancistrus brevipinnis is a freshwater fish, endemic to the Patos Lagoon system, which occurs in all system watersheds located in the state of Rio Grande do Sul, Brazil. The species type locality is uncertain and its diagnosis and description are poorly detailed. For these reasons, traditionally, all specimens of the genus in that system are identified directly as A. brevipinnis, even when some polymorphisms are detected. Facing the taxonomic problem related to this species, partial results of a Master's dissertation are presented, which propose the species taxonomic review through morphometric, morphoanatomical and genomic analyzes, where preliminary results related to morphometric variation in populations of this aroup are described. A total of 120 specimens from five different river basins (Sinos River, Gravataí River, Vacacaí-Vacacaí/Mirim River, Alto Jacuí and Mirim-São Goncalo basins) deposited in the Museu de Ciências e Tecnologia, Pontíficia Universidade Católica do Rio Grande do Sul (MCP) and in the Ichthyology Laboratory reference collection, Universidade do Vale do Rio dos Sinos (UNIC) were analyzed. Only specimens measuring 40mm standard length (SL) or more, considered as adults, were chosen for analyzes. On the left side of each specimen, 24 linear measurements were taken using a precision digital caliper. Descriptive morphometric and meristic tables were elaborated. Morphometric characters are expressed as percents of SL, except for subunits of the head, which are expressed as percents of head length (HL). The measurements were transformed into decimal logarithms in order to normalize the distribution and homogenize the data variances. In addition, Burnaby's isometric correction was applied for size effect reduction. Differentiation among populations was evaluated using Principal Component Analysis (PCA) on the R platform. Scatterplot graphs generated from the ordination analysis demonstrate the variation among populations due to morphological variation. The first component explained 46% of the variation and the second 15%. The most influential measures in the analysis result were related to the caudal peduncle, these being the distance between the dorsal-fin base and the adipose-fin origin, length of caudal-fin spines and distance from the adipose-fin base to the caudal-fin origin. It was observed a separation between the Sinos River basin population and the others by the axis of the principal component 1 (PC1). Using advanced morphometric techniques is essential to quantify differences between fish populations, detect differences between groups and differentiate morphologically similar species, since information on the biology and population structure of any species is a prerequisite for developing management and conservation strategies, especially in insufficiently studied groups, such as the genus Ancistrus. Analyzes using aeometric morphometrics data are being performed to evaluate which analysis is the most effective for identifying groups, considering that the literature indicates that geometric morphometrics improves discrimination by shape variation.

Keywords: Ancistrini, multivariate morphometrics, taxonomic review

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### Molecular phylogeny and biogeographic history of the Neotropical tribe Glandulocaudini (Characiformes: Characidae: Stevardiinae)

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The tribe Glandulocaudini comprises three genera, Lophiobrycon, Glandulocauda, and Mimagoniates, and ten species, distributed in freshwater environments of eastern and southern Brazil, Paraguay, and northeastern Uruquay. Its members include small Neotropical fishes, whose degree of morphological and behavioral specialization, as well as the distributional patterns of the species are of great importance for evolutionary studies and understanding of biogeographical patterns of South American freshwater fishes. Although studies on systematics and biogeography carried out represent considerable progress on the knowledge of the Glandulocaudini, none was grounded on molecular evidence. Thus, we proposed herein the first hypothesis of relationships for the tribe based on phylogenetic analysis of DNA sequences. We used maximum likelihood (ML) and Bayesian (IB) methods to infer the phylogeny of Glandulocaudini, using two mitochondrial (16S rRNA and COI) and one nuclear (RAG2) genes to generate a concatenated matrix of 1,829 base pairs. In this matrix, all genera and 80% of the valid species of the tribe (missing only Mimagoniates barberi and M. pulcher) were included besides the outgroup (representatives of all the other tribes of the Stevardiinae, Argopleura chocoensis, considered incertae sedis in the subfamily, and three species of non-Stevardiinae characids). DNA sequences of Glandulocauda caerulea and Mimagoniates sylvicola were analyzed for the first time. A coalescentbased species tree approach was implemented using the sequence-based method \*BEAST. Also, two fossil calibration points and relaxed-clock Bayesian analyses were used to estimate the origin of the Glandulocaudini and the approximate timing of cladogenetic events within the group. Our analyzes recovered Glandulocaudini as monophyletic with strong statistical support, as previously suggested by morphological and other molecular studies. The phylogenetic hypotheses inferred by the IB and ML methods are identical to relationships within the Glandulocaudini, but differ on the proposal of its sister-group. According to the former, Glandulocaudini is sister-group of Stevardiini, but according to the hypothesis inferred by the ML method, this tribe is more related to (Hemibryconini (Creagrutini, Diapomini)). No hypothesis recovered Glandulocauda as monophyletic, since G. melanopleura is proposed as sister-group of Lophiobrycon weitzmani while G. caerulea is more related to the Mimagoniates species, which form a monophyletic group. The relationships within the genus Mimagoniates were resolved and the results indicated (M. inequalis, M. rheocharis) as sister-group of M. lateralis and this clade as related to (M. microlepis, M. sylvicola). The molecular clock results indicated an origin of the Glandulocaudini during the late Miocene and the estimated diversification dates in the group were within the Neogene (Miocene and Pliocene) to Pleistocene (10.8–1.4 million of years ago). These results plus the available geomorphological information suggest the origin of the Glandulocaudini in the Brazilian crystalline shield, from which possibly several dispersal events took place to adjacent river basins during the Neogene. Thus, the occupation of the Atlantic Coastal drainages was posterior and probably due to several river capture events associated with strong tectonic activity during the Tertiary. Apparently, Pleistocene sea-level fluctuations also influenced the current distribution pattern of some glandulocaudin species, especially within the Mimagoniates genus and at the population level.



#### Integrative taxonomy of *Astyanax* Baird & Girard from the rio Paraguaçu basin, Bahia, Brazil

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Delimiting species taxonomically is a crucial step in the process of building biological knowledge. In some taxa, especially in groups of species with recent divergence, it can be a very complex task. In these cases, the traditional morphological methods may be not elucidative and the use of an integrative taxonomy approach is helpful. Astyanax is considered one of the most taxonomically complex group of the family Characidae, with difficult species delimitation mainly due to its high diversity, overlap of morphological characters between lineages, and unknown distribution of lineages. Particularly to the rio Paraguacu basin, more than ten species of Astyanax has been cited in the literature, the great majority not identified at specific level. In the present study, an integrated taxonomic analysis was performed, based on morphological and molecular data, to delimit biological units. The biological material used is from the ichthyological collections of Museu de Zoologia da Universidade Federal da Bahia, Museu de Zoologia da Universidade de São Paulo, and Museu Nacional, Universidade Federal do Rio de Janeiro. A total of 431 specimens were examined for morphological analyses, focusing on color, meristic, and morphometric features, besides the sexual dimorphism analysis of the specimens. The preliminary results reveal that beyond the three endemic species of the rio Paraguacu basin, named A. epiagos, A. hamatilis, and A. brucutu, ten different morphotypes can be proposed, considered herein as initial hypotheses of species. To the molecular analysis, 141 specimens of Astyanax had their total DNA extracted, 110 had the mitochondrial gene COI amplified, and 58 have already been sequenced. A total of 1,300 sequences archived in Genbank® were used in the analysis in addition to the 58 sequences cited. The maximum likelihood method was used to propose a hypothesis of relationships for Astyanax and revealed two highly distinct radiations in the rio Paraguacu basin, each one with a series of distinct lineages. Apparently, one of the two radiations is more restricted to the high course of the basin and comprises four distinct lineages, and the other is widespread along the basin, with lineages occurring in sympatry with the first radiation. The analysis of DNA Barcoding also corroborated the two large radiations and the lineages within them. A preliminary analysis comparing morphological and molecular results reveals partial agreement in delimiting of lineages. The molecular data will be complemented to enable the use of molecular methods for species delimitation (i.e., Bayesian Poisson Tree Process (bPTP), Generalized mixed Yulecoalescent model (GMYC)) and more refined integrative study will be provided.

Key words: Chapada Diamantina; Morphological characters; Molecular data Financial support: CAPES, CNPq



### Phylogeography of species of the genus *Hypostomus*: standards in the karyotype and molecular variation

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The Hypostomini tribe is composed of the genus Hypostomus, and the possibility that this genus consists of some monophyletic groups is suggested from the presence of significant morphological variation and several diploid numbers and karyotypes formulas. The objective of this work was to trace the phylogeography of various Hypostomus species and analyze the genetic diversity of several populations of Hypostomus ancistroides, with subsequent identification of the relationship between chromosomal and molecular variations present in this group. Phylogenetic trees for all species were inferred using Maximum Likelihood and Bayesian analysis, while a haplotype network was built for the species H. ancistroides using the Median Joining method. Network analysis revealed 18 haplotypes and the formation of two haploaroups for H. ancistroides, the first deriving from the Paranaíba river basin and the second from all other basins investigated, namely Tietê, Paranapanema and Grande. Phylogenetic trees combined with the available gendered chromosomal data showed the formation of three haplogroups by Bayesian analysis for all species considered. Here, the first group was formed by H. ancistroides belonging to the Tietê and Paranapanema river basins, and virtually all individuals exhibited 68 chromosomes. The second group was formed by various species from the Paranaíba and Grande rivers, which exhibited 72, 74, or 76 chromosomes. The third haplogroup was formed only by H. faveolus with 64 chromosomes. The presence of high diploid numbers as well as a large number of subtelocentric and acrocentric chromosomes relative to complement and multiple nucleolus organizer regions (NORs) suggested that Hypostomus derives from the family Loricariidae. Phylogenetic analyses also confirmed chromosomal symplesiomorphy between Hypostomus species, while morphological, molecular, and karyotypic data supported the existence of at least two groups within the genus Hypostomus.

Key-words: Bayesian, Haplotype network, Maximum Likelihood Financial support: CAPES (Fellowship for M.Sc. degree) and CNPq (process 484626/2013-2)



## A new species of *Hemiodus* Müller (Teleostei: Characiformes: Hemiodontidae) from Rio Xingu, Brasil

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Hemiodus is the largest genus in the family Hemiodontidae containing 21 valid species. The genus is defined by a single shared synapomorphy: the presence of a ventrally angled depression on the medial surface of the preopercle, which receives the posteroventral portion of the hyomandibular. Additionally, the genus is diagnosed from other hemiodontids by: presence of small multicuspid teeth in the upper jaw (vs. teeth absent in Anodus; unicuspid and pedicellate in Micromischodus; and tricuspid in Argonectes and Bivibranchia). In this study, a new Hemiodus is described from the Rio Xingu. The new species can be readily distinguished from its congeners, except H. ternetzi, H. thayeria and H. tocantinensis, by the presence of a dark longitudinal stripe extending from behind the head to the tip of the lower caudal-fin lobe (vs. dark longitudinal stripe absent in H. amazonum, H. argenteus, H. huraulti, H. immaculatus, H. iratapuru, H. jutuarana, H. langeanii, H. microlepis, H. orthonops, H. parnaguae, H. guadrimaculatus, H. sterni, H. vorderwinkleri and H. unimaculatus; or variably originating near vertical through dorsal-fin to the end of the lower lobe of the caudal fin in H. atranalis, H. gracilis, H. goeldii and H. semitaeniatus. Hemiodus sp. n. differs from H. thayeria and H. ternetzi by the presence of a dorsal-fin oblique blotch (vs. blotch absent). The new species is diagnosed from H. ternetzi, H. thayeria and H. tocantinensis by the higher number of scales rows above lateral line (12–14 vs. 9–11, 6–8 and 8–9, respectively). Additionally, the new species differs from H. thayeria and H. tocantinensis by the higher number of perforated lateral line scales (60–64 vs. 46–49 and 52–54, respectively). Hemiodus sp. n. is further distinguished from H. tocantinensis by the longitudinal stripe originating behind the eye (vs. longitudinal stripe originating on the body), and the higher number of branched pelvic-fin rays (10 vs. 9). Hemiodus sp. n. belongs to the H. quadrimaculatus group by presence four vertical bars in the body. Additionally, Hemiodus sp. n. shares with H. ternetzi, H. thayeria and H. tocantinenses the presence a dark longitudinal stripe extending from head to the tip of lower caudal fin lobe, which would suggest a close relationship among these species. Furthermore, Hemiodus sp. n. share only with H. tocantinensis the presence of a dorsal-fin oblique blotch.

Key-words: Neotropical region, taxonomy, systematics Financial support: CNPq (ISS 148559/2016-6; ALN-F 313404/2015-1; GMD 300066/2016-3)



### Landmark data tested in phylogeny of the Pseudopimelodidae (Teleostei: Siluriformes)

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The relationship between morphometric and phylogenetic analysis has been quite controversial and discussed over the years. Regardless, there is a growing effort to use morphometric characters in phylogenetic reconstruction without discretization. In this sense, the use of landmark data in phylogenetic analyses of Neotropical freshwater fish remains largely unexplored. With the advancement of new techniques for the use of geometric morphometry in systematics, implementations in the TNT program allow the incorporation of quantitative data directly into cladistics analysis. This allows to translate the data obtained from geometric morphometry into phylogenetic characters capable of reflecting homologies in the form of species. Herein the method of Landmark Analysis Under Parsimony (LAUP) was tested in a phylogenetic analysis of Pseudopimelodidae, a monophyletic family of Siluriformes composed of seven genera and 48 species. This analysis establishes ancestral states for a character that changes in two or three dimensions, choosing for each ancestral point the positions that minimize the displacement of landmarks along all ancestral/descendant. That is, the similar landmark positions in different taxa can be explained by common ancestry. The analysis was based on external morphology data, using fourteen discrete characters and three configurations representing different structures. Thirty-eight biologically homologous landmarks were selected for each species, producing the first hypothesis of phylogeny using LAUP in the Neotropical freshwater fish systematics. The influence of dynamic and static alignment and its effects on the improvement of the tree score also was tested using matrix combined with discrete and geometric morphometry data. The data confirmed that the alignment method used may influence LAUP results. In addition, landmark settings from external morphology, are unreliable when treated independently, but provide useful information when combined with other data. A symmetric resampling was necessary for the collapse of the over-estimated nodes revealing that the landmark data aid in supporting the branches. Morphometric analysis using body shape as a character revealed that it is possible to use morphometric characters without being discretized. We have also shown that the landmark data can compose a new character font for the Neotropical freshwater fish systematics.

Key words: Catfish; Systematics; Geometric Morphometric; Morphometry



### The Aspidoras Ihering, 1907 (Siluriformes: Callichthyidae) armoured catfishes: a taxonomic review, with description of seven new species

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Aspidoras was described by Ihering as a monotypic genus harboring the new species, A. rochai. According to Ihering, the genus can be recognized by the presence of the following combination of features: (I) two pairs of dorsolateral body plates between the parieto-supraoccipital and dorsal-fin origin; (II) parieto-supraoccipital nearly hexagonal, with poorly developed posterior process; (III) head conspicuously deep, not depressed, rounded anteriorly and slightly laterally compressed; (IV) scapulocoracoid entirely covered by skin on ventral portion of the body; (V) barbels short, not reaching anteroventral limit of gill opening. Currently, the most accepted diagnosis is the one that was provided by Britto, which is based on the presence of the following synapomorphies: (I) posterior portion of mesethmoid wide; (II) frontal fontanel reduced; (III) supraoccipital fontanel present; (IV) opercle compact; (V) ossified portion of pectoral spine strongly reduced, less than half the length of the first branched pectoral-fin ray. Aspidoras harbors 24 valid species, and is relatively well distributed along Brazilian territory, occurring from São Paulo, its southernmost record, to Ceará. After the taxonomic review provided by Nijssen & Isbrücker 40 years ago, no extensive work to elucidate the taxonomy of Aspidoras was conducted. The main objective of this work is to provide a comprehensive taxonomic review of Aspidoras, redescribing species that were described before 2014, and additionally describing nine new species. The gathered material comes from several Brazilian and foreign collections, comprehending specimens from the states of São Paulo, Mato Grosso do Sul, Goiás, Minas Gerais, Mato Grosso, Bahia, Tocantins, Alagoas, Pernambuco, Pará, Piauí, Ceará and Maranhão. Measures, counts and osteological analysis were performed following proper literature. We improved the diagnosis proposed by Britto, including the following exclusive feature: base of first pectoral-fin branched rays with small laminar expansions on inner margin, forming structures similar to serrations in some specimens. Some new synonymies were proposed: A. eurycephalus and A. taurus with A. albater; A. menezesi and A. spilotus with A. raimundi; A. carvalhoi with A. rochai; and A. microgaleus and A. marianae with A. poecilus. Aspidoras pauciradiatus and A. virgulatus were not considered as Aspidoras and should be allocated in Corydoras and Scleromystax, respectively. Aspidoras sp. 1 is described from the upper rio Araguaia basin, Mato Grosso; Aspidoras sp. 2 is described from the upper portions of the rivers Paraguay and Araguaia basins, Mato Grosso; Aspidoras sp. 3 is described from the rio Itapicuru basin, Bahia; Aspidoras sp. 4 is described from the rio Paraguaçu basin, Bahia; Aspidoras sp. 5 is described from the rio Tocantins basin, Tocantins; Aspidoras sp. 6 is described from the São Francisco and Jequitinhonha river basins; and Aspidoras sp. 7 is described from the lower rio Xingu basin, Pará. An identification key to the species was also provided.

Key words: Aspidoradini; Neoptropical; Osteology; Taxonomy

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#### A new long-snouted species of *Corydoras* Lacépède, 1803 (Siluriformes: Callichthyidae) from the Madre de Dios River basin, Peru, with the first pseudotympanum description for Callichthyidae

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The Callichthyidae are small- to medium-sized armored catfishes, which can be clearly recognized by the presence of two longitudinal series of dermal plates on the flanks. Corydoras, its largest genus, comprises around 170 valid species, being thus the most speciose genus of the Siluriformes. The genus is widely distributed in cis-andean South America, predominantly occurring in the Amazon basin, where more than the half of the known species can be found. The Madeira River is a tributary to the Amazonas River, and it harbors 43 species of Corydoras, which represents nearly one quarter of the species of the genus. The examination of material from some tributaries to the Madre de Dios River revealed a new long-snouted species of Corydoras, which is described herein. Additionally, the analysis of several Corydoradinae species allowed the recognition and description of a peculiar pseudotympanum in this subfamily that was never described for Callichthyidae. The new species can be distinguished from all congeners by having the following combination of features: mesethmoid overall long (vs. short); upper tooth plate with three series of teeth (vs. two); area at the corner of the mouth, ventral to the maxillary barbel, with a fleshy flap (vs. fleshy flap absent); infraorbital 2 contacting compound pterotic by means of a posterodorsal secondary laminar expansion (vs. not contacting); pectoral-spine serrations strongly well-developed (vs. clearly less developed), conical (vs. laminar), and directed towards origin of spine (vs. perpendicularly directed or directed towards tip of spine), and a lonaitudinal dark brown or black stripe along flank midline, which is variably fused with a roundish or irregular dark brown or black blotch on caudal-fin base (vs. blotch and/or stripe absent). The pseudotympanum in Corydoradinae is longitudinally elongated and delimited by the compound pterotic and first dorsolateral body plate; its anteriormost portion is characterized by an irregularly roundish depression on middle portion of compound pterotic, which continues towards posterior margin of lateral opening of swimbladder capsule; the posterior expansion of compound pterotic is basically formed by the postotic main canal, which becomes clearly widened just posterior to the pterotic branch, almost entirely covering the lateral opening of the swimbladder capsule, leaving only a slender area on its dorsal margin, that is covered only by thick layer of skin. Despite the existence of descriptions depicting the peculiar morphology of the postotic lateral line of the Callichthyidae, and the recent advances in osteology of the family, this is the first formal description of a pseudotympanum for the group.

Key words: Laterosensory canal; Osteology; Systematics; Taxonomy

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#### A new genus and species of the Stevardiinae (Characiformes: Characidae) from Paraguay Basin, Central Brazil

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The Characiformes is one of the most diverse orders in the neotropical ichthyofauna. About 65% of its species are included into the Characidae family. Stevardiinae is one of the 13 Characidae subfamilies and its monophyletic status is well-suported by many researchers. Therefore, here we aim to describe a new genus of Stevardiinae and understanding their phylogenetic relationships with other members of this subfamily using cladistics methodology. The taxonomic study was carried out through the examination of 17 samples that underwent 17 measurements and 15 counts. Hypothesis of phylogenetic relationships were elaborated from analysis of 48 terminal taxa and 175 characters with basis on osteology, external morphology, and sperm ultrastructure. The ovary of a sexually mature female was checked by histological analysis revealing presence of spermatozoa. The geographic distribution of the species is the Sepotuba River, Rio Paraguay basin. The phylogenetic study showed that this genus is part of the Stevardiinae family with sister-group relationships to the genera Diapoma, Acrobrycon, Hysteronotus, Pseudocorynopoma, Corynopoma, Pterobrycon, Gephyrocharax, Chrysobrycon, Argopleura, Phenacobrycon, Ptychocharax, latobrycon, Tyttocharax, Xenurobrycon and Scopeocharax. This new genus may be distinguished from other genera included into the Stevardiinae by having scales of equivalent size and development in the ventral lobe of caudal fin both in the male and female individuals, except for Diapoma, Planaltina and Acrobrycon. It differs from Diapoma and Acrobrycon by showing two modified scales in the ventral lobe versus 3-6 modified scales observed in Diapoma, and 4-8 scales in Acrobrycon. Its complete lateral line is what differs it from Diapoma which has an interrupted lateral line. It also differs from Planaltina by having 5 or 6 teeth in the inner row of premaxilla and exhibiting the beginning of the dorsal fin posterior to that of anal fin, while Planaltina has only 2 teeth in the inner row of premaxilla and shows the beginning of dorsal fin parallel to that of anal fin. Sperm ultrastructure is also described for the new genus.

Key words: New Genus Systematics; Freshwater; Neotropical Financial support: CAPES; FAPESP (07/52756-0)



### Taxonomic revision and geographic distribution of two species of the genus *Jenynsia* (Cyprinodontiformes: Anablepidae)

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The ichthyofauna of continental freshwater and coastal seas currently represents about 27% of the world's total fish fauna, with recent estimates exceeding more than 9,100 species. It is subsequently considered the most diverse fish fauna on Earth. The Neotropical Region is known for its vast diversity of freshwater fish, with more than 8,000 species thought to reside there. The Neotropical Region is characterized by its high endemism, a fact that occurs due to its immense density and richness of fish species. Such a level of endemism is seen in less than 12% of the continental surface of the world. Fish have the greatest richness among vertebrates, however, sweet ecosystems are gradually getting more vulnerable, a fact that occurs as a consequence of the uncontrolled use of environmental resources by humans. That way, the sampling of freshwaterichthyofauna, its analysis, composition, and group review, is of paramount importance for the preservation of these taxa. The genus Jenynsia is very controversial and needs studies to uncover its diversity. Jenynsia multidentata and Jenynsia lineata, target species of this study, have historically passed by many changes in their composition. Through the inconsistency in the species classification, the objective of this work was to reveal geographic distribution and to solve taxonomic incongruities of J. multidentada and J. lineata, and verify the existence of morphological variations between these species populations. This study analyzed the amount of 250 females and 165 males, from different hydrographic basins. A Principal Component Analysis and a multivariate analysis of variance by permutations, bifactorial cross, followed by paired tests were carried out in order to assess possible differences between morphological measures from both species in different populations. The Bray-Curtis index was used, being performed from 9,999 random permutations. The meristic data were compared with the data from the description of both species. The coloring patterns and distribution of chromatophores were observed and compared among the populations. The results showed no morphometric and meristic differences between the populations analyzed. The inconstancy in the diagnoses that differ J. lineata and J. multidentada is an aggravating factor in correct differentiation of this species. From the results obtained in the present study, it is not possible to affirm that J. lineata and J. multidentada are different species. In that way, J. multidentada is proposed as a junior synonym of J. lineata. J. lineata species show a large geographic distribution being present from Rio Negro in Argentina to the southeastern region in Brazil. Factors as: high salinity tolerance, anoxic conditions and reproductive advantage, can contribute to explain a higher genic flux between the populations and by consequence a wider distribution pattern presented by Jenynsia lineata.

Keywords: Taxonomy; Synonymy; Morphometry; Anatomy

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### Heterochronies and developmental pattern of the neurocranium of three species of Characidae (Teleostei: Ostariophysi: Characiformes)

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The relative change in the developmental timing, or heterochrony, is an important mechanism in evolution, because minor shifts in relative timing of onset, offset or rate of process, may have significant phenotypic effects. The analysis of the order of discrete events in a developmental sequence has proved to be useful in detect heterochronies among taxa, demonstrated when the position of an event within a developmental trajectory change relative to other events. There are few studies aiming to detect heterochronies among fishes, in part due to the difficult in obtain complete ontogenetic series for comparable data. Here I compare the sequence of ossification of the neurocranium of three species of Characidae, Moenkhausia pittieri, Paracheirodon innesi, and Salminus brasiliensis, a large-sized basal species in the family. Skeletogenesis of the neurocranium of M. pittieri and P. innesi was established through analysis of 90 and 97 specimens, respectively, preserved everyday during the first 15 days and every other day from days 16 to 60, maintained at the same conditions. The specimens were measured, clear and stained, in order to detect individual length in the onset of each ossification (a total of fifteen events). Data on skeletogenesis of S. brasiliensis and of other species for further discussion were taken from literature or personal observations. Events were ranked according to the order of ossification. The results show dramatic heterochronies in the onset of ossification of the mesethmoid and pterotic. The mesethmoid is the fifth bone to ossify in the neurocranium of S. brasiliensis whereas it is the last (fifteenth) to appear in P. innesi and M. pittieri. The pterotic is one of the last bone to ossify in the neurocranium of S. brasiliensis (fourteenth) whereas it starts to ossify in early stages of P. innesi and M. pittieri (the ninth bone to appear). Apart from differences, the comparisons revealed sets of bony elements whose ossification sequence appears to be relatively conserved across the family, which is (basioccipital + parasphenoid) - prootic - pterosphenoid - lateral ethmoid - vomer. Species of Nannostomus, Pyrrhulina and Lebiasina (Lebiasinidae, Characiformes) and Danio rerio (Cyprinidae, Cypriniformes) have similar sequence of ossification, with few discrete shifts, whereas Ancistrus cf. triradiatus (Loricariidae, Siluriformes) present a quite distinct sequence of formation of such elements, possibly related to the highly specialized morphological condition of loricariids. Another find is that the miniature Paracheirodon innesi lacks one neurocranium component, the nasal. Bone losses are a common result of the evolution of small body size, in which structures that have late ossification within a lineage are lost by terminal deletion. Bones of the olfactory region of P. innesi and M. pittieri have relative late ossification, even considering the whole skeleton. Therefore, it is possible that heterochronic shifts toward a late formation of the olfactory region within the Characidae, along with terminal deletion, may be the evolutionary mechanism of nasal loss in *P. innesi* and possibly in other miniature characids.

Key words: ontogeny; miniaturization; evolution; ossification sequences Financial support: FAPESP 2009/15075-0, 2011/50282-7



#### Phylogenetic relationships of Copella and developmental truncation in Lebiasinidae (Ostariophysi: Characiformes)

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The genus Copella comprises six small and colorful species distributed in the rio Amazonas and Orinoco basins, and coastal drainages of Guyana, French Guiana, Surinam, and Venezuela. A hypothesis of phylogenetic relationships of the genus is proposed on the basis of the parsimony analysis of 120 morphological characters, including the six species of the genus as ingroup and 17 as outgroup taxa, most of them members of the family Lebiasinidae. Thirty-eight synapomorphies support the monophyly of the genus, of which 13 are exclusive, 19 homoplastic, and six dependent of optimization. Several synapomorphies of Copella are related to jaw modifications, refrecting the typical configuration of the mouth in the genus, such as maxilla strongly curved anteriorly and bearing teeth along its entire anterior border in males, premaxilla with ventral profile gently convex and with lateral tip rounded and toothless. A large amount of synapomorphies for Copella is related to reduction or loss of ossification around sensory canals, bone losses or simplifications. Other shared modifications for the genus are related to the pectoral and pelvic girdles. The result shows that Copella arnoldi is the basal taxa, sister of a clade including the remaining species of the genus. The clade represented by Copella nattereri and C. callolepis is sister of the clade (C. eigenmanni (C. compta and C. vilmae)). Copella is sister of Pyrrhulina, and, along with Copeina, form the subfamily Pyrrhulininae, which is also supported by several bony losses and reductions related to the complexity of the sculpturing of their skeleton. Larger representatives of the family belonging to the genus Lebiasing and Piabucing lie basally. Based on the results of the phylogenetic analysis and on the observation of the ontogenetic series of representatives of Lebiasinidae (species of Lebiasina, Copella, Nannostomus and Pyrrhulina), it is supposed that many morphological traits present in members of the family have evolved through developmental truncation of characters, i.e., they are observed in larval stages of larger related taxa. Character transitions indicative of developmental truncation plotted in the tree topology indicate that bone reductions are specially concentrated within the Pyrrhulininae and in the miniature Nannostomus and uzei. Skeleton simplifications seem to have occurred gradually within Lebiasinidae, resulting in terminal taxa with distinct "levels" of reductive features in their bodies. The presence of developmental truncated characters is not always accompanied by size decrease.

Key words: miniaturization; evolution; systematics Financial support: FAPESP 2014/11911-7; 2011/50282-7



### Genetic differentiation in Neoplecostomus (Hypoptopomatinae, Neoplecostomini) in tributaries of Paraná River

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Neoplecostomus constitutes a fish group known as armoured catfishes, endemics of South American and their representatives are distributed in the south and southeast regions of Brazil. The genus includes currently 16 species described for the Paraná River, São Francisco and Atlantic Coastal basins, being 8 of these belonging to the Paraná River basin and there are evidences of occurrence of new species from this basin. Therefore, this work aims to investigate the genetic divergence in Neoplecostomus from the Iguacu, Itararé, Cinzas and Tibagi rivers with highlight in the biodiversity description. For this purpose, sample collections were performed in four localities: Pinhão river (Neoplecostomus sp. 1), Samambaias river (Neoplecostomus cf. botucatu), das Pedras river (Neoplecostomus sp. 2), and São João river (Neoplecostomus yapo). Twenty one fragments of Cytochrome Oxidase C Subunit I (COI) were amplified and sequenced. The data analysis shows a genetic distance ranging from 4,7% to 9,9%, haplotypic diversity 1,0 and absence of gene flow among studied species. The sequences were also compared with those deposited in the BOLD Systems. According to this comparison, the species from das Pedras and Samambaias rivers presents similarity around 95 % with N. botucatu. The sequences from the São João and Pinhão rivers has similarity above 95% with N. yapo. The analysis of neighbor-joining tree shows some non-consistent branches with bootstrap values ranging from 15 to 97%. In addition, the Bayesian Analysis considering the evolutionary model HKY+G generate by Modeltest, showed four consistent branches. The values of posterior probability ranged from 55 to 100 %. Through the Analysis of Molecular Variance (AMOVA) it was possible to verify a value of  $\Phi_{st}$ =0,33450. Regarding the analysis of the population structuration, no introgression was demonstrated in the BAPS analysis. Hence, two groups: (Samambaias + Pedras) and (Pinhão + São João) were determined. Structure analysis considering K = 2 were observed in the same groups. The attribution likelihood from the sequences was close to 90 %. When considering K = 3, it was observed the group (Samambaias + Pedras), (São João) and (Pinhão), in which some sequences were not defined in distinct groups, mainly between Pinhão and São João rivers. Sequences from Pinhão presented 50-60% of attribution to São João group and sequences from São João river with up to 40% to attribution to Pinhão group. In conclusion, the Bayesian analysis, the haplotypic network and the Fst values shows the absence of gene flow among Neoplecostomus populations and indicates a non-described species for *Neoplecostomus* sp. 1 in the Pinhão river.

Key words: armored catfishes, Cytochrome Oxidase C, population structuration Financial support: CNPq, CAPES, Fundação Araucária, SETI, FAPESP



#### Preliminary inferences of molecular variation and phylogeography of Isbrueckerichthys spp. (Siluriformes: Loricariidae) in First and Second Plateaus hydrographic systems of Paraná State

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The regions from First and Second Plateau of Paraná State have important biologic, historic, geologic, and evolutionary features that evolve during millions of years and present processes that should be investigated and preserved. The hydrographic basins included in these regions are the Upper Ribeira de Iguape River, the Upper Iguaçu River and Paranapanema River. These systems present a fluvial dynamism as a cause from the geologic events, such as rifting and uplifiting processes, erosive retreats, magmatic intrusions, among others. These events had a major impact in the biodiversity, especially in the distribution and in evolutionary events due to interconnection and isolation of headwaters during the geologic time. The aim of this work was established the phylogeographic pattern between Isbrueckerichthys populations among the sample locations that range from the Tibagi and Ribeira de Iguape basins, and to infer geological, evolutionary and historical events through the molecular data arrangement. The data from Cytochrome Oxidase C Subunit I (COI) from 13 individuals of Isbrueckerichthys (5 from the da Areia stream; 3 from the Açungui River - Ribeira de Iguape basin; 3 from the Charqueada River and 2 from the Jacutinga River – Tibagi basin) were sequenced and analyzed. Subsequently, the original sequences were gathered with correlated sequences from Genbank to offer a broader perspective. Nucleotide statistics were inferred with Mega 6 and Geneious 7.1. Phylogenetic analyses were performed using PAUP\*, MrBayes and PhyML. Analysis of Molecular Variance (AMOVA) were performed using Arlequin and haplotypes estimations using DnaSP. For haplotype network construction were used the Network and the TCS softwares. Final alignment contained 485 base pairs of length, from 13 original sequences. The haplotype diversity (Hd) was 1,00 and the Nucleotide diversity ( $\pi$ ) was 0,003. The  $\Phi_{st}$  index considering all population isolated (da Areia, Acunqui, Charqueada and Jacutinga) was 0,67. Although a higher value of  $\Phi_{st}$  (0,73) was obtained when considering the structuration (Charqueada+Jacutinga, da Areia+Açungui). Using the taxonomy tool in Barcode of Life Systems (Bold Systems) databank it was possible to identified individuals from the da Areia and Acungui as I. duseni (average of 99,23% of hit for Acungui specimens and 99,53% for specimens from the da Areia), and specimens from the Chargueada River as I. saxicola (with 99,86% of hit), while the specimens collected in Jacutinga River returned no match hit in BOLD Systems, but were morphologically classified as I. saxicola, and have more similarity with sequences of I. saxicola in less specific databases like BLAST/Genbank. Phylogenetic inferences (Maximum Likelihood and Bayesian analysis) also corroborated the closest relationship between I. duseni from da Areia and Acungui, and the relationship between *I. saxicola* from the Chargueada and the specimens from Jacutinga River. Subsequently, for next steps, we will add more specimens from other locations in Paraná, covering other drainage systems and other Isbrueckerichthys species, with the purpose to investigate a more broadly frame from *Isbrueckerichthys* phylogeography and to infer more accurate assumptions about the geological and evolutionary history of Siluriformes fishes in the context of the basins of Paraná State.



### What DNA Barcode can clarify on the challenging evolutionary question of a widespread Neotropical freshwater fish?

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One of the most current themes of biology involves investigating processes generating the diversification of organisms and elevating them to the category of species. Increasingly analytical methods to delimit species have been implemented, inferring that species can be considered a hypothesis based on data that assume evolutionary independence from other lineages. This evaluation has become extremely important, mainly in regions with high biological diversity that may harbor cryptic lineages. The Neotropical region show the highest level of biodiversity in world in freshwater fish, and understand species limits and processes that underlie diversification of such diversity has been a challenge for biologists. We evaluated presence of independent evolving lineages in the Hoplias malabaricus ("traíra"), one of the few fish species having wide distribution in the neotropics. For that, mitochondrial sequences of COI (DNA barcoding) were obtained and analyzed comparatively with chromosomal and geological data for 150 individuals of 12 locations covering the natural distribution of the specie. We addressed the following questions: 1) Are there genetic lineages of H. malabaricus found in distinct hydrographic basins in Brazil? 2) What are the historical relationships among hydrographic basins and ecoregions? 3) What is the relationship between mtDNA lineages and previously described karyotypes? The magnitude of genetic diversity encountered among different H. malabaricus populations was greater than 2% and we found four cryptic lineages. The recognized cytotypes (based in chromosome number and presence/absence of sexual pairs) did not form monophyletic groups, suggesting that karyotypic macrostructure could be a homoplastic character. The haplotype relationships suggest secondary contacts between the ecoreaions of northern-northeastern Brazil molded by coastal links between adjacent watersheds during the Pleistocene and probable exchanges between their ichthyofaunas. This indicates that multiple factors drive the diversification of H. malabaricus – from ancient geological events linked to the reactivation of tectonic faults to more recent occurrences related to eustatic changes in ocean levels. Our data suggests that an intricate combinations of geomorphological and climatic factors have molded the species diversity of H. malabaricus, and that the magnitude of its genetic diversity suggests the necessity of revising its taxonomic status.



#### Redescription of the Hypoptopomatinae catfish *Hisonotus leucofrenatus* (Miranda-Ribeiro, 1908) (Siluriformes: Loricariidae) from rio Ribeira de Iguape basin, southeastern Brazil

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Otocinclus leucofrenatus Miranda-Ribeiro, 1908 was described on basis of a six-centimeter specimen found among other Loricariidae collected by Ricardo Krone on the rio das Pedras, a tributary of the rio Ribeira de Iguape at Iporanga, São Paulo, without indication of a holotype. At that time, the genus Hisonotus Eigenmann & Eigenmann was considered by Regan as a synonym of Otocinclus Cope. Regan considered Hisonotus a synonym in view of the temporal plate (=compound-pterotic) perforate. The Regan's study, as others on the freshwater fish fauna from that period was done when the Hypoptopomatinae were only briefly known and Hisonotus was not properly defined relative to other members of the subfamily. But the genus was resurrected by Schaefer and the species herein studied is now referred as Hisonotus leucofrenatus. Although many specimens from the rio Ribeira de Iguape basin were collected and identified as Hisonotus leucofrenatus, the species was never properly characterized and the specimen that served as basis for the original description was lost. Otherwise, H. notatus type species of the genus (type locality now restricted to Rio de Janeiro State, Santa Cruz, rio Guandu, Santa Cruz farm), was recently redescribed by Martins and Langeani, with a lectotype designation and the description of H. thayeri. The genus Hisonotus was considered by these authors as polyphyletic and comprising 32 valid species. The recognized characters to distinguish the genus Hisonotus in Hypoptopomatinae include: snout plates in the anterior portion of the nostril reduced or absent, the rostrum having enlarged odontodes, and the lateral rostral margin composed of thickened plates. Britski and Garavello considered the rostrum with enlarged odontodes quite variable in Hypoptopomatinae, suggesting that rostral thickened plates and other known characters are not satisfactory to properly define Hisonotus. The purpose of the present work is to redescribe H. leucofrenatus based on fresh specimens of the type locality and its surroundings, deposited in the collections of LISDEBE and MZUSP. Comparisons will be made with other southeastern species of the genus, especially discriminating it from H. notatus with which it closely resembles. Through redescription of Hisonotus leucofrenatus, a neotype will be designated for the species. The distribution of the species will be discussed, taking into account that it is considered as occurring along the coastal rivers of southeastern Brazil and according to Carvalho and Reis in the rio Tramandaí, Lagoa dos Patos, RS.

Key words: Systematics; Taxonomy; Freshwater; Fishes, Neotropical Financial support: FAPESP (process 2011/50213-5)



## New species of the genus *Leporinus* Agassiz, 1829 (Characiformes: Anostomidae) from Venezuela

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Anostomidae is one of the most diverse families of Characiformes and the number of known species is still increasing rapidly as field expeditions are carried out and museum's old collections are revisited. Here, a new species of Leporinus is described and illustrated using morphological data and compared to all other species of the family. Specimens were illustrated using digital photograph, measured using digital calipers, and had teeth, scales and fin rays counted under a stereomicroscope. The morphological data, partially summarized in tables, were compared to original data obtained from specimens of other species or to data available in the literature. The new species is distinguished from all other Anostomidae, except Anostomus anostomus, A. brevior, A. ternetzi, Hypomasticus despaxi, Leporinus arcus, and L. striatus, for having four dark longitudinal stripes on body. The new species is distinguished from that species by having 12 series of scales around caudal peduncle (vs. 16), from the species of Anostomus by having terminal mouth (vs. upturned); from Hypomasticus despaxi and Leporinus striatus by having four teeth on premaxilla (vs. three) and from Leporinus arcus by having 35 or 36 scales on lateral line (vs. 36 to 38). The new species is allocated in Leporinus for having mouth terminal and incisiform teeth with blunt distal edge (unicuspidated) gradually decreasing in size from symphyseal tooth. The new species is apparently restricted to highlands of east portion of the Orinoco river basin in Venezuela. The new species is most similar in coloration, body shape, and teeth morphology, and possibly closely related to Leporinus arcus. The diagnostic features and distribution of L. arcus is evaluated based on examination of specimens from distinct fish collections, the latter is currently known only from the Essequibo river in Guyana, and the upper Jari river in Brazil.

Key words: Systematics; Taxonomy; Anostomoidea Financial support: FAPESP (10/51250-9), CNPq



#### Phylogenomics of the Neotropical family Serrasalmidae (Ostariophysi: Characiformes) based on ultraconserved elements

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Serrasalmidae is a monophyletic family of Neotropical freshwater fishes including 16 genera and 94 species that possess ecological specializations to either herbivory or carnivory. Represented by the popularly known pacus, the famous piranhas and the large tambagui, serrasalmids are broadly distributed through major South American basins. They can be identified by a combination of a deep, laterally compressed body, ventral keel composed by spines derived from modified abdominal scales and presence of a prominent predorsal spine, the last character present in all genera except Colossoma, Mylossoma and Piaractus. Several morphological and molecular phylogenies agree to recover the monophyly of Serrasalmidae. Nevertheless, intergeneric relationships are still conflicting, which demands a further study dealing with reminiscent polytomies, non-monophyletic groups, as well as generic and specific assignments of various members. We used the new methodology of sequence capture of ultraconserved elements (UCEs), highly-conserved nuclear loci that have hipervariable flank regions, from 52 specimens covering all genera of Serrasalmidae. To analyze the data we used the Phyluce package that implements several analytical pipelines and we estimated a maximum likelihood tree using RAxML. The 75% complete matrix contains 1327 UCE loci with a total of 345.179 bp after alignment. Our results recover a well-supported topology that is highly congruent with previous molecular hypotheses. We corroborate the arrangement of the socalled Pacu clade, composed by Colossoma, Mylossoma and Piaractus as the sister group of all remaining serrasalmids, which, in turn, presented two larger clades. The Myleus clade includes Acnodon, Myleus, Mylesinus, Myloplus, Ossubtus, Tometes and Utiaritichthys, and the Piranha clade contains Metynnis as sister to Catoprion, Pristobrycon, Pygocentrus, Pygopristis and Serrasalmus. In addition, our results corroborated the non-monophyletic Serrasalmus in which some species appeared more related to Catoprion and Pygopristis while others are closer to Pygocentrus and Pristobrycon calmoni.

Key words: freshwater fish, piranha, UCE, systematics Financial support: CNPq, FAPESP



### Signature of population expansion in Salminus brasiliensis from the Pantanal region during the Pleistocene

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Pleistocene climate changes are one of the major historical events that impacted the South America biodiversity, driving extinction episodes, speciation, intraspecific divergence, and demographic oscillations. Although their effects are well-documented in several biomes, it is poorly known how these climate shifts affected the Pantanal floodplain biodiversity. Fish is one of the most diverse groups in the Pantanal floodplains and represents a suitable biological model in reconstructing paleoenvironments scenarios. Trying to identify any effect of such Pleistocene changes in the Pantanal's ichthyofauna, we used genetic data from multiple populations of a top predator long-distance migratory fish, Salminus brasiliensis. We specifically asked if Pleistocene climate changes affected the past species demography. If so, we expected to find changes in population size over time. Thus, we assess the genetic diversity of S. brasiliensis to trace the demographic history of nine population (a total of 52 specimens) from the upper Paraguay basin, that formed one genetic group, employing approximate Bayesian computation (ABC) to test five scenarios: constant population (null hypothesis), old expansion, old decline, old bottleneck following by recent expansion, and old expansion following by recent decline. All drawn scenarios assumed an initial ancestral population (Na), demographic changes occurring during the Late Pleistocene (2-120 thousand years, ka, before present; t1 and t2), and the recent populations represented in t0. Based on two molecular markers (Cytochrome B and Dloop), our inference from the ABC analysis, the Bayesian Skyline Plot result, the star-like networks, and the patterns of genetic diversity (high haplotype diversity and lowmoderate nucleotide diversity) indicated a sudden demographic expansion of population. The ABC approach allowed us for making strong quantitative inferences about S. brasiliensis demographic history. We estimated a small ancestral population size that underwent a drastic 12-fold expansion, probably associated with colonization of newly formed habitats. The point estimated from this expansion time (55 ka ago) was consistent with humidity and warm phase inferred by speleothem growth phases and travertine records during Pleistocene interglacial periods. The strong concordance between our genetic inferences and this historical data could represent the first genetic record of a humid and warm phase in the Pantanal in the period since the Last Interglacial to 40 ka ago.

Key words: Approximate Bayesian computation; mitochondrial markers; Interglacial period; Neotropical fish; Upper Paraguay basin Financial support: CAPES and CNPq



#### Phylogeography of the species of *Deuterodon* Eigenmann, 1907 (Characiformes: Characidae)

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Freshwater fishes are at the mercy of various processes, either natural or not, that may occur to the ambient that they inhabit. This way, fish are excellent models for phylogeographic studies, since the distribution of the populations may reflect the historical and ecological changes resulting from the geological processes of the basins that they occupy. Deuterodon Eigenmann, 1907 is a genus of Neotropical fishes with species described from different regions of South America, but recently restricted to seven species endemic of the south/southeastern Brazilian Atlantic drainages. The aim of this study is to do a phylogeographic analysis of the populations of these seven species of Deuterodon sensu stricto. In order to accomplish that, 34 specimens had they DNA extracted by the CTAB method and four genes were amplified. Two mitochondrial genes, Cytochrome Oxidase c Subunit 1 (COI) and the NADH dehydrogenase 2 (ND2), and two nuclear genes, the nuclear Alpha-myosin 6 (MYH6) and the SH3 and PX3 domain-containing 3 like protein (SH3PX3). The genes tree and the haplotype network were built to resolve the phylogenetic relationships of the species and the phylogeographic patterns of distribution of the populations. The existence of a biogeographic pattern in the structuring of the phylogenetic tree of Deuterodon species was evidenced. Deuterodon singularis and D. longirostris are a sister group of Deuterodon stigmaturus and formed the south clade. These three allopatric species belong to the same paleodrainage, that represents the southernmost distribution of the genus. Its sister group, the north clade, contains the remaining species. The species in the north clade include D. langei, that forms the sister clade to D. iguape, D. rosae and D. supparis. These later species are distributed in other three paleodrainages that comprise the northernmost distribution of the genus. The histories of connections and separation between paleodrainages due to marine regression and transgression processes during the Pleistocene culminated in the patterns of structuration of Deuterodon species and populations. Thus, it is possible to conclude that the phylogeographic history of Deuterodon species reflects the history of connections and splits in the south/southeastern Brazilian drainages, recovering a scenario similar to those observed in other groups as for example in Hollandichthys multifasciatus.

Key words: Paleodrainages; Biogeography; Freshwater; Neotropical Financial support: CNPq scholarships to MGL (process 143962/2016-7), PCS (process 141407/2013-1; 406776/2017-2) and LRM (process 307890/2016-3)



### Redescription and osteology of Astyanax fasciatus (Characiformes: Characidae)

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Comparative and classical osteology are important sources of characters to recognize differences among Ostariophysan fish. Descriptions of species of Characidae, however, include a limited number of osteological characters, despite the widespread use of clearing and staining techniques over the last decades, and comparing osteological features among species remains a challenging task. The genus Astyanax includes more than 150 valid species widely distributed from Texas (EUA) to northern Argentina. The genus is defined by the presence of two series of teeth in the premaxilla, the first series with a variable number of teeth and the second series with usually five premaxillary and mandibular teeth usually ridged and denticulated, maxilla with 0-9 teeth, a complete lateral line, absence of predorsal spine, and caudal fin not covered with scales. Most of these characters are broadly distributed among characids, and the genus lacks apomorphic characters. Many species are morphologically very similar to each other and the lack of adequate species delimitation contributes to the complexity of their taxonomy. Some are part of species complexes (e.g., A. scabripinnis species complex, A. fasciatus species complex). Phylogenies of Characidae sensu Mirande and of species of Astyanax of Central America sensu Soto included osteological characters of a limited number of species of Astyanax. The purpose of this study is to describe the osteology of A. fasciatus (Cuvier) based on specimens from the São Francisco river basin, where the type locality is situated. Counts were made as described by Fink & Weitzman. The specimens were cleared and stained following the technique of Taylor & Van Dyke. Cleared and stained material belong to MNRJ, Museu Nacional, Universidade Federal do Rio de Janeiro. Bones were drawn with the aid of a camera lucida attached to a stereomicroscope. Terminology follows Weitzman, Fink & Fink, Weitzman & Fink with the modifications proposed by Vari. Astyanax fasciatus is distinguished from other species in the genus by possessing a vertically elongated umeral spot, 20 to 27 ramified anal-fin rays, one or two maxillary teeth, a silvery lateral band becoming black on the caudal peduncle and continued as a black streak to the tips of the middle fin rays, origin of dorsal fin equally distant from snout to caudal fin, pronounced fold on the anterior margin of the maxilar, short upper jaw length (36.0-43.8, mean 40.3 % HL), an elongated dorsal fin in mature males (25.9-37.0; mean 29.2 % HL). Osteological characters that distinguish A. fasciatus from other species include teeth on suspensory pharyngeal 3, foramen in posterior region of metapterygoid, two epurals, one pair of uroneurals, posterior gill rakers on four gill arches, lower pharyngel tooth plates single, ventral apex of urohyal somewhat closer to caudal end with ventro-rostral edge angled, anterior fontanel longer, supraoccipital caudal side undulate to straight, supraoccipital in dorsal view longer and narrow-based. Astyanax fasciatus closely resembles A. mexicanus in most details of its neurocranium.

Key words: Brasil; Neotropical Region; Characiformes; systematics, morphology Financial support: CAPES (BEX0644/02-1), FAPESP, CNPq (307610/2013-6; 476822/2012-2)



# Molecular, mitogenomic, and morphological complementary approaches analyzing the Cis-Andean Trichomycterinae — cryptic diversity and candidate species

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The generic name Trichomycterus and his type species, Trichomycterus nigricans, were made available in 1833 on the basis of one specimen from Santa Catarina, Brazil. A general description of the type species of the genus and other seven Trichomycterus species were briefly given in 1846. Two of the species—T. areolatus and T. maculatus—were described based on specimens captured in "la rivière de San-Jago" or the Maipo River, in Chile. Differences between them were based on a few individuals highlighting meristic characters such as dorsal (13 vs. 15) and anal fin-rays (8 vs. 9), and coloration (with traces of longitudinal band vs. bluish grey spotted body). These two species were valid until 1975, when a detailed osteological analysis of over 400 specimens concluded that skeletal variation between putative species was not significant, synonymizing them. Later on, the endemic genus Bullockia was discovered in Central Chile and more recently the Patagonian genus Hatcheria in southern Chile. A population genetic study with T. areolatus in 2010 found strong molecular differences among watersheds in central Chile, opening the question if another cryptic species remains hidden. Based on the aforementioned study, 90 specimens were sequenced in the Maipo River considering one mitochondrial gene and several anonymous nuclear loci. A number of individuals from the Maipo River grouped by genotypes were cleared and stained searching for diagnostic characters. Complementary with these approaches, several individuals along the distribution of T. areolatus in Chile were photographed alive and sequenced considering other mitochondrial genes. Finally, the Cis-Andean species Bullockia maldonadoi, Hatcheria macraei, T. areolatus and the putative T. maculatus were subjected to next generation sequencing, assembling, aligning and comparing their mitogenomes. In the type locality of T. areolatus, the morphological analysis of cleared and stained individuals shed lights on their cryptic diversity, identifying two characters of the lateralis system that separates between genotypes: numbering of pores of the supraorbital canal as well as the discontinuity of the canal in the nasal region in T. areolatus vs. its continuity in T. maculatus. Strikingly, phylogenetic results clustered the species Bullockia maldonadoi and T. maculatus, plus T. areolatus and Hatcheria macraei. A number of candidate species belonging to populations ascribed to T. areolatus farther from the type locality were identified based on this molecular approach. We discuss the significance of these findings in the context of the subfamily Trichomycterinae, proposing taxonomical rearrangements that are based on these results.

Key words: Systematics; Taxonomy; Freshwater; Andean Region; Siluriformes; Trichomycteridae; NGS; Morphology Financial support: Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT), grant number 3160659 (CQR)



#### Deep barcode divergence in Neotropical freshwater dwarf catfishes (Siluriformes: Scoloplacidae)

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Currently the Neotropical family Scoloplacidae is composed of six species within the genus Scoloplax, endemic to South America and widely distributed in the Amazon and Paraguay-Paraná basins. Scoloplax specimens inhabit small water courses with flooded areas in substrates with leaves and debries. Scoloplacids are characterized by their small size and the largest known specimen is just under 2 cm SL. Because of their small size, low activity, and occurrence in small streams, Scoloplax species are good models for biogeographical studies. This study is part of a larger investigation on the Biogeography and Evolution of this family in which nuclear and mitochondrial genes will be used involving their populations throughout their range. Here the diversity of CO1 is examined among Scoloplax samples, comparing divergences between populations. The Kimura two-parameter (K2P) distance model showed the following divergence between populations: Scoloplax empousa (Pantanal) vs. S. empousa (Ivinheima) (type locality), 11%; Scoloplax dolicholophia (Negro) vs. S. empousa (including all populations), 19-22%; Scoloplax distolothrix (Upper Xingu) vs. S. empousa (Ivinheima), 13%; Scoloplax distolothrix (Upper Xingu) vs. S. dolicholophia, 22-23 %; comparisons among populations of Scoloplax dolicholophia from São Gabriel da Cachoeira to Taruma-Mirim stream close to Manaus showed a 0,00% divergence. Scoloplax distolothrix (Lower Xingu) vs. S. empousa (Ivinheima), 12%; Scoloplax distolothrix (Lower Xingu) vs. S. dolicholophia, 21%; Scoloplax baskini (Aripuana, type locality) vs. S. baskini (Negro), 18%, whereas Scoloplax baskini (Aripuana, type locality) vs. S. baskini (Purus), showed a 9% divergence. Divergence between Scoloplax baskini (Aripuana, type locality) and the following populations are: S. dolicholophia, 18%; S. empousa, 20%; S. distolothrix, 21%; S. dicra, 22%. When compared to the outgroup, the divergence between the Scoloplacidae and the loricarids Hemipsilichthys papillatus and Hemipsilichthys gobio is 7 % and Neoplecostomus and Isbrueckerichthys is 10%. Even within Scoloplacidae, the divergence between populations of S. baskini is almost equal to that between the morphologically distinct S. baskini and S. dolicholophia (18%). The highest mean value divergence among species within Scoloplacidae is 25 %, while between Nematogenys and Scoloplax is 30%, Hoplosternum and Scoloplax is 28%, and Diplomystes is 30%. Divergence between the very morphologically distinctive and phylogenetically distant Astroblepus and Nematogenys is 24%, and between Diplomystes and Hemipsilichthys is 27%, while some populations of Scoloplax shows 18% of divergence within the genus. Morphological studies show variation in anatomy and some meristics between populations of S. empousa, S. distolothrix and S. baskini, whereas molecular data show a very deep divergence indicating a long isolation time for these populations. The S. empousa lvinheima population is separated from the Pantanal by the Serra de Maracajú that is acting as a barrier since Pliocene and although no natural barrier exist today among the populations of S. baskini, the distance now among populations and the low vagility of these specimens prompted higher levels of divergence.



#### Biogeographic analysis of the ichthyofauna from the middle portion of the Southern Espinhaço Mountain Range, Minas Gerais, Brazil

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The middle portion of the Southern Espinhaço Mountain Range (SdEM), located in the central region of Minas Gerais state, Brazil, is characterized by the scarcity of taxonomic and biogeographic studies related to the ichthyofauna. This is especially evident in the headwaters of the upper rio Paraúna (rio das Velhas drainage, rio São Francisco river), in the western portion of the SdEM, and upper rio Santo Antônio (rio Doce basin), in the east portion. With the goal of evaluating the role of the SdEM middle portion as a geographical barrier for fishes, a cladistic biogeographic analysis was performed through the Parsimony Analysis of Endemicity method (PAE). The diagnosis of the middle portion of the Southern Espinhaço Mountain Range (SdEM) ichthyofauna was carried out in tributaries of six main drainage areas in the upper rio Paraúna and upper rio Santo Antônio. For this purpose, field work and visits to scientific collections were performed. The field work was carried out on March and July 2017, and the ichthyological collections in Brazil were verified. The specimens were identified to the lowest taxonomic possible category. A total of 59 fish morphospecies were recorded, of which 33 were associated with the upper rio Paraúna, 40 with the upper rio Santo Antônio, and 14 with both sub-basins. Of these, 18 were biogeographically informative and were included in the analyses. Four equally-parsimonious area cladograms were obtained. The strict consensus revealed a higher pattern of relationship between the drainages belonging to the upper rio Santo Antônio region. The results indicated that the SdEM middle portion acts as an effective geographical barrier for fishes, although the sharing of some taxa by rio das Velhas and Santo Antônio drainages has been confirmed and the distribution expansion for two valid species were suggested. It was also verified that the rio das Velhas and Jequitinhonha drainages share some species, as well as between Santo Antônio and Paraíba do Sul basins.

Key words: rio das Velhas; rio Santo Antônio; hydrographic basin; stream; distribution Financial support: CAPES; CNPq



#### A new species of *Trichomycterus* (Siluriformes: Trichomycteridae) from the Doce river basin

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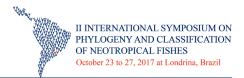
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Trichomycteridae currently comprises 298 small catfish valid species, arranged in eight subfamilies, of which 217 valid species belongs to the genus Trichomycterus Valenciennes, 1832. This is the most species-diverse genus of the Trichomycteridae and has a wide distribution, covering almost all South America and lower Central America, from Costa Rica to Argentina. A new species of Trichomycterus is recorded from the eastern portion of the Southern Espinhaço Mountain Ranae medium stretch, and the first time related to the headland region of the Santo Antônio drainage, rio Doce basin, Minas Gerais state, Brazil. The new species can be easily distinguished from its rio Doce basin congeners (Trichomycterus alternatus and T. argos), and from T. pradensis and T. immaculatus, by the presence of a great amount of dark blotches with a weakly defined outline all over the light brown body (vs. light brown body with sequential or slightly rounded sequential spots in the lateral midline in T. alternatus; body with rounded spots that do not overlap each other throughout the body in T. argos; pale yellow body with weak clear spots, grayish to light brown in T. pradensis; no spots in T. *immaculatus*); further more differs from T. argos by the number of pectoral fin rays (i+7 vs. i+6). Additionally, it can be distinguished from the species occurring in the western portion of the Southern Espinhaco Mountain Range (rio São Francisco basin) by the absence of transversal stripe or elliptic dark spot in the base of caudal fin rays (vs. presence in T. novalimensis and T. trefauti, respectively); little dark rounded spots on the body (sometimes overlapping each other) in T. brasiliensis. It can be further distinguished from T. trefauti by the shorter length of the barbels (maxilary barbels 41.0-57.8% in HL vs. 84.2-93.0%, nasal barbels 42.8-63.2% vs. 69.8-74.3% and rictal barbels 30.5-51.6% vs. 67.6-74.3%); from T. alternatus and T. pradensis by greater number of opercular odontodes (16-28 vs. 14-15 in T. alternatus and 10-14 in T. pradensis), and by greater number of branchiostegal rays (eight vs. seven in T. alternatus and T. pradensis). The new species further distinguishes from T. alternatus by the position of the anal-fin origin, in a vertical posterior to the last dorsal-fin ray, at the 24th vertebral center (vs. in a vertical through the 9th dorsal-fin ray, between 21st and 23rd vertebral center); and strongly truncated caudal fin (vs. rounded in T. brasiliensis and T. novalimensis, rounded to weakly truncated in T. argos, T. pradensis and T. trefauti).

Key words: Trichomycterinae; Neotropical region; Espinhaço Mountain Range Financial support: CAPES; CNPq-PCI DA grant (process 302248/2016-1); USP (Edital Programa Institucional de Pesquisa nos Acervos da USP)



#### South American percichthyids and percilids. What is new about them?

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The family Percichthyidae was erected, without diagnosis, about 90 years ago to contain one genus of fishes—Percichthys—of the Austral Region of South America. It was nearly 50 years before the family was expanded, adding another Austral genus, Percilia, three Australian genera, and a variety of marine perciforms, such as Acropoma, Ctenolates, Lateolabrax, and Polyprion. In the beginning of the 1980's, the family was interpreted as a monophyletic group containing fishes of only Gondwanan distribution such as the South American Percichthys and Percilia and nine Australian genera; however, at the end of the 1980's, another study only included a few of the Australian percichthyids and marine perciforms (e.g., Acropoma) in this family, omitting South American forms. This omission was corrected in the "Catalogue of Genera of Recent Fishes", published in 1990, which retained Acropoma, Coreoperca, and Ctenolates within Percichthyidae. Yet another study based on morphological evidence proposed a new family, Perciliidae, for the Austral species of South America. In contrast to morphological studies, the most recent molecular one suggests that the Suborder Percichthyiodei (= Family Percichthyidae) is not a member of the Order Perciformes, but of the Centrachiformes, and Percichthyiodei contains Percichthys and other Australian forms, such as Maccullocchella, Gadopsis, Nannoperca, and Maguaria ambigua. This last genus (Macquaria) appears nonmonophyletic, with two species of Macquaria placed in a new family, Percalatidae, within a new suborder, Percalatoidei and one species within Percichthyiodei. An examination of morphological structures (e.g., bones, muscles, sensory canals, and scales) of the proposed percichthyid genera and most of their extant and fossil species is in progress. Simultaneously, a molecular analysis including part of these proposed percichthyids has been executed. Another analysis of the species included in this molecular analysis has been performed based on morphology. Our preliminary results reveal that the South American Percichthys and Percilia are more speciose than previously thought and may represent more than two genera, with a complex pattern of cryptic species arising, similar to that found in other fishes in the Southern Hemisphere. There are disagreements among the various phylogenetic analyses using different data sets, as well as outgroups, which polarize the characters.

Key words: Systematics; Taxonomy; Freshwater; Estuarine; Gondwanan distribution Financial support: Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT), grant number 3160659 (CQR)



## Evolution of body size and headstand behavior in Anostomoidea (Characiformes)

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The Anostomoidea includes Anostomidae, Chilodontidae, Curimatidae and Prochilodontidae and it is the most diverse group of Characiformes (excluding Characidae), encompassing more than 300 valid species. The diversity in morphology, feeding tactics, and reproduction exhibited by anostomoid fishes is great. Some species are large size migratory fishes that form large schools whereas others are minute in size and live in restricted areas of slow water associated with trunk and plants. Some species of Anostomidae and Chilodontidae are known as headstanders since specimens rest with head down with the body forming a 45° angle with the substrate, a behavior well known among aquarists. Here, we investigate the evolution of the body size and headstand behavior in Anostomoidea. Data on headstand behavior were obtained from the literature. Standard Length in mm was used to represent Body Size. Data of body size of all species of Anostomoidea were taken from species descriptions and taxonomic revisions, or eventually from examination of specimens deposited in fish collections. Untransformed body size data was typically skewed, and therefore log-transformed body size data were used in all analyses. Species assignment and phylogenetic partitioning of the data for subclade analyses within Anostomoidea followed a compilation of the most recent and comprehensive hypotheses available in the literature. A phylogenetic tree was constructed using Mesquite, and statistical analyses were performed using R software. Evolution of body size and headstand behavior were reconstructed using parsimony. Phylogenetic signal was calculated using Pagel's Lambda Correlation Structure, showing strong correlation among body size and behavior, with phylogeny, especially in the least inclusive taxa, such as species. Our results evidenced independent cases of size reduction, as for example in Anostominae or Chilodontidae, and increase of size, as for example in Megaleporinus. Correlation between body size and head standing behavior was analyzed using Pagel's and Grafen's Correlation Structure exhibiting significant and negative correlation, regardless of the phylogeny history, showing that this behavior is strongly linked to small size fishes. Headstand behavior evolved three times independently, two in Anostomidae and one in Chilodontidae.

Key words: Systematics; Anostomidae; Prochilodontidae; Curimatidae; Chilodontidae Financial support: CAPES



#### Taxonomic assessment of *Anostomoides* (Characiformes: Anostomidae) based on morphological and molecular data

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The taxonomic history of Anostomoides started before its description by Pellegrin in 1909. Steindachner (1876) described Leporinus nattereri based on several specimens from the Amazon basin, a species that remained poorly documented until now. Anostomoides atrianalis was described by Pellegrin (1909) based on three specimens collected in the Orinoco river in Venezuela. Eigenmann (1912) described Schizodontopsis laticeps, based on specimens from the Essequibo river in Guyana. In 1950, Myers reviewed the genera of Anostomidae recognizing Schizodontopsis as a junior synonym of Laemolyta. In 1974, Géry transferred Laemolyta laticeps to Anostomoides. More recently, Santos & Zuanon (2006) described A. passionis as a species endemic to the rapids of the middle Xingu river in Brazil. Anostomoides was only rarely mentioned in the literature, and specimens of Anostomoides were tentatively identified in ichthyological surveys. This study aims a comprehensive taxonomic assessment of Anostomoides based on morphological and molecular data. 245 specimens were morphologically examined including those preserved in alcohol, cleared and stained, and dry skeletons. Type specimens were personally examined or through photographs and illustrations were prepared using a stereomicroscope with multifocal software. Meristic data were taken under stereomicroscope and morphometric data taken with a digital caliper and analyzed using Analysis the Canonical Variables (CVA). The COI gene were generated for four specimens of A. atrianalis and six A. nattereri and used to estimate intra and interspecific genetic distances. A maximum likelihood tree was generated including available sequences of all anostomid genera and all known genetic lineages of Leporinus. Our taxonomic review demonstrate that A. laticeps is a junior synonym of A. atrianalis, while A. passionis is a junior synonym of Leporinus nattereri, and the latter name should be transferred to Anostomoides forming a new combination. The molecular results evidenced a very small genetic differentiation among analyzed specimens of A. atrianalis and A. nattereri (<0.5%). CVA analysis showed that examined specimens form two distinct morphotypes, corroborating the validity of both A. atrianalis and A. nattereri. Our results also provide an updated and precise generic diagnosis for Anostomoides: a slightly upturned mouth with four premaxillary teeth with, symphyseal tooth bicuspid, and remaining teeth slightly tricuspid with medial cusp distinctly larger or with blunt cutting edge, four dentary teeth with two medial teeth with truncate cutting edge without cusps, and two lateral teeth with three to five small cusps. Anostomoides nattereri is distinguished from A. atrianalis by having four branchiostegal rays (vs. three), four pores in infraorbital one (vs. three), 38 pored lateral-line scales (vs. 42 or 43), three dark rounded midlateral blotches (vs. three or four vertically elongated blotches and/or a faded dark longitudinal stripe), dark lines between scale series on posterior half of body in specimens smaller than 150 mm SL (vs. dark lines always absent), a greater body depth and higher interorbital distance. Anostomoides nattereri occurs in tributaries of the Amazon river, including the Araguaia, Negro, Tapajós, Tocantins and Xingu rivers, whereas A. atrianalis is widespread throughout the Amazon, Orinoco and Essequibo basins.



# Development of the caudal-fin skeleton in *Lophiosilurus alexandri* (Siluriformes: Pseudopimelodidae) and insights on its evolution in the family

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The caudal-fin skeleton is one of the most studied osteological complexes in systematics and evolution of fishes. Observations on ontogenetic changes often allow tracing back the homology of each element in the adult caudal-fin skeleton. Lophiosilurus alexandri is the largest species of the catfish family Pseudopimelodidae, reaching up to 720 mm total length, and is endemic to the rio São Francisco basin in northeastern Brazil. During its development, impressive changes in the body morphology occur, including enlargement of body width and consequent reduction of body depth. Accordingly, L. alexandri is an ideal model to observe morphological changes during development and to seek solid interpretations on the polarity of phylogenetic characters. Elements of the caudal-fin skeleton in 26 specimens of distinct larval periods (yolk sac, flexion and postflexion; n = 19 specimens), juvenile stages (n = 4) and adult specimens (n = 3) were studied. Specimens were cleared and double stained, except for large adults, which were prepared as dry skeletons. Illustrations were prepared using a stereoscopic microscope and multifocal software. In L. alexandri, the greatest morphological changes in the development of the caudal-fin skeleton occur during the postflexion larval stage: formation and ossification of uroneural, fusion of paripural to hypurals 1 and 2, fusion and ossification of preural centrum 1 plus ural centrum 1, formation of procurrent and principal caudal-fin rays. Lophiosilurus alexandri has Type C hypurapophyses, a condition shared with most catfishes. The hook-like epural, and the robust hemal and neural spines present in early juveniles (60 days old) are synapomorphies for L. alexandri plus Cephalosilurus fowleri. Finally, two autapomorphies for L. alexandri are identified: the shallow (reduced) diastema between hypurals 2 and 3, and the fusion of hypurals 3, 4 and 5, both features present exclusively in large adults.

Key words: Ontogeny, anatomy, osteology, axial skeleton, caudal fin Financial support: CAPES; CNPq



#### Species validation and cryptic diversity in the Geophagus brasiliensis complex (Teleostei: Cichlidae) from Brazilian coastal basins as revealed by DNA analyses

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Reliable information about species richness is essential to any biodiversity study and conservation policies, which represents a challenge when it refers to the Neotropical ichthyofauna. This is the case of cichlids of the tribe Geophagini, particularly of the genus Geophagus, historically recognized as a taxonomically controversial group. Many allopatric populations of the pearl cichlid Geophagus brasiliensis show remarkable morphological, chromosomal and molecular divergence. As a result, Geophagus brasiliensis is regarded as a species complex, combining recent description of new species and several uncertainties about their actual richness. In the present work, we evaluated the number of evolutionary units in this fish complex along hydroaraphic basins in the state of Bahia, northeastern Brazil by using DNA barcode based on sequencing of Cytochrome c Oxidase subunit I (COI). Moreover, species delimitation algorithms such as the Barcode Index Numbers (BINs), the Automatic Barcode Gap Discovery (ABGD), an automatic procedure that separates the sequences of hypothetical species based on the barcode gap, the Phylogenetic Map PTP (PhyloMap PTP), which combines Principal Coordinate Analyses (PCoA) and the Poisson Tree Process (PTP) model, both sensitive to recent speciation events were used as well as phylogenetic analyses based on Maximum Likelihood (ML) and Bayesian Inference (BI). A total of 12 evolutionary units were identified with high support values and mean differentiation within and among groups of 0.4% and 13.8%, respectively. Besides confirming Geophagus itapicuruensis as a valid species, we provided further evidence of additional species yet to be properly described. Moreover, the evolutionary units in coastal basins of northeastern Brazil were clearly differentiated from Geophagus brasiliensis stricto sensu. The present data are useful to infer the evolutionary units in a cryptic and widespread fish group from the Neotropical region. Finally, the presence of undescribed taxa in coastal basins of northeastern Brazil, currently threatened by environmental degradation, introduction of exotic species and construction of dams have direct implications to the conservation of regional ichthyofauna diversity.

Key words: Barcode, Cichlids, COI, Genetic diversity, Ichthyofauna Financial support: FAPESB (RED 0009/2013)



## A new hypostomine (Loricariidae) genus-level taxonomy incorporating molecular and morphological data

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The current state of genus-level taxonomy of the Hypostominae is chaotic. This has been caused both by describing genera based on few or no characteristics and by relying on morphological phylogenies. Molecular phylogenies thus far have shown that morphological homoplasy is common and rates of morphological evolution are high within Hypostominae. Even recently diverged sister species can differ by characters that have been used to define genera. For example, in the Venezuelan sister species Peckoltia lujani and P. wernekei, P. lujani has long dentaries almost forming a straight line, which was previously indicative of Hemiancistrus, and P. werneki has shortened dentaries that form an acute angle and have few teeth, which is more consistent with the standard definition of *Peckoltia*. The two species differ by as little as 0.3% CytB sequence divergence, suggesting very recent speciation. Unexpected relationships between species groups such as this is increasingly common in molecular analyses, necessitating a near complete structural breakdown of Hypostominae genera. Initial strange relationships posited by early mitochondrial gene phylogenies have largely been upheld first by rigorous multi-locus phylogenies including nuclear genes, and now also by phylogenomic techniques examining hundreds of exons. In a separate talk, we will present a phylogeny based on 810 exons and 172,794 base pairs that is used to constrain a five gene, multi-locus dataset to obtain a new picture of relationship throughout the Hypostominae. From this phylogeny, it is clear that numerous clades require description at the genus level, several genera must be expanded, and several genera must be recognized as synonyms. Use of the molecular phylogeny to examine morphological evolution reveals extensive homoplasy in various character suites, including some that were previously believed to have strong phylogenetic signal (e.g., evertible cheek odontodes, jaw morphology). Such homoplasy in traditionally heavily weighted character suites should motivate a deeper examination of morphology and a search for diagnostic characters in less thoroughly examined regions of loricariid anatomy, such as neuroanatomy and musculature, to construct a more robust morphological scaffold for loricariid taxonomy.

Key Words: Classification, Freshwater, Neotropical, Phylogenomics, Systematics



#### Systematics of Pareiorhina Gosline, 1947 (Siluriformes: Loricariidae)

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Pareiorhina currently shelters seven species: P. rudolphi (Miranda-Ribeiro, 1911), its typespecies from the Rio Paraíba do Sul drainage, and six other species described since 2003, P. carrancas Bockmann & Ribeiro, 2003 (Rio Grande drainage, Upper Rio Paraná); P. brachyrhyncha Chamon, Aranda & Buckup, 2005 (Rio Paraíba do Sul drainage); P. cepta Roxo, Silva, Mehanna & Oliveira, 2013 (Rio São Francisco) drainage); P. hyptiorhachis Silva, Roxo & Oliveira, 2013 (Rio Paraíba do Sul drainage); P. pelicicei Azevedo-Santos & Roxo, 2015 (Rio Grande drainage, Upper Rio Paraná); and P. rosai Silva, Roxo & Oyakawa, 2016 (Rio São Francisco drainage). Preliminary analyses have shown that genus real diversity may be larger than that described by literature, what led to the present research, aiming the taxonomic revision and the systematic analysis. The taxonomic revision examined specimens from various fish collections, including many Southeastern Brazil drainages and all current nominal species. The phylogenetic analysis was performed using Winclada v. 1.7 TNT (Nixon) to run TNT v.1.1 (Goloboff, Farris & Nixon). All characters were considered non-additive and received equal weights. To find the most parsimonious tree, a heuristic search with new technologies (ratched, with 200 iterations, and drift, with 50 cycles), random addition sequences, 1,000 replicates and maximum retention of 10,000 trees was performed; the tree was rooted between Astroblepus and Loricariidae taxa. Biogeography hypotheses were generated after superimposing the known areas of distribution for each of the loricariid species analyzed to its respective branch. Twenty four most parsimonious trees were found (1947 steps, CI=0.25, RI=0.68). The monophyly of Pareiorhina was confirmed based on 12 synapomorphies; the genus resulted as more related to genus Kronichthys and the Hypoptopomatinae, with the Neoplecostominae, Pareiorhaphis, Isbrueckerichthys, and Neoplecostomus as its sister-group. Pareiorhina was divided in two clades: "Pareiorhina rudolphi" group, with P. rudolphi and some new species, and its sister clade, divided in two sub-clades: one with P. carrancas and P. hyptiorhachis and the other with ((P. cepta + P. pelicicei) + (P. brachyrhyncha + "P. rosai group")). The phylogenetic relationships suggest the occurrence of a first major cladogenetic event splitting "P. rudolphi" group and the remaining species. In both groups various headwater streams capture were responsible for the current diversity throughout the upper lands of Southeastern Brazil harboring in the headwaters of the Doce, Paraíba do Sul, Paraná, and São Francisco river drainages. Putative new species were recognized in the "P. rudolphi" group (upper Rio Paraná, Rio Paraíba do Sul, and Rio de Janeiro State coastal river); P. cepta was also registered in the upper Paraná, and P. rosai was also registered in the upper Rio Paraná and in the Rio Doce.

Key words: Brazilian crystalline shield; mountain streams; headwaters capture Financial support: CNPq (process 306566/2014-1 to FL) and FAPESP (process 2013/2404706 to BNA)



#### Redescription of Schizodon dissimilis (Garman, 1890) and evaluation of the dark vertical banded Schizodon species from northeastern Brazil (Characiformes: Anostomidae)

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Although species of the Anostomidae family are relatively well known, many of them, especially those of the genus Schizodon still need to be better diagnosed and their distribution better studied. Schizodon dissimilis (Garman, 1890) is one such species which is here redescribed on the basis of morphological data and is adequately illustrated from the syntypes and fresh specimens from the type locality and surroundings. Schizodon dissimilis was described by Garman in an abridged way and without any illustration, on basis of specimens of the Rio Poti River, a tributary of the Rio Parnaíba, Piauí. In this contribution, Garman presents Anostomus dissimilis (=Schizodon dissimilis) and Anostomus platae (=Schizodon platae). Posteriorly, Borodin in a little informative article, accepts Schizodon as subgenus of Anostomus and describes Anostomus (=Schizodon) rostratus from Rio Puty (=Rio Poti). For many years, specimens of S. dissimilis from rivers of the Northeastern Brazil were scarcely mentioned in the literature because specimens of this species were identified as S. fasciatus in 1941, by Fowler with material from the Rio Jaguaribe and 1973 by Roberts that examined specimens of the Rio Parnaíba. In this study, the syntypes of Anostomus dissimilis (MCZ 19381, 19382, 19383, 19384), USNM (120238) and specimens of S. fasciatus (MCZ, 851, 19329, 19336 and 19365) have been examined together with recent specimens of S. dissimilis and S. fasciatus. Schizodon dissimilis is distinguished from all other species of the genus except S. fasciatus, S. vittatus, S. borellii, S. intermedius and S. corti by the presence of four dark vertical bands on body (vs. color pattern without vertical bands). It is also distinct from the species with vertical bands (above cited) by having the end of the caudal peduncle devoid of a dark rounded blotch (vs. with a rounded blotch or a longitudinal stripe on caudal peduncle). Populations of S. dissimilis, S. intermedius and S. borellii are also compared through principal component analysis and size-free canonical variate analysis of Bookstein et al., applied to selected measurements of combined samples of the three species. The analysis revealed they have similar body shape, but differ in corporal proportions in order to reappraise S. dissimilis as distinct of those two species. Besides differing from S. fasciatus, S. borellii and S. intermedius by the lateral line scale counts, color pattern or corporal proportions, S. dissimilis also differ from them by the karyotype pattern and the DNA barcode molecular sequence.

Key words: Systematics; Taxonomy; Freshwater; Fishes, Neotropical Financial support: FAPESP (process 2011/50213-5)



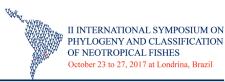
# The facial muscles of the neotropical electric-eels (Ostariophysi : Gymnotiformes): descriptive morphology, synonym and phylogenetic inferences

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The Gymnotiformes comprises ca. 240 living species restricted to continental waters of Americas. Although Gymnotiformes have been the focus of phylogenetic studies based on morphological and/or molecular data, the use of myologycal characters is scarce across the order. Thus, the aim of this project is to undertake a thorough analysis of the facial myology of Gymnotiformes, filling out a large gap in the anatomical knowledge of an important component of the freshwater ichthyofauna and assist in the understanding of their morphological diversity and evolution. The present study redefined hypotheses of homology and nomenclature of the facial myological complex with a detailed documentation of the phylogenetic variations relevant to each gymnotiform subgroup. The anatomical descriptions were based on 121 specimens, comprising around 87 species, including representation all genera, except Akawaio and Procerusternarchus (Hypopomidae), Iracema (Rhamphichthyidae), Tembeassu and Megadontognathus (Apteronotidae). Descriptive morphology included all dorsolateral head muscles identified (sections of adductor mandibulae, levator arcus palatini, dilatator operculi, levator operculi, adductor arcus palatini, adductor hyomandibulae and adductor operculi), and was used to compose a synonymic list of all names currently utilized, resulting in ca. 30 names for 16 muscles. Additionally, descriptions were also utilized as basis for phylogenetic inference, resulting in the proposal of 56 characters from facial muscles, of which 50 are new. Data were synthetized into discrete characters, coded using Mesquite 3.2 and analyzed for parsimony with the program TNT 1.5. An analysis based only on myologycal was performed as a test of congruence with the hypothesis resulting the entire data set (e.g. external morphology, bones, electrophysiology, or molecules). The myological analysis resulted in 40 equally parsimonious trees with 192 steps (IC: 0.35; IR: 0.86). Results corroborated previous hypotheses such as Gymnotidae as sister-group of all other Gymnotiformes, the monophyly of Rhamphichthyoidea (Rhamphichthyidae + Hypopomidae) as the sister-group of Sternopygoidea (Apteronotidae + Sternopygidae), in addition to the recovery of natural status of Eigenmanniinae, Platyurosternarchus + Sternarchorhynchus, Sternarchorhamphus + Orthosternarchus, Magosternarchus nested in Sternarchella, and the monophyly of almost every genus in the order. A discussion about incongruences between the present results and previous studies is presented.

Key words: Anatomy, Musculature, Phylogenetic, Nomenclature Financial support: FAPESP (process 2013/09926-3)



## Two new species of *Odontostilbe* Cope, 1870 (Characiformes: Cheirodontinae) from Andean Amazon piedmont

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There are currently 16 valid species of Odontostilbe in the Neotropical region, six of which are restricted to the Amazon basin: Odontostilbe dierythrurg Fowler, O. ecuadorensis Bührnheim & Malabarba, O. euspilura (Fowler), O. fugitiva Cope, O. nareuda Bührnheim & Malabarba, and O. parecis Bührnheim & Malabarba. Specimens of Odontostilbe species of the Andean Amazon piedmont are examined, and two new species are described. Horizontal measurements were made point to point on the body, counts traditionally used in Cheirodontinae studies and morphological characters were revised using diaphanized material and scanning electron microscopy (SEM). Statistical analyzes to determine which morphometric variables differed significantly among species were performed using the VARSEDIG statistical package, a function free from the R program. Odontostilbe sp. n. P differs from all species of the genus, except from Odontostilbe pequira and Odontostilbe sp. n. K, by the presence dentary with four anterior teeth with three large and equally longer compressed cusps and 2-3 lateral small cusps (vs. anterior dentary teeth with five cusps, being central cusp larger and longer than laterals cusps). Odontostilbe sp. n. P differs of Odontostilbe pequira by the absence of a conspicuous spot on the distal part of the dorsal fin (vs. present). Odontostilbe sp. n. P is distinguished from Odontostilbe sp. n. K by the presence first unbranched pectoral fin ray more longer than remaining rays, extending posteriorly the base of the pelvic fin (vs. first pectoral-fin ray reaching anterior edge of pelvic bone). It additionally differs of Odontostilbe sp. n. K by the number of arranged lamellae on both sides of central elongated raphe in the olfactory rosette (20 vs 15 respectively). Olfactory rosette in Odontostilbe is characterized by being slightly oval, with number of lamellae that varies between species, allowing species to be differentiated, with outer margins free, and inner margins attached to median raphe. Central median raphe slightly elongated. Lamellae present a convex ventral and concave dorsal surface. Dorsal surface attached to the wall of the olfactory chamber by long connection. The length of lamellae varies according to their position; posterior lamellae very developed, with more convex ventral surface that ventral surface of anterior lamellae. The apical parts of the lamellae are flat and wide but the middle and bases are slender. Herein we provide a description of two new species of Odontostilbe and comment on new diagnostic characters.

Key word: Amazon basin, Lamellae, Freshwater, Olfactory rosette, Taxonomy Financial support: CNPq (process 141479/2017-5)



## Taxonomic revision of Awaous tajasica (Lichtenstein, 1822) and putatively related forms (Actinopterygii: Gobiiformes)

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The gobiid genus Awaous has pantropical distribution. It is characterized by the presence of sensory papillae over much of the gill structure and it belongs to the subfamily Gobionellinge. Awaous tajasica, known as "Amborê" or "River goby", inhabits sand bottoms of the rivers. This species reaches a moderate size (up to 168.8 mm SL), is omnivorous and has amphidromous habits. Literature and collection records indicate that A. tajasica has a wide distribution, from Florida to the State of Rio Grande do Sul, Brazil, occurring in all Caribbean islands. The aim of our work is to provide characters that can help in the delimitation of A. tajasica as a valid species, highlighting the diagnostic features separating it from all other representatives of genus Awaous that occurs in Western Atlantic. Based on morphological data A. tajasica can be distinguish from A. flavus for having larger size, 168.8 mm of standard length (vs. 84.0 mm SL in A. flavus), posterior oculoscapular canal continuous between pores H and K (vs. discontinuous in A. flavus), body with brownish blotches (vs. body with vertical bars in A. flavus). Awaous tajasica can be distinguish from A. banana for possessing a single pore F of the anterior oculoscapular canal (vs. pore duplicated in A. banana), and less scales in transversal series, 15-18 (vs. 16-26 scales in traversal series in A. banana). These diagnostic characters allow to restrict the distribution area of A. tajasica to the region from the mouth of rivers of the Maranhão State to Rio Grande do Sul State, Brazil. In addition, A. flavus is recognized to occur in freshwaters from the Orinoco Delta to the mouth of the Amazonas River, while A. banana is found from State of Florida to the Orinoco Delta, including the Caribbean Islands. Phylogenetic studies in progress may help elucidate the biogeography of these fishes, as well as provide clues about the historical and ecological factors involved in delimiting their present distributions.

Key words: goby, taxonomy, distribution Financial support: Fapesp (process 2015/14323-1)



### Phylogenomics of Gymnotiformes: estimating improved partitioning schemes for UltraConserved Elements (UCEs)

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UltraConserved Elements (UCEs) have become one of the most popular markers for phylogenomic studies. They are relatively simple to collect from distantly-related organisms, and contain sufficient information to infer relationships at almost all taxonomic levels. Most phylogenomic studies of UCEs use partitioning methods to account for variation in molecular rates among sites, for example by estimating an independent model of molecular evolution for each UCE. However, rates and patterns of molecular evolution vary substantially within as well as between UCEs, which suggests that there is an opportunity for improvement in how UCEs are analyzed for phylogenetic inference. Here, we propose and evaluate two new partitioning methods for phylogenomic studies of UCEs: Sliding-Window Site Characteristics (SWSC), and UCE Site Position (UCESP). The first method uses site characteristics to generate partitions that account for heterogeneity in rates and patterns of molecular evolution within each UCE. The second method groups nucleotide sites based on their locations in the UCEs. We examined the new methods with a UCE dataset of Gymnotiformes. We demonstrate that **SWSC** generates partitions that account for the heterogeneity rates among structural regions and lead to large increase in the fit of partitioning schemes to the data. The UCESP method generates partitions that are worse than other strategies used to partition UCE datasets (e.g. one partition per UCE alignment). We then, examined the impact of the new partitioning methods on the phylogenetic inference of Gymnotiformes, and suggest the use of SWSC for phylogenomic studies of UCE markers to improve the accuracy of inferences made from UCE alignments.

Key words: Phylogenomics, UCEs, partitioning, partitioning methods, Sliding-Window Site Characteristics, PartitionFinder Financial support: PNPD (16003012001P4 - PNPD)

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### FishFinder: tracking species on the road

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The metamorphosis of the cell phone into a mobile computing platform has opened an avenue of opportunities to enhance the productivity scientific endeavors. Here, we present a GPS-based application including maps of species distributions with additional features, leading users to type localities or other points of interests. The aim of the application (app) FishFinder is to assist researchers in the fieldwork by helping them to locate species nearby using mobile phones. In its current version, FishFinder includes 6460 unique GPS coordinates of 241 described aymnotiform species (order Gymnotiformes), that covers their known geographic distributions. The data available in the app were assembled primarily from papers of species descriptions and museum databases, and from two meta-databases: GBIF and FishBase. The dataset presented in the app was thoroughly inspected to minimize obvious error of locations and taxonomy. The app also contains information on maximum body-size of species, locations of holotypes, indices of species vulnerability and uniqueness, among others. A remarkable feature of the FishFinder is its interactively with Google Maps. This interactiveness lets users locate species on maps and specify road routes to points of interests, using a mobile phone with internet connection. FishFinder app was built on a dual platform that allows prospective collaborators to update data of the app in their personal computers. FishFinder app is expandable to include other groups of Neotropical fishes. This app is available for Android e iOS.

Key words: Smartphones, Applications, Neotropical Fishes, Knifefishes, Species Distributions, Biogeography Financial support: PNPD (16003012001P4 - PNPD) to VAT; NSF (0741450 and 1354511) to JSA



#### Comparative and Functional Anatomy and Evolution of Feeding Apparatus and Diet of Serrasalmidae (Teleostei: Ostariophysi: Characiformes)

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The feeding behavior is subject to different selective pressures and the feeding apparatus varies widely within the evolutionary history of different groups of animals. Fishes of the family Serrasalmidae are endemic to the Neotropics and are widely distributed in the major river basins of South America, except in coastal rivers of Brazil and the rivers west of the Andes. This fish group shows extensive variations in feeding apparatus and also in feeding strategies. There are carnivores, herbivorous and lepidophagous Serrasalmidae species, making this fish group an excellent model for evolutionary studies. The present study sought to understand, by ecomorphological, anatomical, functional and evolutionary approaches, the changes in feeding strategies and in feeding apparatus of Serrasalmidae. Thus, variations related to muscles, bones and digestive tube were shown and associated to feeding behaviors. Additionally, phylogenetic inferences were made with data obtained, showing a probable evolution of such features. In this study, we found a craniobranchial bone and muscle rearrangement correlated with hardness, vagility and size of food items. In addition, we also found different proportions of regions of digestive tube correlated with nature of food items (animal or vegetal items). Fishes like durophagous carnivorous piranhas Serrasalmus maculatus, S. elongatus and Pygocentrus nattereri that feed on basically hard, vagile and animal items have supraterminal mouths, elongated lower jaws, one row of mandibular and premaxillary blade-like teeth, mandibular tendon inserted posteriorly in the lower jaw, short snouts, large adductor mandibulae with several expansions of its origins, short gill rakers, relatively short intestines and elongated stomachs. Catoprion mento, a non durophagous lepidophagous piranha, that feed mainly on scales, is morphologically very similar to other piranhas, but it has reduced adductor mandibulae with no expansions of its origins and elongated gill rakers. On the other hand, fishes like durophagous herbivorous pacus Mylossoma duriventre, Piaractus brachypomus, Utiaritichthys sennaebragai and Acnodon senai that feed on basically hard, sessile and vegetal items have subterminal mouths, two rows of incisiviforms or molariforms premaxillary teeth, one or two rows of mandibular incisiviforms or molariforms teeth, short lower jaws, elongated snouts, relatively elongated intestines and short stomachs and mediumsized adductor mandibulae with several expansions muscle origins. Metynnis maculatus, a non durophagous herbivore, feed basically on soft vegetal matter, is morphologically very similar to other pacus, but it has reduced adductor mandibulae with no expansions of origins of this muscle. Finally, the reconstructions of ancestral character states allowed us to understand the forms and to infer behaviors of a hypothetical Serrasalmidae ancestor and, accordingly with our data, this fish probably was a durophagous omnivorous with a tendency to herbivory. This fish would have a subterminal mouth, two or more rows of mandibular and premaxillary teeth, elongated digestive tube, short lower jaw, elongated snout and medium-sized adductor mandibulae.



#### The Otophysi fossil record and the Weberian apparatus through time: new considerations on some synapomorphic characters

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The Otophysi fossil record is well known by complete species since the Paleocene, from which the characteristic anatomy of the group was already established. However the description of some species in marine sediments from the Cretaceous of Europe, Africa and South America led to a heated debates since the 80's years about the origin and diversification of the Otophysi clade. Mostly of the discussions dealt with the primary environment of the early diversification of the clade as well the erection of possibly missing link in this history. Despite the publication of enumerous articles related to the evolutionary origin of the Otophysi based on the molecular date and no longer the enhance of the knowledge of certain osteological key characters specially based on ontogenetic studies the known mesozoic fossil record was never reviewed or even observed by the neontologist. Recently, we started to review these fossils and surprinsigly none of them correspond to a crown taxa Otophysi. It seems guite obviously that the biogeographical history of the Otophysi clade as a whole and its subgroups is complex. And moreover, the comprehension of the acquisition of certain key characters, especially the Weberian Apparatus, remains obscure. The Weberian apparatus is a complex structure compounded of soft and bony elements including the modified anteriormost vertebrae. It includes the modified peri- and endolymphatic spaces of the inner ear, the modified anteriormost centra, spines and arches, the resulting Weberian ossicles, and the swimbladder divided into two chambers. It includes two series of small bony elements (claustrum, scaphium, intercalarium, tripus) linked by the interossicular ligament. Its presence is an otophysan character, but its morphology and anatomy vary depending on the taxa. Moreover, the homology of certain ossicles is still debated in modern taxa. In the fossil species, only the bony elements (centra and ossicles) are preserved and their homology with structures observed in modern fish is hard to establish when the Weberian apparatus does not correspond to a modern type. Except by Grande and de Pinna (2004) in a publication concerning the phylogenetic perspective about the evolution of the Weberian Apparatus (without observing most of the fossils), no one else has worked on this structure and even treated together extant and the controversial taxa from the Cretaceous. The presumed presence of this structure on these fossil taxa was used as an argument to considered them as primitive Otophysi and the probable responsible for the first radiation of the group. The aim of this work is to compare the typical outline of the Weberian Apparatus of extant species, which display the transformation series in the first four occipital vertebrae, with some fossil species otophysanlike. The preliminary results here presented suggest that the rise of the Weberian ossicles or loss of some components (e.g. 1° supraneural) may be independent and that such transformation/ arrangement cannot be treated as an unique complex structure.



#### Taxonomy of Scyliorhinus boa Goode & Bean, 1896 and S. haeckelli Miranda Ribeiro, 1907 (Elasmobranchii: Carcharhiniformes: Scyliorhinidae), with notes on their distribuitions

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The genus Scyliorhinus Blainville, 1816 currently includes 16 valid species of catsharks identified mainly on the basis of differences in color pattern. However, differences in color pattern are also due to sexual dimorphism, ontogenetic, individual and geographic variation. In addition there is a large degree of overlap in meristic and morphological characters of specimens from different localities, raising uncertainties in the identification of Scyliorhinus species. The Western Central Atlantic is the most diverse area for this genus, with six species of Scyliorhinus that are not clearly defined in revisionary works and identification keys. Scyliorhinus haeckelii Miranda Ribeiro, 1907 was considered a junior synonym of S. boa Goode & Bean, 1896 by some authors. More recently, Springer & Sadowsky, followed by Cadenat & Blanche, classified S. boa, S. haeckelii and S. meadi Springer, 1966, as subespecies of S. retifer Garman, 1881, considering only two species for the Western Central Atlantic Ocean: S. retifer and S torrei Howell-Rivero, 1936. Later, Springer erected S. boa, S. haeckelii and S. meadi to species rank, although without detailed descriptions. The lack of proper diagnoses of S. boa and S. haeckelii allied to uncertainties in their distribution has resulted in their misidentifications. In this study, specimens of both species collected from off New Orleans (Gulf of Mexico) to Suriname (Caribbean Sea) and from Northeastern Brazil to Northern Argentina were examined; measurements and meristics were taken and dissections were performed. Scyliorhinus boa can be distinguished from S. haeckelii mainly by the presence of dark spots on the body, smaller than the spiracle, delimiting and bordering seven to eight transverse bands (saddles), and absent within those bands (vs. dark spots of varied sizes on the body, not bordering saddles, and located predominantly within the saddles in S. haeckelii). Other characters such as shape of nasal flaps (anterior nasal flap with a concave posterior edge vs. straight edge in S. haeckelii), clasper morphology and morphometric characters (interdorsal space, internarial space and mouth width slightly greater in S. boa) also help to distinguish both species. Differences in clasper morphology include dermal denticle cover (dorsomedial surface and envelope not covered by dermal denticles vs. clasper completely covered in S. haeckelii), exorhipidion development (poorly developed in S. boa and well-developed in S. haeckelii), and presence of accessory marginal cartilage (absent vs. present in S. haeckelii). Tooth and vertebral counts overlap between both species. Scyliorhinus haeckelii occurs from Northern Argentina to Northeastern Brazil and S. boa is endemic to the Gulf of Mexico and Caribbean Sea. These results are part of an ongoing taxonomic review of the genus Scyliorhinus.

Keywords: catsharks; West Central Atlantic Ocean Financial support: Fapesp (process 2014/20316-5)



### Morphology and phylogenetic significance of the siphon sac in Scyliorhinidae (Chondrichthyes: Elasmobranchii: Carcharhiniformes)

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The family Scyliorhinidae is a diverse group of sharks that includes approximately 153 species and some waiting to be described. The systematics of the group is still controversial and recent works based on morphological and molecular data points out the paraphyly of this taxon. Since the last morphological hypothesis involving the interrelationships within Scyliorhinidae, few taxonomic reviews of its genera have been made but none of them significantly increased the number of morphological characters employed. A recent effort is being made to increase the number of characters, which are being reevaluated, expanded and redescribed. In this way, the present study highlights the morphological variation and importance of secondary sexual characters in elucidating the interrelationships within Scyliorhinidae employing the morphology of the siphon sac. Siphon sacs are subcutaneous muscular bladders under the abdominal region and are involved in the transferring of sperm products to the female. The siphon sacs open through the apopyle to the clasper groove but are coalesced anteriorly. The anterior extension of the siphon sac varies in terms of its length, and in some taxa, it does not surpass much of the anterior margin of the pelvic girdle (Cephaloscyllium, Holohalaelurus, Poroderma and Scyliorhinus), not reaching the level of the pectoral girdle as observed in other shark species within the family (Asymbolus, Atelomycterus, Figaro, Halaelurus and Haploblepharus). The siphon sac is also an inverted "Y" shaped structure that is bifurcated posteriorly and anteriorly coalesced or not. In cases in which the sac is not anteriorly coalesced, a thin longitudinal conjunctive tissue delimits each half of the sac. The anterior tip is blind and areatly enlarged is some species and occupying most of the abdomen/ belly, but narrow in others. Furthermore, the siphon sac narrows as it reaches the posterior portion of pelvic fins and enters the clasper. Similarly, the muscular walls of the siphon sac vary considerably, being visibly muscular in Holohalaelurus, Poroderma and Scyliorhinus for instance but thin and nearly transparent in Atelomycterus and Halaelurus. The morphology of the siphon sac is being analyzed in shark representatives outside Scyliorhinidae (Eridacnis, Hemigaleus, Iago, Proscyllium, and Loxodon), to indicate if some of the conditions observed so far may be apomorphic for some of the genera within the family. Lastly, characters related to the reproduction are proven to be relevant and should be employed in future phylogenetic studies concerning the interrelationships of elasmobranchs.

Key words: Scyliorhinidae; morphology; siphon sac; phylogeny Financial support: Fapesp (processes 2010/52677-6; 2012/22692-9; 2014/20316-5)



### Miniaturization in Trichomycteridae (Ostariophysi: Siluriformes)

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Neotropical freshwater miniature fishes are arbitrarily defined by the reduction of body size, with species not exceeding 26 mm of standard length or when the sexual maturity is reached in specimens with less than 20 mm SL. Moreover, when compared to its congeners, the miniaturization is usually accompanied by several paedomorphic characters, which are usually related to fusion, reduction or even loss of bones, reduced number of fin rays and truncate development of laterosensory system. Among the Neotropical fishes, the family Trichomycteridae shows many miniature species. Despite that, information about the evolutionary process that led to so many reduced or miniaturization of the species is scarce and was never subject of a major review. In this study, 37 species representing the eight subfamilies of Trichomycteridae, as well as the geners Scleronema and Ituglanis and the species included in the Trichomycterus hasemani clade were analyzed, including the 13 small species around 26 mm SL. It was identified, described and discussed the characters related to size reduction and miniaturization, which are strongly associated to osteological characters such as, reduction or absence of metapterigoid, reduction of frontals, parieto-supraoccipital, lower counts of odontodes in the opercular series, branchiostegal rays, ribs and fin rays counts. As results, we observed that Trichomycteridae presents species with a combination of structural simplifications associated to truncated development, mainly in the TSVSG clade, with several reductions and losses of bone elements, as well as branches and pores of the laterosensory system. However, reductions in this system may not necessarily be related to miniaturization in the family, since many non-miniature species of "Trichomycterinae", Tridentinae, Sarcoglanidinae, Stegophilinae and Vandelliinae show a pattern of pore and branch reductions very similar to each other, although the extreme truncation of the laterossensory system in many species of Glanapteryainae has been observed. Aditionally, several paedomorphic characters are identified and discussed and the variation evaluated in light of the three major phylogenetic hypotheses proposed for the Trichomycteridae.

Key words: Miniature; Paedomorphosis; Morphology; Neotropical; Freshwater Financial support: CNPq (WBW, processes 300940/2015–7 and 405144/2013–0), CAPES (ISC, process 2471906/14), and PROAP-CAPES (ISC, process 23073.017704/2015–58)



### Taxonomic study of *Moenkhausia cotinho* Eigenmann, 1908 (Teleostei: Ostariophysi: Characiformes)

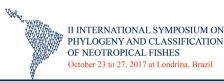
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Moenkhausia cotinho Eigenmann is currently considered a widely distributed species in the Amazon and Orinoco river basins and in the rivers of the Guyana. The species presents morphological variation throughout its distribution range, and previous authors have specifically reported variation in the number of perforated scales in the lateral line (lateral line complete vs. incomplete). In order to understand the taxonomic limits of M. cotinho, 1650 specimens of M. cotinho and other species similar morphologically, from Brazilian (INPA; MPEG; MZSUP; UNIR; ZUEC) and North American ichthyological collections (ANSP, CAS, FMNH, INHS, MCZ; and ROM) were analyzed based on data from external morphology, morphometric and meristic data. Moenkhausia cotinho differs from its congeners in the presence of a dark spot on the caudal peduncle (vs. dark spot absent), body depth 2.9 or greater in standard length (vs. body deeper, depth up to 2.7 in SL), three rows of scales below (vs. two or more than three) and five scales above the lateral line (vs. more than five). All specimens possess a complete lateral line and dentary teeth with five cups. Many specimens from drainages of Cuyuni, Mazaruni and Potaro have an incomplete lateral line and dentary teeth with seven cusps, with those two features varying independently from each other. In the absence of additional information that could indicate the recognition of distinct species, we tentatively identified all specimens from the Guyana as M. cotinho. In addition to M. cotinho, two new species were recognized: Moenkhausia sp. 1 from the Rio Beni, Rio Guaporé, Rio Madeira and Rio Madre de Dios drainages, and Moenkhausia sp. 2 from Rio Madeira, lower Rio Tapajós and lower Rio Trombetas. M. cotinho is still widely distributed in rivers of the Amazon and Orinoco basin. The new species are diagnosed on the basis of differences in color pattern, relative length of the pectoral fin, and number of teeth on the dentary. Hemmigrammus newbodi Fernandez-Yépez, previously suggested to be a synonym of M. cotinho, is herein considered a valid species based on differences in color pattern, by having an incomplete lateral, and a relatively long pectoral fin. This species presents a peripheral distribution in rivers of the upper Amazon and Orinoco basins. The synonymy of Knodus albolineatus Holly with M. cotinho is herein corroborated.

Key words: Taxonomy; Characidae; Freshwater; Neotropical Financial support: CAPES (PROEX) Project: 33002010027P5 - USP - Ciências Biológicas (Zoologia)



#### Morphological characterization of representatives of *Trichomycterus* Valenciennes from Rio Todos os Santos, Minas Gerais (Teleostei: Siluriformes: Trichomycteridae)

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Trichomycterus is the most diverse genus among approximately 40 in the family Trichomycteridae, with estimates pointing to a large number of unknown species. The great diversity of the genus and the potentiality of new species reflect the high degree of endemism of these fishes. Although Trichomycterus is not considered monophyletic, many species have been described and assigned to the genus in the last 15 years based on general morphological features. In Brazil, Trichomycterus is conspicually diverse with many species recently described from coastal streams between Southern Bahia and Northern Espírito Santo States, in addition to new species discovered in other major neotropical basins. In 2010, many specimens assigned to Trichomycterus were collected in seven sampling transects in Rio Todos os Santos, one of three tributaries of the upper Rio Mucuri in the city of Teófilo Otoni, MG. These individuals have a wide variation in body color, which did not allow the determination of how many species could be recognized in the samples at the time. To address this issue, 80 specimens were studied based on 30 morphometric measures using a digital caliper and 8 meristic data, under stereomicroscope. All measures were taken point to point, on the left side of specimens, whenever possible. Body color pattern was also observed. The data analysis was realized with MYSTAT 12 Version 12.02.00. Our data suggests the existence of a single form with no apparent sexual dimorphism. In comparison to literature referring to other species of Trichomycterus from northeastern coastal basins, the form found in Rio Todos os Santos is very similar to Trichomycterus pradensis. From the comparison of 13 morphometric data between specimens examined herein and those reported in the literature for Trichomycterus pradensis, 11 show values overlapped, but with subtle differences in many of them nonetheless. In general, even with the overlaps, specimens from Rio Todos os Santos are slightly longer and more slender, head length is longer, preanal, prepelvic and predorsal lengths are longer, and anal-fin and dorsal-fin bases relatively shorter. Snout length and width, however, are measures that differ considerably between the studied specimens and Trichomycterus pradensis, representing potential diagnostic characters between both forms. Moreover, the region where specimens of this study were collected is distant from the regions with confirmed records of T. pradensis which are generally closer to the shore and do not include the drainage of Rio Mucuri. Considering that species of Trichomycterus usually have a limited dispersal capacity and high degree of endemism, information gathered so far suggest that the form found in Rio Todos os Santos is an undescribed species and will be better investigated in the next steps of this study through an osteological approach.



#### Molecular and morphological phylogeny of the annual fish genus Austrolebias Costa 1998

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The anual fish genus Austrolebias is composed by 41 valid species mostly distributed in the La Plata basin and the Patos-Merin drainage, an a recently described species from the Amazon basin in Bolivia. Its monophily is based on morphological and molecular evidence. Austrolebias was originally described to include some species of the genus Cynolebias. Posteriorly, species of Megalebias (also originally in Cynolebias) were included in Austrolebias. In spite of extensive research, phylogenetic relationships within the genus are still unclear, where morphological differ from mitocondrial and nuclear phylogenies. Discrepancies may not only be originated by source of data but also from the number of species included in the analyses. For example, large size specimens form a monophyletic clade in morphological analyses, while they appear in two unrelated clades in mitocondrial trees. In this work we analysed the phylogeny of Austrolebias using two mitocondrial (16s, citochorme b) and two nuclear (RAG1, Glyt) markers (total 2600 bp), and 171 morphological characters, from 39 species of Austrolebias and 11 outgroups with representatives of most Rivulidae big clades. Trees were constructed based on each molecular marker and morphological characters analyzed separately; concatenated molecular markers; and total evidence using Bayesian (MrBayes) and Parsimony criteria (PAUP, TNT). Mitochondrial trees differed from nuclear trees, and both from morpholocal trees. In some analyses, the position of A. nigripinnis questions the monophyly of Austrolebias, since it appear in a basal polytomy together with species of the Cyolebias, Simpsonichthys, Spectrolebias, Simpsonichthys, and the remaining Austrolebias. On the other hand, the monophily of the large sized species is also questioned, these species are located in two different branches of the tree, suggesting an independent evolution of large size in the genus. Also species of higher altitudes from southern Brazil, previously considered to form a monophyletic clade, seem to be unrelated based on the present study. Differences in the trees inferred from different markers suggest that introgressive events may have a played an important role on the evolution of the genus. These results indicate that the phylogeny of Austrolebias is not resolved and more markers (nuclear, SNPs) have to be included. A complete analysis, using most of the species of the tribe Cynolebiasini, seems crutial to improve the knowledge of the evolution of these fishes.



#### Hidden diversity in Odontocharacidium Buckup, 1993 (Ostariophysi: Characiformes: Crenuchidae) based on the Cytochrome c Oxidase 1 gene

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Odontocharacidium has a single valid species, O. aphanes (Weitzman & Kanazawa, 1977), which is widely distributed in the Amazon basin, also occurring in the upper Río Orinoco basin. Its typeseries was originated from a Terra Firme stream, tributary of the Rio Negro, in Santa Isabel of Rio Negro. Given the increasing representativeness of the genus in collections from several additional localities in the Amazon and Orinoco basins, it was possible to identify considerable variation on color pattern and body shape among samples. However, it is still necessary to test if those differences are the result of geographical, phenotypic variation or if those samples could represented distinct evolutionary lineages. To test which scenarios was responsible for the variation observed among groups of specimens, Cytochrome c Oxidase 1 (COI) gene sequences of twenty Odontocharacidium samples originating from the Rio Branco, Rio Madeira, Rio Negro and Rio Solimões, and covering the variation observed in the different basins, were obtained, and compared with other Characidiinae genera as outgroups. Approximately 680 base pairs (bp) were amplified for the COI matrix, and analyzed employing the neighbor joining/Maximum likelihood algorithm. In the resulting distance tree four well supported (100% bootstrap values) distinct lineages of Odontocharacidium were recognized and arouped in two main clades: The first aroup includes specimens from the Rio Madeira (=Odontocharacidium sp. 2), itself the sister group of the specimens from the Rio Solimões near Tabatinga (=Odontocharacidium sp. 3). The second clade formed by individuals from the Rio Japurá (=Odontocharacidium "mancha umeral"), with the third, clustering specimens from the Rio Branco (upper Rio Negro), corresponding to the morphological form recognizable as Odontocharacidium aphanes, the type species. In the distance analyses the number of base substitutions in all sequences was 13.2% with a standard deviation of 1.1%. The clade distances values for all four evolutionary units recognized herein are above 2%, and are considered indicative of cladogenetic diversification. The genetic distance within the groups was 0.2% for O. aphanes; 0.6% for O. "mancha umeral" and 0% for O. sp.2 and O. sp.3, indicating that there is no significant structure at the species level within each species recognized. In the maximum likelihood tree all clades were retrieved as in the genetic distance analysis, indicating the separation of lineages in the genus. The support values of the first clade formed by O. "mancha umeral" was 100%, the clade being sister to all remaining Odontocharacidium. The clade recognized as O. aphanes is strongly supported with a value of 100% bootstrap, and closely related to the clades of O. sp.2 and O. sp.3, both with a value of 100% bootstrap. However, the node that supports the clades of O. aphanes, O. sp.2 and O. sp.3 has 24% bootstrap values, and is not a strongly supported relationship. A phylogenetic analysis with concatenated genes may recover this relationship with stronger bootstrap support. Despite the different interrelationships obtained in both analyzes, the same Odontocharacidium lineages were recognized, demonstrating that the diversity of the genus was poorly known until the present study.

Key words: Teleostei; Freshwater; Neotropical; Mitochondrial; Barcode Financial support: CNPq (process 130950/2016-5), FAPESP (process 2014/26508-3)



### Phylogenomic insights into the evolution of Trichomycteridae (Ostariophysi: Siluriformes)

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Trichomycteridae is one of the most diverse family of the suborder Loricarioidei with about 284 species divided in eight subfamilies (Copionodontinae, Trichogeninae, Trichomycterinae, Glanaptervainae, Sarcoalanidinae, Steapphilinae, Tridentinae, and Vandelliinae) and 41 aenera. Its members are widely distributed through the freshwaters of Central and South America, showing an exceptional ecological and phenotypic disparity. Morphologically the family can be distinguished from all catfishes, and even other teleosts by the synapomorphic condition of a highly modified opercular system, involving the interopercular and opercular bones. The family has been recognized and corroborated as monophyletic by different authors, as well as, its position within Loricarioidei. Different studies using morphological characters have been addressed to understand the phylogenetic relationships within each subfamily corroborating their monophyletic status; nevertheless, Trichomycterinae and its more diverse genus, Trichomycterus, have been historically recognized as non-monophyletic. Also, Ituglanis and Scleronema have been proposed as more closely related to other subfamilies, occupying phylogenetic positions that justify different subfamily status. Additionally, the increased knowledge of the taxonomic diversity of Glanapteryginae and Sarcoglanidinae has revealed a series of slightly differentiated taxa that are even difficult to confidently assign to one of these subfamilies. In order to assess the phylogenetic relationships of Trichomycteridae, we collected sequence data from ultraconserved elements (UCE) of the genome from 139 members of Trichomycteridae and 11 species of the outgroups. The data set included 852 UCE loci and the alignment contained 25% missing taxa per locus. We used a concatenated matrix to infer the relationships by Bayesian (B) and Maximum Likelihood (ML) inference. The results show a highly resolved phylogeny with broad agreement between B and ML trees. The results provide overwhelming support for the monophyletic status of Trichomycterinae including Ituglanis and Scleronema. Previous hypotheses of relationships among subfamilies, as the sister relationship between Copionodontinge and Trichogeninge as the sister clade to the remaining trichomycterids and the intrafamilial clade TSVSG are corroborated, while the monophyly of Glanaptervainae and Sarcoalanidinae is not recovered and unexpected novel relationships between members of both subfamilies are found with an astonishing biogeographic correspondence. Our results reveal that phylogenomic approximations offer enormous potential for resolving phylogenetic relationships within catfishes.

Keys words: catfishes, ultraconserved elements; evolution Financial support: FAPESP (Process 2014/06853-8, 2015/13382-4)



# From genome to chromosomes: insights into the evolutionary relationships and biogeography of Gondwana fishes (Teleostei: Osteoglossiformes)

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The order Osteoglossiformes has at least one representative in each of the southern hemisphere continents, except for Antarctica. While some families (Gymnarchidae, Mormyridae and Pantodontidae) are restricted to Africa, other ones (Osteoglossidae, Notopteridae and Arapaimidae) present a disconnected distribution, with species endemic to different continents. The investigative model fostered by this group, associated with its wide geographical distribution and its basal position in fish phylogeny, makes it an important object for systematic and evolutionary studies. However, cytogenetic and genomic records, in addition to being scarce, are restricted to a few species, with most studies characterizing only one or a few genomic regions, making it impossible to determine the evolutionary trends that occurred in this order. This lack of data is also associated with its wide distribution, with endemic aroups from different continents, hindering an integrative study that allows a globalized view of its evolutionary process. Here, we focused on the biogeography, genomic and chromosomal evolution of Osteoglossiformes species belonging to Arapaimidae and Notopteridae families. These fishes have a South America/Africa and Africa/Southeastern Asia distribution, respectively. Conventional (Giemsa staining and C-banding) and molecular cytogenetics approaches were used, including the chromosomal mapping of different repetitive DNA sequences, comparative genomic hybridization (CGH), and whole chromosome painting (WCP) with probes obtained from chromosome microdissection procedures. Additionally, a significant step in the exploration of genetic variability present in both families was given, using DART-Seq (Diversity Array Technology Sequencing) markers. The results highlighted contrasting modes of evolution between both families, since while Notopteridae fishes presented conserved karyotypes in almost all its species, both Arapaimidae species presented highly divergent ones. However, principal component analysis (PCA) revealed that Arapaimidae species present very low genetic differentiation among its species, different from the scenario found for Notopteridae ones. We also highlighted the impact of continental drift on the current diversity presented by these fishes, as well as the proposition of new biogeographical models to explain their actual geographical distribution.

Key words: Notopteridae, Arapaimidae, Dart-Seq Financial support: FAPESP (process 2016/22196-2) and CNPQ (304992/2015-1)



### Genetic characterization and phylogenetic relationships of the complex Gymnotus carapo

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Molecular analyzes recognized six clades in Gymnotus: pantherinus, coatesi, anguillaris, tiger, cylindricus and carapo. The carapo complex includes a variety of morphologically similar species or cryptic species of difficult taxonomic separation. There are few molecular studies for the carapo complex, including in particular the representatives of the floodplain of the upper Parana River The objective of this work was to characterize genetically and to establish the phylogenetic relationships between Gymnotus of the carapo complex of the upper Paraná River basin, using the molecular marker cytochrome C oxidase I (COI), comparing these specimens with specimens from the Doce River basin. Specimens of G. inaequilabiatus (5), G. sylvius (5), G. pantanal (8) was collected in the Paraná River Basin and Gymnotus sp. 1 (6) was collected in the Doce River Basin. Were included in the study 46 sequences of Gymnotus (BOLD) and 17 sequences of Gymnotus (Genbank) totaling 13 species. The DNA of the specimens was extracted using phenol/chloroform based methodology, the COI gene was amplified by PCR and the determination of the nucleotide sequences proceeded in ABI 3500 automatic sequencer. The 626 bp sequences were aligned and edited in the BIOEDIT and MEGA6 programs and phylogenetics analyzes performed on the RAXml from the maximum likelihood and on the BEAST from the Bayesian method. Analyzes of species delimitation were performed by GMYC and PTP models. The greatest interspecific genetic distance value was 20% between G. ineaguilabiatus and G. jonasi and between G. jonasi and Gymnotus sp. The lowest interspecific genetic distance value was 2% between G. chaviro and G. sylvius and between Gymnotus sp.1 and G. ineaguilabiatus The PTP delimited 12 possible OTUS and the GMYC ten possible OTUS. The specimens of Gymnotus sp.1 from the Doce River basin were grouped with species Gymnotus sp. of the Guaíba River being considered the same species by the GMYC method and different species by the PTP method with genetic distance of 4.9%. Still within this first cluster of the clade carapo, together with the species G. ineaguilabiatus there are four specimens sequenced in this work and morphologically identified as G. sylvius, but present COI gene sequences similar to those presented for G. inaequilabiatus species and are characterized by the delimitation of species PTP and GMYC as G. inaequilabiatus. The species G. sylvius, G. inaequilabiatus, G. carapo, G.pantanal, G. sylvius, G. chaviro, Gymnotus sp.1 from the Doce River and Gymnotus sp. of the Guaíba River represent the clade Carapo. The data obtained corroborate some previous studies of the literature that showed evolutionary relations and the monophyly among the members of the genus Gymnotus. Based on our data, the Coatesi clade (formed by G. Coatesi and G. jonasi) represents the oldest group among the Gymnotus analyzed here, followed by the clade pantherinus, and later the clado cylindricus. The clade carapo shows itself formed in a recent divergence as already proposed by other works.



### Geomorphology influencing the diversification of fish in small-order rivers of neighbouring basins

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Trichomycterus species occur in the main drainage basins of southern Brazil. T. davisi have a restricted distribution, specifically in the Iguaçu and Ribeira de Iguape river basins. However, specimens with the morphological characteristics of T. davisi has been reported in the Paranapanema River basin and at various sites in the Ivaí, Piquiri and Tibagi river basins. To test whether the uplift of the Serra da Esperança and the Ponta Grossa Arch in the Serra Geral resulted in ichthyofaunistic changes in adjacent basins, we describe the phylogeographic structure among populations of Trichomycterus collected from five hydrographical basins in southern Brazil: Iguacu River basin; Ivaí River basin; Piguiri River basin; Tibagi River basin and Ribeira de Iguape River basin. DNA from the specimens was extracted with commercial Invitrogen kit, the COI gene was PCR amplified and nucleotide sequence determination performed on the ABI 3500 automatic sequencer. The 603 bp sequences were aligned by algorithm Clustal W 1.6 implemented in MEGA 6. An ultrametric tree was built with BEAST2 and the program SplitsTree was used to construct a neighbour-net. Analyzes of species delimitation were performed by GMYC model. According to the ultrametric tree, most of the samples from the Iguaçu and Piquiri river basins were mixed. However, a group with three haplotypes of the Iguacu River basin proved to be different from the other samples. All samples from the Ribeira de Iguape site were grouped with high support precisely where the T. davisi site type haplotype was included. Samples from the Tibagi and Ivaí river basins also formed haplogroups with good support, although two haplotypes from Ivaí River basin were grouped with samples from the lauacu and Piquiri river basins. Neighbour-net also revealed the distinction between the sampled river basins and evidenced the high variability of sequences in the Iguaçu and Piquiri river basins. Characteristics diagnosed in the species T. stawiarski could be identified in some specimens from the Iguaçu + Piquiri haplogroup. The other haplogroups featured morphological characteristics of T. davisi and thus confirmed previous analyses. Six possible OTUs were delimited by GMYC model and estimated time of diversification was 1.74 My. Molecular analyses show that, beside T. davisi sensu stricto, which only occurs next to the type-locality and in the Ribeira do Iguape basin, at least five new species occur in the hydrographic basins analysed. The results actually increase the number of Trichomycterus species already described for the regions under analysis. Further, they also show the occurrence of T. stawiarski in the Piquiri and Ivaí river basins and thus widen the distribution of this species that was thought to be endemic to the laugeu River basin. Molecular analyses support the interpretation that genetic structure in T. davisi is explained by tectonic events intrinsic to the areas of influence of Serra da Esperança and the Ponta Grossa Arch.



#### Development and characterization of polymorphic microsatellites loci in the endangered catfish Pseudoplatystoma magdaleniatum (Siluriformes: Pimelodidae)

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The Neotropical freshwater catfish *Pseudoplatystoma magdaleniatum* Buitrago-Suarez & Burr, 2007 is an endemic species that occurs in the Magdalena, Cauca and San Jorge river basins. This species is listed as Endangered (EN) by the UICN mainly because over the last decades has experienced drastic population reductions mainly due to overfishing and habitat degradation. In this work, we developed for the first time a set of 20 microsatellite *loci* by using MiSeq Illumina Next generation platform to explore the genetic structure of *P. magdaleniatum* in populations from Magdalena and Cauca Rivers. Our AMOVA results showed a lack of population differentiation among populations. Besides, we found that average values of alleles per locus (Na) and expected heterozigosity were higher than those reported for Neotropical Siluriformes. This results are important for development of management and conservation policies in this valuable species.

Key words: Molecular marker, Next-generation sequencing, Genetic structure, Population genetics Financial support: Universidad Nacional de Colombia, Integral S.A., 19th September 2013 and Empresas Públicas de Medellín, Grant CT-2013-002443.



#### Contributing to the species delimitation of Neotropical characins: Implications for taxonomists on the classification and phylogeny of complex groups in ichthyological collections

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Accurately delimiting species of characins for identification and cataloging is crucial for studies of phylogeny, phylogeography, ecology, conservation and biogeography in Neotropical basins. The limits of species and genera in the Characidae family are controversial due to its uncertain phylogenetic relationships, high level of morphological homoplasy and the use of ambiguous morphological characteristics for descriptions not based on autapomorphies. This study compared the putative species recovered using barcoding models (Generalized Mixed Yule Coalescent, GMYC; Poisson Tree Processes, PTP; and Automated Barcode Gap Discovery, ABGD) for Bryconamericus, Eretmobrycon, Knodus and Hemibrycon (Stevardiinae: Characidae) with those established by morphology alone to examine species boundaries within the classification and phylogeny framework for Characidae. The results reveal a high species delimitation success rate by the implemented methods and by following a workflow in the context of integrative taxonomy. Circumscriptions to putative species using the three methods were congruent with the morphological sorting for 83 morphospecies, while 48 morphospecies were only molecularly congruent, which indicates that together the three molecular methods recovered the same grouping for 131 OTUs (87%). Sixty erroneous identifications revealed the difficulty and complexity surrounding the delimitation and taxonomy of these genera in biological collections. Based on the obtained results, it is possible to infer the generic boundaries for clusters in the trees (GMYC and PTP) that are consistent with some of the recently proposed taxonomic changes to Characidae. However, we suggest taxonomic changes for thirteen species, and we also find a high degree of hidden diversity, resulting in nine strong candidates from different basins for classification as new species. We propose a workflow as an objective, fast and accurate way to delimit species from mitochondrial DNA sequences and to help clarify the classification and phylogeny of these conflicting taxa.

#### Key words: Characidae; Stevardiinae; Integrative Taxonomy

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# New species of *Microcharacidium* (Ostariophysi: Characiformes: Crenuchidae)

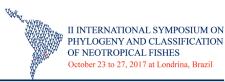
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The genus Microcharacidium Buckup 1993 currently includes three small-sized Characiformes: Microcharacidium electricides, M. gnomus and M. weitzmani. The genus is widely distributed throughout cis-andean South America, with representatives occurring in the upper Orinoco, most Rio Amazonas basin, and adjacent coastal basins draining from the Guiana and Brazilian shields. Herein a new species of the genus is described from the Rio Negro, Rio Tapajós and Rio Trombetas as the result of an ongoing revisionary and phylogenetic project focusing on the genus. The new species shares with all congeners the diagnostic features of the genus: the presence of 17 major rays in the caudal fin, fusion of postcleithrum 1 and 2, the posterior constriction of the gap between the anterior lamellae of pelvic bones and the well defined aspect of the brown medio-lateral band which is wide and with sharp edges. It differs from all congeners by the presence of 12 scales around caudal peduncle (vs. 10 in M. eleotrioides and M. weitzmani, and 14 in M. gnomus) and by the presence of seven black bands on the body connected dorsally and ventrally (vs. 8-9 ventrally incomplete bands). The new species further differs from M. weitzmani and M. gnomus by the presence of two short dark stripes on the ventral margin of the eye, extending to the maxilla (vs. one suborbital stripe). The new species further differs from M. electricides by the absence of lateral cusps on all teeth (vs. teeth tricuspid), and by the fewer perforated scales on the lateral line series (3 - 4 vs. 5); from M. gnomus by the presence of less dorsal-fin rays (10 - 11 vs. 12), the presence of less scales both along lateral line (3 - 4 vs. 7 - 11), and on the lateral line series (21 - 26 vs. 30 - 31); and from M. weitzmani by the presence of fewer anal-fin rays (5–6 vs. 7–8), besides reaching larger sizes.

Key words: Taxonomy; Characidiinae; Neotropical Financial support: CNPq (133586/2017-0)



#### Phylogenetic interrelationships of members of Potamotrygoninae (Garman, 1877): a morphological perspective (Chondrichthyes: Myliobatiformes)

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The family Potamotrygonidae currently encompasses two subfamilies: Styracurinae and Potamotrygoninae. Styracurinae includes a single genus (Styracura) with two species (Styracura schmardae and Styracura pacifica), and Potamotrygoninae four genera (Heliotrygon Paratrygon, Plesiotrygon and Potamotrygon), being the genus Potamotrygon the most speciose (with 27 valid species). Styracurinae embraces marine/euryhaline species that were previously known as Amphi-American Himanturas, whereas Potamotrygoninae includes exclusively freshwater representatives distributed solely in South American river basins. This newly proposed taxonomic arrangement with Styracurinae considered as a sister taxon to Potamotrygoninge was supported by several and recent molecular studies but also by morphological evidence supporting a closer relationship between these two subfamilies in relation to other dasyatids. Also, potamotrygonins are widely accepted as a monophyletic group, and supported by the following morphological and physiological characters: a very elongated prepelvic process in the pelvic girdle, hyomandibular-meckelian ligament bearing angular cartilages, a very reduced rectal gland, and low concentration of urea in the body fluids. Despite the close relationship presented by these two subfamilies, the interrelationships within Potamotrygoninae was never appropriately addressed in species level. Few phylogenies based on molecular data and including only part of the species within potamotrygonins were proposed, however, a morphologically based hypothesis was never conducted. Other morphological hypotheses focused mainly on the order Myliobatiformes and only intergeneric relationships were considered. In recent years a series of revisions of members of Potamotrygoninae and the description of new species of this subfamily not only raised but also increased the number of important morphological characters that were never tested in a phylogenetic analysis. In this manner, several characters of the external morphology and internal anatomy are being raised, codified and incorporated in an exclusively morphological matrix, including coloration, dermal denticles, dentition, ventral lateral line canals, skeleton, musculature, and also morphometric and meristic data. The aim of the present study is to better understand the interrelationships between the species of Potamotrygoninae based solely on morphological characters and including representatives of the subfamily Styracurinae as an outgroup. Previously proposed characters suggesting a close relationship among some of its members will also be tested to prove if they are in fact synapomorphous for these groups. The recovered tree will be confronted with those retrieved from molecular based analyses to identify where they agree or conflict.

Key words: Freshwater stingrays; phylogeny; morphology; Potamotrygoninae; Neotropical Financial support: Fapesp (processes 2007/52193-6; 2010/52677-6; 2011/23420-0; 2012/22692-9)



### Diversification times in the Pimelodidae: An assessment of fossils as node calibrations in a Neotropical fish group

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The Pimelodidae is a family of Neotropical catfishes inhabiting freshwater habitats through most of South and Central America. The family has one of the best-preserved fossil records in the Neotropics comprising most of the Cenozoic that has been applied to studies of divergence time and biogeography. Its phylogenetic relationships have been extensively studied, and ongoing studies are further improving our knowledge on its taxonomy and systematics. Given the these advantages, the Pimelodidae is one of the best Neotropical freshwater-fish models for studying divergence times under a bayesian framework as it behaves as a bestcase setting in contrast to most other Neotropical freshwater fish groups that usually have one or two fossil occurrences available. Estimates of divergence time are possible when coupling prior knowledge of dated fossil occurrences along with diversification models and phylogenetic inference since the fossil information can be trated as a prior on time provided that a probability density function (PDF) is incorporated into the analysis of time calibration. One of the most popular PDFs is the lognormal distribution, that postulates a minimum time of diversitication on a node but allows inference to proceed back in time when coupled to the diversitication model. However, definition of such priors directly on the node is inconsistent with the meaning of fossil dating as it asssumes that fossil age uncertainty applies to the node being dated. In addition to being logically inconsistent, its effect on prior sensitivity is seldom discussed in studies of divergence time. The goal of the present study is to explore the impact of prior especification on several aspects of inference such as: Fossil age prior and effective time prior, posterior sensitivity to effective time priors, the effect of different PDFs specified on the nodes, and the behavior of fossil calibration priors given their position in a phylogenetic tree. It has been found that Pimelodid calibration priors tend to differ from effective time priors as suggested in theoretical general studies, showing that the diversitication model has an important effect on the time prior. Using only one calibration point generally results in strong posterior sensitivity to the effective prior, implying that an analysis using one calibration point is subject to strong bias. There are difference between specifying different PDFs on a given node, what implies that such choice is not trivial and should be carefully addressed in a study of divergence time estimates. A possible solution to these difficulties is the specification of a hyper-prior setting in which the uncertainty of fossil age best agrees with its logical role as affecting the location parameter instead of the node itself. These results imply that studies where only one or two calibration fossil occurrences are available should be done only when exceptional justifications are taken into account, as there seems to be no analytical or empirical solution to the strong effect of priors on the posterior estimates.

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### Evolution of the cranial musculature in the Carangidae (Teleostei: Percomorphacea: Carangiformes): preliminary results

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The Carangidae has been historically recognized as a monophyletic lineage, a hypothesis supported by both morphological and molecular data. Species of the family are currently allocated in four tribes: Trachinotini, Scomberoidini, Naucratini, and Caranaini. The most recent cladistic analysis of the Carangidae based on morphological data was published almost 30 years ago and employed a surprisingly low number of characters. A single character from the musculature is included that study, the presence of A<sub>1</sub>' division on the adductor mandibulae, which was recovered as a synapomorphy for the clade Naucratini + Carangini. The total amount of available data on the carangiform musculature is negligible when compared with the osteological information reported in the literature for the family. The present study aims to conduct a comparative analysis of the cranial muscles of representatives of the four carangid tribes plus some closely related outgroups (Nematistiidae and Coryphaenidae) and generate new myological data to be included in a combined cladistic analysis. The mandibular, opercular, and hyopalatal musculature were described and illustrated in most taxa and some preliminary phylogenetic inferences can be advanced. The presence of a ventromedial section of the levator arcus palatini attached to the medial face of the suspensorium seems to be a synapomorphy for the Carangidae. Number of divisions in the cheek section (segmentum facialis) of the adductor mandibulae varies from two, in Trachinotini and Scomberoidini, three, in Naucratini and Carangini, and up to four in some members of the Carangini. The so-called A,' subdivision of the adductor mandibulae corresponds to the promalaris and its presence is confirmed to be diagnostic for naucratins and carangins. An explicit phylogenetic analysis including all new myological data along with the osteological characters reported in previous studies will be conducted later. The results of this project are expected to contribute for future studies on both myology and systematics of carangids and other related groups.

Keywords: Myology, Comparative Anatomy, Systematics Financial support: CAPES



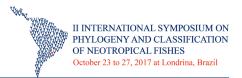
### Molecular phylogeny of the superfamily Loricarioidea (Ostariophysi: Siluriformes) using ultra conserved elements

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The Loricarioidea is the largest monophyletic group of catfishes endemic to the Neotropics with about 1,556 valid species assigned to six family-level taxa: Astroblepidae (81 species), Callichthyidae (219 species), Loricariidae (951 species), Nematogenyidae (1 species), Scoloplacidae (6 species) and Trichomycteridae (298 species). Loricarioid fishes are widely distributed throughout freshwater habitats in tropical South America and southern Central America. In addition to their exceptional species richness, loricarioids exhibit a wide range of morphological, physiological and ecological specializations, occupying many habitats and trophic levels, including obligate herbivores, parasites (e.g., Vandellia) and the only known wood-eating fish species (Panague). The sucker-shaped mouth and spoon-shaped teeth morphologies of loricariids allow many species to forage on algae and cling to substrate surfaces. Within Loricarioidea the family-level phylogeny is well established from both morphological and molecular datasets, however the species-level relationships within each family, mainly at subfamilies level, remain poorly documented. In the present study, we included 263 specimens representing all the six families of Loricarioidea (i.e., Loricariidae, Scoloplacidae, Astroblepidae, Callichthyidae, Trichomycteridae and Nematogenyidae). Poptella paraguayensis and Hyphessobrycon compressus (order Characiformes) were used as outgroups. All analyzes were performed using the package "phyluce" that implement a wide range of phylogenomic programs. We used "abyss" algorithm to assemble the data and the "matt" aligner to do the alignment among the UCEs (Ultraconserved Elements) loci. All data were edge trimming including the maximum variation found in UCEs sequences. We used the RAXML algorithm to estimate the phylogenetic tree. About 2,500 loci of UCEs were sequenced using the Illumina method generating a matrix with about 700,000 characters. The bootstrap method was used to estimate the support of all nodes of the phylogeny. Our results showed that the six families analyzed formed monophyletic groups, with Scoloplacidae close related with Astroblepidae, and these two families forming sister group to Loricariidae. The members of the family Trichomycteridae were the first to diverge inside Loricarioidea, followed by members of Nematogenyidae. The family Callichthyidae formed sister group to Scoloplacidae+ Astroblepidae+Loricariidae. All nodes of the tree have high values of statistical support (100% of bootstrap for all node of the phylogeny). The close relationship of Astroblepidae forming sister group to Scoloplacidae, and of Trichomycteridae being the first aroup to diverge inside Loricarioidea contradict previous morphological hypothesizes which Astroblepidae form sister group to Loricariidae and Trichomycteridae form sister group with Nematogenyidae.



# Phylogenomics of the family Pseudopimelodidae (Ostariophysi: Siluriformes)

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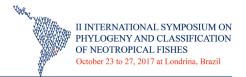
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The family Pseudopimelodidae is a monophyletic group of catfishes widely distributed in Neotropical freshwaters with roughly 50 valid species assigned to seven genera: Batrochoglanis (four species), Cephalosilurus (four species), Cruciglanis (one species), Lophiosilurus (one species), Microglanis (31 species), Pseudopimelodus (four species), and Rhyacoglanis (five species). The species-level relationships within this aroup remain poorly documented. In the present study, we analyzed 30 species of Pseudopimelodidae representing six out of seven genera. We used a new class of molecular markers based on ultraconserved elements (UCEs) that contains variable flank regions with high phylogenetic informativeness. All phylogenetic analyzes were performed using the package Phyluce that implements a wide range of phylogenomic programs. We used ABYSS algorithm to assemble the data and the MAFFT to align around 2.500 UCE loci. All data were edge trimmed including the maximum variation found in UCEs sequences. We use the RAXML algorithm to estimate the best phylogenetic tree and used replicates of bootstraps to test support of nodes. The matrix contains 546.835 characters. The phylogeny corroborates the monophyly of Pseudopimelodidae being the sister group of Pimelodidae in agreement with previous morphological and molecular hypotheses. Pseudopimelodidae forms three main clades: the first clade to diverge is composed by Pseudopimelodus and the monophyletic Rhyacoglanis. The second clade to diverge contains Cephalosilurus and Lophiosilurus, however Cephalosilurus did not appears as a monophyletic group, with C. fowleri more closely related with L. alexandrii than C. apurensis. The third and most derived group within Pseudopimelodidae is composed by Batrochoglanis and Microglanis. The first species to diversify within B. is B. raninus and the species B. villosus did not form a monophyletic group, suggesting that more species within this complex may exist. There are four aroups within Microglanis: the first is composed by two species from Orinoco basin, the second is composed by species from Atlantic coastal rivers (M. cottoides, M. paraybae, M. cibelae, M. malabarbae), the third group is formed by M. oliveirai and M. leptostriatus and the fourth group is composed by Microglanis gr. poecilus from the Amazon basin. The phylogeny of Pseudopimelodidae presented herein permits to recognize monophyletic units and advances our overall knowledge of evolutionary relationships within the family.

Key words: Systematics; Catfish; Ultraconserved elements Financial support: FAPESP and CNPq (process 150027/2017-6)



#### Phylogenetic analysis of the subfamily Stevardiinae, Gill 1858 based on morphological and molecular data (Teleostei: Characiformes: Characidae)

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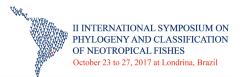
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Stevardiinae is currently considered monophyletic based on molecular studies (Oliveira *et al.*, 2011; Thomaz *et al.*, 2015). To perform the analysis aiming at testing the monophyly of the subfamily and the internal relationships of the included taxa using a different approach we used 175 characters from external morphology and also from primary and secondary sexual characters observed in 49 specimens. These data were concatenated with the molecular characters provided by Thomaz *et al.* (2015). The combined matrix was analysed under maximum parsimony and the resulting tree has 5,439 steps and "fit"= 130. The topology obtained confirms the monophyletic condition of the Stevardiinae, represented by 7 major clades having *Markiana nigripinnis* as the sister taxon of all stevardiines. Within Stevardiinae, *Phenacobrycon* and *Argopleura chocoensis* are the successive sister taxa to all remaining stevardiine clades. *Mimagoniates* and *Glandulocauda*, representing the "Glandulocaudinae" of Menezes & Weitzman (2009), were recovered as the sister group of the remaining stevardiines where the "Stevardiinae" of Menezes & Weitzman are the basal group of the other stevardiin clades. Based on the unequivocal synapomorphies at the nodes a hierarchical classification of the clades at different taxonomical levels is proposed.

Keywords: Systematics, Phylogeny, Parsimony, Neotropical region Financial support:



#### Shortfalls in the knowledge on Neotropical Auchenipteridae fishes

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The Neotropics harbors the greatest diversity of freshwater fishes on Earth. Despite recent advances in characterizing this high-diverse group, key shortfalls in the knowledge of the total number of species, geographical distribution and evolution of this group remain poorly explored. Quantifying the extent of each of these shortfalls is essential for planning future research and guiding conservation efforts. This study aims to guantify three knowledge shortfalls regarding: 1) taxonomical knowledge (named Linnean shortfall), 2) geographical distribution (Wallacean shortfall), and 3) evolutionary relationships (Darwinian shortfall) of the Auchenipteridae catfishes. This family is widely distributed across the Neotropics and presents considerable morphological and niche variability. The current status of the Linnean shortfall was assessed by analyzing the historical accumulation of 123 valid species name. For addressing the Wallacean shortfall, we first compiled a database containing 10,336 records of Auchenipterids catfishes collected over ca. 250 years. From these, only 5,393 (52.2%) were deemed suitable after a stringent data filtering. Using these data we estimated inventory completeness by calculating the ratio between the number of species observed with the estimate obtained through species richness estimators and species accumulation curves. Inventory completeness was calculated using a grid of one degree resolution and cells with at least 10 records. The Wallacean shortfall was also evaluated by the accumulation of records. of the most recorded species – such as Trachelyopterus galeatus, Auchenipterichthys longimanus, Ageneiosus ucayalensis, and A. inermis - over time. Finally, to quantify the Darwinian shortfall, we measured the dissimilarity between available phylogenies of Auchenipteridae (Britski, Ferraris, Curran, Royero, Akama, Ribeiro, and Birindelli). For each pair of phylogenies, we explore the correlation of patristic distance among common genera. Our results showed a steep slope in the historical accumulation curve for valid species name over the whole time period (1766 to 2017), indicating that a substantial proportion of taxa may remain to be described. The analysis of inventory completeness revealed that Auchenipterid catfishes remain largely under-collected; only 30% of the cells that have inventory completeness reliably estimated reached a completeness of 75%. Most of these well-sampled areas occur near wellestablished research centers, such as Manaus and Belém (Brazil). Moreover, the temporal variation in the accumulated number of records for the most recorded species indicated that the yearly rate of increase in the number of database records is generally slow before 1980, increasing conspicuously after this date. Finally, analysis of dissimilarity between phylogenies showed that recent topologies tend to be more similar to each other, suggesting a tendency toward a robust phylogenetic hypothesis of this family. However, despite this tendency there is still much work to be done, as more species are expected to be described in the future and most of the already described species lack reliable information regarding their geographical distribution. Reducing these gaps will require a concerted effort of taxonomists, ecologists, and biogeographers.

Key words: Taxonomy; Geographic distribution; Evolution; Catfish; Knowledge shortfalls Financial support: TMSF - CAPES (process 88881.134719/2016-01); JS - CNPq (process 150824/2015-7); JH – CNPq (PVE process 314523/2014-6); LFAM – CNPq (process 305017/2016-0) and CAPES (process 88881.119097/2016-01)



### Evaluating the taxonomy of the Auchenipteridae Family in online repositories

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Free and open access to primary biodiversity data is essential to enable effective decision-making concerning the biodiversity conservation. In this sense, taxonomic accuracy should be carefully treated, because is crucial to data quality in online repositories of species occurrence data. However, taxonomy is often unreliable, especially for Neotropical fishes, which may lack critical revisions of voucher specimens. In this study we focused on the errors and inaccuracies contained in the Global Biodiversity Information Facility (GBIF) and Sistema de Informação Distribuído para Coleções Biológicas (speciesLink) about taxonomy of the Auchenipteridae family, which has recently been targets of taxonomic reviews and descriptions of new species. First, the records identification was evaluated for different taxonomic level: family, genus, or species. Second, records which were identified to the species level were classified as valid (when no taxonomic error was found) or inaccurate. Third, and finally, inaccurate species records were categorized into: 1) Inaccurate: presence of arguments such as "aff.", "cf.", "ar."; 2) Nonexistent combination: uses of a nonexistent combination, but clearly assigned an undue genus to one species, or vice versa, when in fact it belongs to another taxon; 3) Nonexistent species: non-existent combination and when it was not possible to associate with possible spelling mistakes or misattribution of species or genus; 4) Not Auchenipteridae: assigned to a species that was not belong to the Auchenipteridae Family; 5) Outdated combination: a given taxonomic designation that no longer belongs to the cited genus. This occurs when a taxonomic revision revalidates a genus or describes a new one, relocating existing species. 6) Species inquirenda: species of doubtful identity needing further investigation; and 7) Synonym: when a given taxon has two or more distinct nomenclature proposed by different authors. In this case, the first published name is considered valid. A total of 9,148 records were obtained for Auchenipteridae fishes, of which 4,983 were from speciesLink and 4,165 from GBIF. Records identified at species level summed 7,276, which represented 79.5% of the total, followed by identification at genus (1,837 records; 20.1%) and family level (35 records; 0.4%). Within identified species 6,039 records were considered valid (83.0%) and 1,237 (17.0%) had some taxonomic inaccuracy. These inaccurate records were composed by: Outdated combination (720 records; 58.2%), Synonym (190; 15.4%), Inaccurate (165; 13.3%), Species inquirenda (94; 7.6%), Nonexistent combination (39; 3.2%), Not Auchenipteridae (28; 2.3%), and Nonexistent species (1; 0.1%). Combining all categories that somehow do not contemplate the most current taxonomy, inaccurate records totaled more than 3,000, representing more than 30% of the total records. Despite the essentiality of the repositories online data in recent macroecological studies, these deficiencies demonstrated how important it is for biodiversity researchers to be cognizant of current scientific literature, nomenclature and species identification. Despite considering one fish family, our results may demonstrate that zoological collections should be constantly updated for the taxonomic purpose, especially when dealing with megadiverse groups such as fish. We also argue that more investments should be made in training new specialists, and in promoting taxonomic review projects.

Key words: Fish; Systematic; Biodiversity data; Taxonomic accuracy Financial support: TMSF – CAPES (process 88881.134719/2016-01); JH – CNPq (PVE process 314523/2014-6); LFAM – CNPq (process 305017/2016-0) and CAPES (process 88881.119097/2016-01)



# Addressing the Achilles heel of biodiversity: UCEs indicate cryptic speciation in the neotropical electric fish *Eigenmannia macrops*

Brandon T. Waltz, Fernando Alda, Maxwell Bernt, Prosanta Chakrabarty, James S. Albert

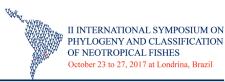
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Understanding biodiversity is fundamental to elucidating the relationships of life on Earth and understanding evolutionary patterns and processes. Evolutionary analyses and reliable measures of species richness depend on accurate alpha taxonomy; therefore, it is necessary to understand the nature of variation as the basis for correct species delimitation. However, the vast majority of species have been described based solely on morphology, leaving out a key source of variation and leading to the "Achilles heel" of biodiversity: undocumented cryptic diversity. Metapopulations face a variety of evolutionary processes contributing to lineage divergence and independence, including processes traditional morphological practices would be unable to recognize. Environmental conditions may exert strong stabilizing selection on aspects of morphology, resulting in maintenance of similar morphology throughout and after speciation. Additionally, and likely alongside stabilizing selection, the diversification of non-visual mating cues can result in undetected diversity. Eigenmannia macrops is a freshwater fish present in abundance throughout three large Neotropical river basins and possesses a weakly-electric signal used for non-visual communicative, reproductive, and foraging behaviors, making E. macrops an excellent candidate species to assess cryptic diversity. Furthermore, preliminary genetic analyses suggest the presence of undocumented diversity within the nominal taxon E. macrops. Here we present a case study examining the evolutionary patterns associated with cryptic speciation. Utilizing 101 samples from across the Amazon, Orinoco, and Essequibo basins, we examine the evolutionary history of Eigenmannia macrops using next generation target enrichment methods coupled with Bayesian species delimitation and validation methods. We also utilize high resolution computed tomography scans to examine the osteology of specimens from across the current species range, detecting internal morphological divergence. We find that there is substantial genetic evidence that cryptic species exist within what is currently considered E. macrops. We also conclude that studies involving species exhibiting non-visual signals and/or constituting widespread species would strongly benefit from molecular analyses, especially using genomic markers.

Key words: biodiversity; morphological stasis; phylogenomics; phylogeography

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#### Reproducibility crisis of species delimitation

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Species richness – the number of distinct evolutionary lineages that comprise a regional assemblage or higher taxon – is a fundamental measure of biodiversity. Accurate identification of species boundaries is a logical prerequisite to documenting genetic, physiological, and ecological diversity in fields as varied as infectious disease, physiological ecology, and conservation genetics. Proper taxonomic practices are important, as species names have legal status in conservation and management. Additionally, the Linnaean shortfall describes the state of modern taxonomy in which a large fraction of living species on Earth have not yet been formally described, and there is increasing urgency for rapid designation of species names as the biodiversity crisis accelerates in the Anthropocene. Taxonomists therefore face the enormous challenge of documenting undescribed species before they go extinct. Modern molecular and computational methods allow taxonomists to test hypotheses of species boundaries with greater precision. However, the urgency to identify undescribed species must be balanced against the rising concern among biologists regarding the reproducibility of results in scientific research, and precision in species delimitation has been evaluated only for a few taxa. Recent estimates indicate that results from roughly 70% of all scientific studies cannot be replicated, whether due to methodological inconsistencies, investigator biases, or other statistical/investigator errors; therefore, reproducibility remains a central issue in metaanalyses using datasets from disparate sources. More than 100 new species of Neotropical fishes have been described every year since 2000, and total species richness shows no sign of approaching an asymptote. At the same time, Neotropical fishes are increasingly impacted by a host of human activities. Given their diversity, dynamic taxonomy and vulnerability, Neotropical fishes are excellent candidates for assessment of taxonomic reproducibility. Here we examine the reproducibility (i.e. precision, not accuracy) of species delimitation in Neotropical fishes based on morphological and molecular datasets for four genera across four orders, analyzed by 40 taxonomic experts. We found a high level of discord (33%) in species delimitations among the experts. This finding highlights the need for greater transparency, rigor, and resources for taxonomists undertaking the monumental task of documenting biodiversity in the Anthropocene.

Key words: Taxonomy, Precision, Neotropical, Freshwater fishes Financial support: US National Science Foundation awards DEB 0614334, 0741450 and 1354511



#### Diversification of lineages and functional phenotypes in the Neotropical electric fish Apteronotidae

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Neotropical freshwaters constitute the world's largest continental aquatic biota, with more than 8,000 fish species, or approximately 12% of all vertebrate species on Earth. Yet the ecological and evolutionary processes underlying the formation and maintenance of this exceptional ecosystem remain poorly understood, in particular how functional characteristics promote and constrain the processes of biotic diversification. One widely-cited model of adaptive diversification in African rift-lake cichlids (Kocher) proposes diversification in three stages; habitat specialization (H), trophic specialization (T), and sexual communication (S). This model makes explicit predictions about the sequence of evolution in these phenotypes. Here we explore adaptive diversification in the Neotropical electric fish Apteronotidae (Teleostei: Gymnotiformes). We use a combination of morphological (osteological and morphometric). behavioral (electric signal), ecological (trophic positon, habitat utilization), biogeographic, and phylogenetic datasets to test alternative hypotheses regarding the sequence and rates of evolution of lineages and functional phenotypes. Analyses were also performed using Navajini (a clade of riverine-adapted apteronotids) to evaluate if rate differences are correlated with particular phenotypes. We use Blomberg's K and rate analyses to propose a diversification model for Apteronotidae. Apteronotids exhibit a range of phenotypes with functional roles in H, T, and S, including aspects of head and jaw morphology, and electric (i.e. sexual) signals, and also occupy a range of habitat and trophic categories. In Apteronotidae, the relative rates of evolution of H, T, and S related traits broadly overlap in time, and the temporal sequence of peak rate-values is ordered: T-H-S. Similarly, African rift-lake cichlids and Navajini both exhibit a T-H-S sequence. We propose this T-H-S sequence reflects differences in relative rates of trait evolution, such that traits with higher rates are inferred to occur later in the sequence. We interpret these patterns of lineage and functional diversification to arise in part from differences in rates of evolution of mouth size and positon. It is possible that different gymnotiform families will be found to exhibit different patterns of diversification due to different life history and constraints.

Keywords: Adaptation, Allometry, Diversification, Gymnotiformes, Neotropical



### Molecular identification of *Hisonotus leucofrenatus* (Siluriformes: Loricariidae)

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Hisonotus leucofrenatus is widely distributed throughout isolated coastal drainages of southeastern and southern Brazil. Recently, a morphological review of H. leucofrenatus revealed no differences between examined populations from rio Ribeira de Iguape and rio Tramandaí, however genetic studies have shown that many widely distributed species are actually species complexes composed by previously unrecognized cryptic species. The objective of this study was to test the hypothesis that H. leucofrenatus represents a single species. Were analyzed 28 partial sequences of the mitochondrial gene COI of H. leucofrenatus from different regions between the rio Ribeira de Iguape and rio Tramandaí. The consensus sequences were obtained using the program Geneious 7.1.4 and the alignment was generated with the algorithm Muscle under default parameters. We obtained a matrix with 532 characters (478 conserved sites). Nucleotide frequencies were cytosine = 29.8%, thymine = 27.8%, adenine = 23.5% and guanine = 18.9%. The matrix was used to perform analysis with the maximum-likelihood (ML) method and genetic distance based on Kimura-2-parameters (K2P) model through Mega 6.0. The maximum-likelihood and K2P genetic distance identified three well-supported clusters (>95%) (I- rio Ribeira de Iguape, II- south coastal river of Paraná and north coastal river of Santa Catarina and III- rio Tramandaí). The values of aenetic divergence showed a variation between clusters of 3.3% to 6.6% and within each cluster from 0.2% to 0.4%. This study suggests that H. leucofrenatus can represent more than one species (possibly three), and demonstrate the efficacy of molecular identification for the discovery of hidden diversity in Neotropical freshwater fishes. The confluence of the Atlantic Coast river systems during periods of low sea level resulting from climatic changes over the last 2.5 million years and the stream capture may explain the observed pattern of species distribution and genetic divergence among lineages of H. leucofrenatus. Additional molecular and morphological studies will still be conducted to test our hypotheses.

Key words: coastal drainages, catfish, genetic, cryptic species, biodiversity Financial support: CAPES, FAPESP



#### On the production of a field guide to the fish genera of the Amazon, Orinoco and Guianas

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In 2011, we started to work out a bold idea: to produce a field guide that would allow the accurate identification of fishes in the Amazon, Orinoco and coastal rivers in the Guianas, a biodiversity province known as Greater Amazonia. It became quickly evident that a field guide to the fish species would be practically impossible given the tremendous number of species, as well as the anticipated high number of undescribed species. We therefore decided to focus on identifying fish genera and to provide references to relevant literature for species-level identification, if available. With this goal in mind, a complicated and challenging project started. A project that soon after its initiation transformed into a large collaborative undertaking, involving over 50 contributing experts. By collating published information, we brought to light that Greater Amazonian contains at least 3,000 described fish species, belonging to 564 genera in 63 families and 19 orders. Of this great diversity, Siluriformes and Characiformes each have approximately 1,100 known species and together make up two-thirds of the known fish fauna. The field guide has now been printed and will be available shortly, and we therefore take this opportunity to briefly describe how this project came about, how it was shaped, and discuss potential future directions.



# Historical Biogeography of the Ghost Knifefishes (Gymnotiformes: Apteronotidae)

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The Apteronotidae is the most species-rich and morphologically-disparate family of electric knifefishes (Gymnotiformes), with roughly 100 species in 15 genera. Apteronotids are found throughout the humid Neotropics from Panama to northern Argentina, but are most diverse and abundant in the main channels of large rivers in the Amazon Basin. The presence of diverse, sympatric assemblages in deep river channels in conjunction with highly-disparate cranial morphologies is a pattern suggestive of an in-situ Amazonian radiation. Although there have been many recent advances in gymnotiform phylogenetics, no study has yet specifically assessed the temporal and geographical context of apteronotid diversification. We use a species-dense time-calibrated phylogeny and biogeographic model testing to estimate ancestral ranges and ultimately infer the effects of geologic history on apteronotid evolution. Our results suggest apteronotids arose in the early-mid Oligocene, with most diversification occurring during or after the Miocene. Ancestral area estimation placed the origin of the family in the Western Amazon region. Most clades were largely limited to the Amazon and Orinoco basins, with only relatively recent colonizations of other basins. One notable exception is the genus Apteronotus, which is the only clade to exhibit early vicariance or dispersal into the La Plata, Magdalena, and Maracaibo basins. We suggest that this more eurytopic clade's ability to survive outside of deep river channels has allowed it to spread and diversify across the continent. For the majority of Apteronotidae, diversification appears to be inextricably linked to the formation of the modern Amazon River system.

Key words: Dispersal; Diversification; Electric fishes; Phylogenetics



#### Redescription of Astyanax rivularis (Lütken, 1875) (Characiformes: Characidae), a senior synonym of Hyphessobrycon santae (Eigenmann, 1907) and Astyanax turmalinensis Triques, Vono & Caiafa, 2003

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Tetragonopterus rivularis Lütken, 1875 was described from the rio das Velhas, rio São Francisco basin, municipality of Lagoa Santa, Minas Gerais State, Brazil. Lütken mentioned the lateral-line of T. rivularis is variable, and can be complete or incomplete in different specimens or even in different sides of the same specimen. He named these specimens as "var. interrupta", but also stated that they cannot be recognized as a different subspecies. Four specimens of T. rivularis were transferred to USNM by Lütken and latter examined by Eigenmann that concluded they belong to two species based on the completeness of the lateral line. Two specimens with incomplete lateral line were described as Hemiarammus santae Eigenmann, 1907 (= Hyphessobrycon santae). The two remaining specimens were identified by Eigenmann as A. fasciatus, however, the specimens are actually A. rivularis by possessing 17–19 branched anal-fin rays (vs. 20 or more). Astyanax turmalinensis Triques, Vono & Caiafa, 2003 was described from headwaters of the rio Jeguitinhonha basin and subsequently expanded to the São Francisco and Doce rivers basins. The types of A. turmalinensis possess scales on lateral line series variable, being complete, incomplete, or discontinuous, as observed in A. rivularis. Due to the morphological similarity among these three species and their occurrence in the same basin, an investigation about their identities was performed. A morphological analysis was done with 78 specimens including types and non-types of these three species. Twenty-one measurements, sixteen external and five osteological counts were taken. The analyzes allowed us to recognize A. turmalinensis and Hyphessobrycon santae as junior synonyms of Astyanax rivularis, which present specimens with a variable lateral line, characteristic already highlighted by Lütken (1875). Individuals with a discontinuous lateral line have pored scales interspersed with non-pored ones, usually at vertically through the pectoral, dorsal, anal fins and/or caudal peduncle (e.g., discontinuous between scales 11-14, 16-19, 23-26, and 30-33). Variation of the lateral line conformation considering both sides of the same specimen was also observed (e.g., complete on right side/discontinuous on left, incomplete on right side /discontinuous on left, and complete on right side /incomplete on left). Astyanax rivularis presents a combination of characters that points its allocation as part of A. scabripinnis species complex. It differs from congeners belonging to the A. scabripinnis species complex by a combination of the following characters: two humeral blotches (vs. one); vertical humeral blotch (vs. horizontally oval); a reticulate pattern of dark chromatophores below lateral line, at the abdominal region (vs. scattered); a narrow naked area between postero-ventral margin of third infraorbital and margin of preopercle, narrower than 25% of the depth of third infraorbital (vs. margin distant more than 25%); 34–38 longitudinal lateral line scales (vs. 39 or more); 13–15 circumpeduncular scales (vs. 16 or more). The synonyms are justified once all morphological data (including measurements) do not differ these taxa, also statiscally. Thereby, Astyanax rivularis actually ocurrs in the rio das Velhas, rio São Francisco and rio Doce basins, and also in headwaters of rio Jeguitinhonha.



### Taxonomic review of Ancistrus multispinis (Regan, 1912) (Loricariidae: Hypostominae: Ancistrini)

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Ancistrus differs from other loricariids by having the anterior and lateral snout margins lacking plates, bearing elongate dermic expansions known as tentacles. Its representatives occur between Panama and Argentina, with cis- and trans-andean species. They are common in headwaters of Atlantic Forest coastal rivers, between Itabapoana and Tramandaí basins. In this extensive area, Ancistrus multispinis is the only recognized species, with its type locality in Itapocú River, state of Santa Catarina. Lack of precise information concerning its description difficult the species' identification and its accurate distribution. For this reason, populations sampled along coastal rivers of Atlantic Forest are commonly identified as A. multispinis. We analyzed 39 morphometric and 23 meristic data of 379 specimens representing most coastal rivers encompassing Ancistrus distribution. A. multispinis was redescribed and its diagnosis was actualized based on images of lectotype and morphological analysis of 70 topotypes from the same sampling event of type material. The species can be diagnosed from its congeners occurring in adjacent basins by the following features: from A. abilhoai by having a wider premaxillary tooth cup (15.3-18.7 vs. 12.1-15.2% in HL); from A. agostinhoi by having one or two dorsal plates between adipose fin and first procurrent caudal-fin ray (vs. no dermal plates interval); from A. mullerae by having the dorsal-fin with blotches occurring just on rays, not extending on membrane (vs. blotches expanding on dorsal-fin membrane and linking each other, resulting in horizontal lines); from A. brevipinnis and A. taunayi by having a less number of light spots on ventral surface. Besides A. multispinis, we found three new morphotypes: Ancistrus sp. n. "Araranguá" from the southern region, in Araranguá, Mampituba and Tramandaí basins. It differs from A. *multispinis* by having a ventral region brown, without light spots; Ancistrus sp. n. "Ribeira de Iguape", endemic from Ribeira de Iguape basin and distinct from other coastal river species, except Ancistrus sp. n. "Araranguá", by having three anal-fin rays (vs. four analfin rays) and from A. sp. "Araranguá" by having the ventral region with light spots; Ancistrus sp. n. "Itabapoana" differs from A. multispinis by having a smaller mouth width (44.9-53.7%, mean of 49.5% in HL vs. 45.7-73.5% [45.7-53.7% = 3% of 191 specimens], mean of 62.7% in HL); from A. sp. n. "Araranguá" by having light spots on ventral surface and from A. sp. n. "Ribeira de Iguape" by having four branched anal-fin rays (vs. three branched rays). Consequently, the distribution of A. multispiinis is limited by Paraíba do Sul River at North and by Tubarão River in the South.

Key-words: Taxonomy, Siluriformes, Neotropical region, coastal rivers Financial support: CAPES; CNPq



### Phylogeny of pike cichlids (Cichlidae: Cichlinae: Crenicichla and Teleocichla)

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Ninety valid species of Crenicichla and nine of Teleocichla are currently recognized. Together, the target-genera of this study represent almost 1/5 of the diversity of Cichlinae, a Neotropical subfamily of Cichlidae. Although the species of Crenicichla and Teleocichla are part of a monophyletic group, corroborated by phylogenies based on both morphological and molecular data, the relationships among the species included are not well-established. The two previous phylogenetic studies partially disagree with each other and included only part of the diversity of the group. Based only on molecular data, these studies were not supported by a morphological study that would indicate synapomorphies or diagnostic characters for the identification of the monophyletic groups proposed. The primary objectives of the present study are to test the monophyly of the group formed by the species of Crenicichla and Teleocichla and to identify and define monophyletic unities within this group using morphological characters. As a secondary objective, this study also aims to test the recent hypotheses of phylogenetic relationships between the species of Crenicichla and Teleocichla and the remaining Neotropical cichlids. All the valid species of Teleocichla and 59 species of Crenicichla (60% of the valid species and five new species) were included in the cladistic analysis. Twenty representatives of different lineages of Cichlinae were also included, resulting in a total of 88 terminal taxa. Cladistic analyses were performed on a matrix of 211 characters derived from a comparative study of external morphology, including color patterns, and osteology. Besides the equal-weighting analysis, other analyses using different weighting systems were explored, such as successive weighting and implied weighting using different values of the variant k. After comparison and discussion of the results obtained by the different analyses, the topology obtained by the analysis with implied weighting using k=3 was chosen as the base for phylogenetic inferences. Two alternative classifications were discussed and the one based on the choice of subgenera instead of genera to represent the monophyletic groups found was preferred to minimize nomenclatural changes, especially because the position of Crenicichla macrophthalma (type-species of Crenicichla) is still considered instable. The genus Crenicichla is corroborated as a monophyletic group formed by all species of Crenicichla and Teleocichla included, and is supported by 40 synapomorphies. Within the genus, a complex scenario is found with many lineages, as also pointed out in previous studies. However, the relationships between these lineages are instable, being variable according to the different weighting systems used and partially in disagreement with respect to previous studies. Teleocichla is a monophyletic group within Crenicichla and is, consequently, considered one of the eight subgenera of Crenicichla. The subgenus Crenicichla is represented only by C. macrophthalma. The remaining six subgenera (named Wallaciia, Batrachops, Hemeraia, Saxatilia, Lugubria and Lacustria) correspond totally or partially to species groups previously proposed for Crenicichla. Within Lacustria, four species complexes were delimited: C. missioneira, C. scottii, C. jaguarensis and C. lacustris sensu stricto. Nominal species of each subgenus were listed and diagnoses for their identification were performed. A new hypothesis of relationships between Crenicichla within the subfamily is inferred based on the results of the analysis, in which Crenicichla is sister-group to the clade formed by Chaetobranchus flavescens and all the representatives of Cichlasomatini and Geophagini included.

Key words: Systematics; Taxonomy; Morphology; Freshwater; Neotropical; Cichlidae; Labriformes Financial support: (HRV) CNPq 150304/2017-0, Fapesp 2013/17056-9, 2011/14630-0; (NAM) Fapesp 2016/19075-9



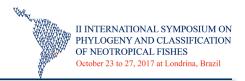
# Reassessment of Probolodini: an expected tribe of Characidae (Actinopterygii: Characiformes)

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Characidae is one of most diverse fish families of the Neotropical region. In the last decades, some phylogenetic studies tried to solve the relationship among the genera included in this family. In most of them, 3 clades are recovered: clades A, B and C. Clade C is the most rich-species and complex because it includes polyphyletic genera. In this clade, some species belonging to the genera Astyanax, Deuterodon, Jupiaba, and Myxiops share a peculiar feature: a special arrangement of gradually decreasing dentary teeth. To test if this feature is homolougous or evolved independently in these taxa we decide to investigate the phylogenetic relationship of these genera inside of Characidae family. Two hundred and nineteen specimens had DNA extracted with extraction kit to access genomic DNA. Four genes were amplified: two mitochondrial, Cytochrome Oxidase c Subunit 1 (COI) and the NADH dehydrogenase 2 (ND2), and two nuclear genes, the nuclear Alpha-myosin 6 (MYH6) and the SH3 and PX3 domain-containing 3 like protein (SH3PX3). Species of Astyanax, Deuterodon, Jupiaba, and Myxiops were also included on a morphological matrix totalizing 233 specimens of Characidae and 412 characters. Both molecular and morphological analyses recovered a major clade named here as Probolodini with high statistical support (22 synapomorphies under parsimony analyses of morphological characters, with one exclusive synapomorphy). This clade is composed by Astyanax species from coastal drainages with gradually decreasing dentary teeth, Hyphessobrycon luetkenii, Jupiaba species with gradually decreasing dentary teeth, Myxiops aphos, Probolodus heterostomus, all Deuterodon stricto sensu species, Deuterodon pedri and two undescribed taxa. Both molecular and morphological analyses were congruent recovering Probolodini. The resurgence of this clade twice and independently with different kinds of data, make the hypotheses of the existence of this unit strong. Most of the species and genera that are part of Probolodini are endemic from coastal drainages of East Brazil. The Brazilian eastern coastal drainages are known as area with the high number of endemic genera and species of Neotropical fish. It is possible to infer that the history of connections, headwaters captures and drainages splits in this area were decisive to Neotropical freshwater fish diversification. The main characteristic shared by the species that are included in Probolodini is the teeth arrangement, but the synapomorphies that support this clade are not exclusively related to dentition. In general, more robust hypotheses emerge with the use of different kinds of data sets that derive from different evolutionary constraints. A combined approach is the best choice to solve polytomies and it should be encouraged. The evolutionary process is better understood when the analyses yield testable hypotheses.

Keywords: Deuterodon, coastal drainages, clade C, phylogeny, parsimony Financial support: CNPq scholarships to PCS (process 141407/2013-1; 406776/2017-2) and LRM (307890/2016-3)



# DNA barcoding in the identification of fish eggs in an important tributary of the Capivara HPP, Paranapanema River Basin, Brazil

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The construction of dams is one of the main factors that promote loss of fish biodiversity in continental aquatic ecosystems, consequently this constructions influence the reproduction of fish species interfering in the composition of the ichthyofauna. Therefore, identify which species complete their recruitment process is essential to trace effective conservation measures. The analysis of the composition and abundance of fish eggs and larvae can bring information about the success of the species permanence through reproduction due to disturbances caused by dams. However, classification in the early stages of development is extremely complex, since many of the characteristics that distinguish the species are not yet developed. Thus, DNA barcoding tool has been used to identify and classify species. The Paranapanema River has along its main channel 11 Hydroelectric Power Plant (HPP), in a cascade system of reservoirs and the largest HPP is the Capivara located in the middle portion of the Paranapanema River, which has one of its largest tributaries in the Tibagi River. Several authors have pointed out the importance of this hydrographic basin for the maintenance of fish diversity in this system. In this context, the present study aimed to identify, through the use of the DNA barcoding technique, eggs captured with their respective species in the Tibagi River. DNA was extracted from 377 eggs collected in the greatest reproductive activity of fish, in the years of 2013 and 2014. The obtained DNA was guantified and sequenced. The obtained sequences were aligned and compared in order to search for unique patterns within the species that differs them from the others. In addition a distance tree was generated based on the Kimura-2-Parameters evolution model including sequences studied and vouchers taken from the database. It was found 11 species belonging two orders, four families and eight genera, with average similarity of 100% to the database. Of the species found, 10 are native to the Paranapanema river basin, and half of them exhibit reproductive displacement. We can highlight the species Pimelodus maculatus and Pinirampus pirinampu (138 and 16 individuals, respectively) because they are long-distance migrators. P. pinirampu also stands out for being a large-size Pimelodidae with diminished population in the Paranapanema river basin. The presence of native species of short and long reproductive displacement in tributaries of the reservoir indicates that these areas are being used for breeding and has a fundamental importance for the maintenance of the ichthyofauna of the Paranapanema river basin.

Key words: Upper Paraná River; Reservoirs; Freshwater; Neotropical Financial support: P&D ANEEL - Duke Energy, FAUEL, UEL, CAPES



#### DNA barcoding in the identification of reproductive products of nonnative fish in tributaries of the Capivara HPP, Paranapanema River, Brazil

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Formation of reservoirs due to construction of dams for energy generation has led to a series of physical-chemical disturbances in the Brazilian watersheds, which end up influencing the reproduction of fish species and directly interfering in the ichthyofaunal composition. Another factor that impacts watersheds is the introduction of non-native species, which may lead to the elimination of some native species. Thus, information about a community becomes more complete when including the life history of species, even in the early stages. The analysis of the composition and abundance of fish eggs and larvae is fundamental to provide information about the composition of the community and how much the environment is already impacted by the dam construction. DNA barcoding technique may help identify specific species, since the taxonomic classification in the early stages of development is complex because many of the characteristics that distinguish the species are not yet developed. Our goal was to identify eggs and fish larvae of non-native species collected in two main tributaries (Tibagi and Cinzas) inserted in the left margin of the Capivara reservoir using DNA barcoding technique. DNA was extracted from 62 eggs and 4 larvae collected in greatest reproductive activity of fish, from the years 2012 to 2015. The DNA was guantified and sequenced. The obtained sequences were aligned and compared in order to search for unique patterns within the species that differentiate them from the others. Three orders, four families, four genera and four non-native species were identified: Triportheus nematurus (62), Serrasalmus marginatus (2), Loricariichthys platymetopon (1) and Plagioscion squamosissimus (1). In comparison to other reservoirs of the Paranapanema River, Capivara HPP presents a low number of non-native species. Due to the imposition of dams, it is expected that general non-native species will be more successful in these environments in relation to native species. However, large tributaries such as the Tibagi River and the Cinzas River have been identified as lotic stretches responsible for the original diversity of the basin, helping to maintain rheophilic populations. The use of DNA barcoding to identify the reproductive products of non-native fish species was shown to be extremely efficient, due to the possibility of inclusion of eggs in the analyzes, making the data more complete.

Key words: Upper Paraná River; Reservoirs; Freshwater; Neotropical Financial support: P&D ANEEL - Duke Energy, FAUEL, UEL, CAPES



## Revision of electric fishes of the *Gymnotus* carapo and *G. tigre* clades (Gymnotiformes: Gymnotidae) from the Southern Neotropics

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The Banded Electric Fish (Gymnotus, Gymnotiformes) comprises the most species-rich genus of Neotropical electric fishes, with 40 species occupying most habitats and regions of the humid Neotropics. Despite substantial alpha-taxonomic work in recent years, however, parts of the genus remain characterized by taxonomic confusion. Here we describe and delimit species of the G. carapo and G. tigre clades (sensu Tagliacollo) present in the southern Neotropics, a region including the Greater La Plata (Paraná-Paraguay-Uruguay) basin, and coastal Atlantic drainages of southeastern South America, extending from the Pantanal of Brazil and Paraguay, to Rio de Janeiro, and South to the Río Salado south of Buenos Aires, Argentina. Members of the G. carapo and G. tigre clades are readily distinguished from congeners by two (vs. one) laterosensory pores at the dorsoposterior margin of preopercle. Members of the G. carapo clade also exhibit: a deep body shape in lateral view (body width/body depth 0.35-0.70); multiple arrowhead-shaped (vs. conical) teeth in the dentary; a clear patch of anal-fin membrane near the caudal end (vs. evenly pigmented anal fin); relatively few precaudal vertebrae (30-34); ovoid scales over whole body surface; and few (20-29) lateral ventral rami, some long and curved. Members of the G. tigre clade also exhibit: a sub-cylindrical shape in cross section (body width/ body depth 0.71-0.90); decurved teeth in premaxilla and dentary with tips oriented away from midline; pigment stripes near the caudal end anal-fin membrane; many precaudal vertebrae (34-45); axially elongate scales at caudal end of body; and many (32-50) short and straight lateral ventral rami. Species are delimited and described using body proportions (traditional morphometrics), fin-ray, squamation and laterosensory-pore counts (meristics), quantitative shape differences (geometric morphometrics), osteological traits, color patterns, electric organ discharges, karyotypes, and ecological data. We examine 153 Gymnotus specimens from 99 different localities in the southern Neotropics, then compare traditional morphometric, meristic and Geometric Morphometric characters of valid and proposed new species in a multivariate statistical framework. These analyses indicate the presence of six species of the G. carapo clade (G. CALA, G. carapo australis, G. chimarrao, G. omarorum, G. pantanal, and G. sylvius), and two species of the G. tigre clade (G. inaequilabiatus and G. paraguensis) in the southern Neotropics. The new species G. CALA is morphologically similar to the broadly sympatric G. c. australis, from which it is distinguishable by a shorter head and deeper head and body, and also similar to allopatrically distributed G. omarorum, from which it is distinguishable by fewer lateral line ventral rami and fewer pored lateral line scales anterior to the first lateral line ventral ramus. This project provides the first comprehensive review of an often-mischaracterized fauna, presenting some of the first records of the exceptionally widespread G. carapo in the South, and better documenting the ranges of often-misidentified species like G. sylvius, G. inaequilabiatus and G. paraguensis. The description of G. CALA also provides formal recognition of a highly abundant and widespread species previously regarded as synonymous with G. carapo.



## Characterization of natural stocks of three *Gymnotus* (Gymnotiformes: Gymnotidae) species explored as live baits in five I locations of Pantanal, Brazil

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Gymnotus is the most representative genus from the Gymnotidae family with 41 species formally described. Popularly known as electric fishes or "tuviras" they represent a major live bait among watersheds in Latin America. In the region of the Brazilian Pantanal of Mato Grosso and Mato Grosso do Sul states it is an important economic resource in fishery for being an excellent bait; adding an high trade value in these regions. Species of this group presents some peculiar traits. In special, an oblong body shape, coloring patterns and an organ that emits shockwaves which are utilized for communication, orientation and group identification. However, morphological similarities between species difficult the taxonomic questions of these organisms group. Based on this, the present work has the objective to characterize the commercial natural livestock of Gymnotus traded as live baits in Pantanal of Mato Grosso and Mato Grosso do Sul states by using data from cytogenetic, genetic, morphometric and color pattern. 242 sequences from barcode gene COI were analyzed by dendrogram using genetic distance (Neighbor Joining - NJ) and haplotype network. A principal component analysis (PCA) was performed from 14 measurements of a group of 66 species suggested from the NJ results. The genetic and morphometric analyses exhibit three distincts taxonomic groups of Gymnotus: Gymnotus paraguensis, G. pantanal and G. sylvius. The results showed G. paraguensis as the most abundant (84.4%) and G. pantanal as the lesser one (7%). The genetic divergence between groups was: G. paraguensis vs G. sylvius = 5.9%; G. paraguensis vs G. pantanal = 7.8% and G. sylvius vs G. pantanal = 8.5%. The haplotype network showed a moderate organization for Gymnotus paraguensis and a possible faunal regionalization. The coloring analyses revealed a homogenous general pattern of stripes in G. sylvius and G.s. pantanal and conspicuous variations between species of G. paraguensis. Our results have shown fragility on taxonomic studies of the group and major importance on integration of chromosomal, genetic and molecular biology data to better distinguish Gymnotus specially found in the Pantanal of Mato Grosso state.

Key-words: COI, live baits, Pantanal, tuviras Financial support: CNPq (process 446925/2014-4)



#### Molecular and morphological diversity of *Brycon* (Characiformes: Characidae: Bryconinae) of the upper portions of the Amazonian drainage of central Brazil

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Brycon Müller & Troschel, 1844 is composed of 44 species, widely distributed in the Neotropical freshwater basins. They are medium to large fish, migratory species, omnivores, important dispersers of forest seeds and riparian forests. They are used in sport fishing and feeding, representing one of the species of great importance in Brazilian fish farming. Although present ecological and commercial importance, it has observed a decrease in their populations as a result of the changes in their natural habitats derived from anthropic actions. These changes have led to the loss of biodiversity and many species have become extinct before being known. The aim of this study was to use an integrative approach based on molecular (DNA barcode) and morphological data to identify how taxonomic units for Brycon in the middle and upper courses of the rivers basins Tapajós, Xingú, Araguaia and Madeira. Were analyzed 201 sequences with a mean of 588 base pairs. Interspecific genetic distances were calculated by the Kimura-2-parameter nucleotide substitution model and ranged from 2.9% to 24.0%. These analyze of Neighbor-Joining, BIN and ABGD, allowed to assume the existence of at least twelve distinct lineages among the fish groups studied herein. The results showed that the species described as B. falcatus corresponds to more than one taxonomic unit, requiring further studies with more genes, along with morphological data to confirm the hypothesis of new species.

Key-words: DNA barcode, Amazon, Brycon Financial support: CNPq (process 446925/2014-4)



#### Population history of the "Geophagus" brasiliensis complex (Perciformes, Cichlidae) helps to unravel diversification processes in Contas river and adjacent basins

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The Northeastern Atlantic Forest (NAF), a hydrographic ecoregion at eastern coast of Brazil, has a unique set of characteristics, such as rich geodiversity and singular hydrographic pattern of parallel and currently isolated drainages with high levels of endemism. Historical connection or isolation of rivers, as well as rivers redrawing, may had been responsible to shape patterns and processes of diversification in freshwater ichthyofauna, as they are restricted to the aquatic environment. Thus, studies that attempt to identify evidence for the interchange of drainages and fauna between adjacent river basins are especially important to understand evolution and biogeography of resident biota. Within the NAF, the "Geophagus" brasiliensis complex comprehends as good model for this purpose due to evidences of high morphological, chromosomal and genetic variation in populations through this region. Herein we investigated the population structure and identified possible past connections among the Contas river and adjacents basins by using sequences of the mitochondrial DNA gene Cytochrome Oxidase Subunit I (COI). We collected specimens on 38 points in Contas, Almas, São Francisco, Pardo, Cachoeira, Almada and Paraguaçu basins. Individuals from Paraguaçu river basin were assumed as G. diamantinensis and the remaining populations as G. brasiliensis. We obtained a median-joining haplotype network and phylogenetic inference to evaluate the spatial distribution of the genetic variability and conducted neutrality tests (Tajima's D and Fu's FS) to detect possible demographic expansion signals. Detailed hydrography, along with Digital Terrain Model (DEM) from Shuttle Radar Topography Mission (SRTM) and other geomorphological data from Geographic Information System (GIS) files were used to infer events of modification on drainage patterns. Our data revealed five distinct lineages (4.0 to 9.2% of divergence) through the Contas basin with all G. brasiliensis populations occurring in two or more adjacent basins and G. diamantinensis being restricted to the upper portion of the Paraguaçu basin. Interestingly, sympatry between different lineages was found in a single site, whereas all lineages of G. brasiliensis were restricted to some portions of the Contas river basin and nearby basins (São Francisco-Contas, Almas-Contas, Lower Pardo-Cachoeira-Almada-Contas and Middle Pardo-Contas) suggesting recent connections between basins, although no sign of demographic expansion was recovered in neutrality tests. In addition, the geological data allowed identifying several elbows of capture (abrupt changes in river's course), paleocourses connecting currently isolated basins and rift systems that are in accordance with the genetic data. The model of connections presented here can be useful to understand the evolution of other freshwater fish fauna in this poorly studied area.

Key words: Cichlids; Freshwater fishes; Neotropical fishes; Stream capture Financial support: FAPESB (process RED0045/2014, JCB0026/2016), CNPq (process 443249/2014-8), CAPES, UFBA



## Taxonomic revision of *Rhamdioglanis* Ihering, 1907 (Siluriformes: Heptapteridae)

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Rhamdioglanis Ihering (1907) is a catfish genus of the Heptapteridae family that occurs in streams of the southeastern Atlantic Forest, Brazil, composed of two species: Rhamdioglanis frenatus Ihering, 1907 and R. transfasciatus Miranda-Ribeiro, 1908. However, doubts about the validity of those species, or even the genus itself, are presented in the literature since shortly after their description, and some authors point out that both would be junior synonyms of Imparfinis piperatus Eigenmann & Norris, 1900. This work aims a taxonomic revision of Rhamdioglanis, comparing its species, as well as Imparfinis piperatus, determining its validity, specific composition and geographical distribution, using morphometric, meristic and osteological data. A total of 129 specimens of Rhamdioglanis were observed, with distribution from Santa Catarina to the north of Rio de Janeiro, of which five were cleared and stained and 11 were radiographed for osteological studies. Comparative material encompass 8 specimens of I. piperatus. A Principal Component Analysis (PCA) was performed as an exploratory analysis for the morphometrical variation observed, in order to acess the components of maximum variance and highlight the similarities and differences among the observed material. Rhamdioglanis frenatus and R. transfasciatus were considered distinct from I. piperatus by 46-48 total vertebrae (vs. 37 in I. piperatus); shorter maxillary barbels, not exceeding the vertical of the adpressed pectoral fins (vs. reaching the origin of the pelvic fins in I. piperatus); and a higher total number of caudalfin rays (52–56 rays, vs. 39–48 in I. piperatus), suggesting that Rhamdioglanis would be a valid genus and distinct from Imparfinis. However, regarding Rhamdioglanis species, no significant osteological, morphometric or meristic differences were found, and the PCA also did not highlight any differences among Rhamdioglanis species' populations. Those results lead us to conclude that, in fact, R. transfasciatus would be a junior synonym of R. frenatus, the only valid species of Rhamdioglanis, with geographic distribution from Santa Catarina to the North of Rio de Janeiro State.

Key words: Anatomy; Morphology; Principal Components Analysis; Southeast Atlantic basin Financial support: PIC-UNISANTA (VE); FAPESP 2013/18623-4 and 2016/01073-0 (VS)



## Phylogenetic relationships of *Pimelodella* Eigenmann & Eigenmann, 1888 (Siluriformes: Heptapteridae)

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Pimelodella Eigenmann & Eigenmann 1888 is an important component of the Neotropical freshwater fish fauna, being the most species-rich among the 24 heptapterid genera, with 79 valid species of the 211 of the whole family. It is distributed throughout cis- and transandean river drainages, from Panamá to Argentina. The identification of Pimelodella species is a known challenge for taxonomists, representing one of the most important taxonomic bottlenecks on understanding the diversity of Neotropical freshwater ichthyofauna. Despite several species have been described, transferred to or removed from Pimelodella since its description, this genus lacks an unequivocal diagnosis, rendering most of these taxonomic actions arbitrary. This work aims to remedy this situation by providing a phylogenetic diagnosis for Pimelodella, as well as elucidating its relationships with the remaining heptapterid genera. For this analysis, a morphological matrix was build with 62 discrete and 15 continuous characters for 27 species currently assigned to Pimelodella and 18 other Heptapteridae, includina all Brachyrhamdia, formerly supposed to be a junior synonym to Pimelodella. A parsimony analysis was performed with TNT program and produced a single most parsimonious tree with score of 214.498, CI 0.44, RI 0.77 and RC 0.34. The results so far indicate that Pimelodella, as presently conceived, is a paraphyletic group, inasmuch as most of Pimelodella species (including its type-species, P. cristata) form a clade which is the sister-group to a monophyletic Brachyrhamdia, and part of Pimelodella species as a sister-group to the clade formed by the rest of Pimelodella+Brachyrhamdia. This more restricted version of Pimelodella is diagnosed by the nasal bone long, 25% longer than autopalatine, an exclusive apomorphy, and it can be recognized by the following combination of characters: 37–51 total vertebrae; supraoccipital process reaching the prenuchal plate; and posterior fontanel opened, among others. Brachyrhamdia is supported by 16 synapomorphies, two exclusive. A new genus is proposed for the remainder species of Pimelodella, being supported by four synapomorphies, two of them discrete characters, namely: the supraoccipital process almost reaching the prenuchal plate; and the absence of tubercle dentations at anterior margin of pectoral-fin spine. Species of this genus can be also recognized by the last dorsal-fin basal radial inserted posterior to neural processes of vertebrae 4 or 5; and dorsal-fin spine a third shorter than first dorsal-fin total length. The relationships of those groups with other Heptapteridae corroborate former works, in which Pimelodella is a basal Heptapteridae and closely related to Rhamdella. The inclusion of more Pimelodella species in the future can increase the tree resolution. The results so far indicate an increase of size and number of vertebrae in more apical clades within Pimelodella.

Key words: morphology; parsimony; catfishes Financial support: FAPESP #2013/18623-4; 2015/26804-4; 2016/01073-0



## Participation and representativeness of women in the Brazilian Society of Ichthyology

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Considering Science adopts the assumption of being 'gender neutral', we aimed to evaluate the female contribution to the Brazilian Ichthyology and designed this study to access the number and representativeness of such contribution, and how it has been developed in the last decade. The potential female representativeness in Brazilian ichthyology was estimated considering the whole membership period of the Brazilian Society of Ichthyology (from Portuguese, SBI). A survey of gender data in the SBI database was based on the entire list of affiliated (not only those who has paid the annual fees) who are Brazilians or researchers in Brazil. To address aspects on that proportion in prominent positions of scientific meetings, we investigated the invited speakers in the Brazilian Ichthyology Meetings (Encontro Brasileiro de Ictiologia, EBI) from the last decade, organized by SBI together with local institutions, and also the announced speakers of the I and II International Symposia on Phylogeny and Classification of Neotropical Fishes. The EBIs' gender data were obtained from Scientific Programs, and Symposium data from "Speakers section" at the Meeting's website to August 16, 2017. Speakers of EBI were counted regarding their number of presentations, split into the following categories: Plenary conferences and Lectures; Discussion tables; Workshops; and Symposia. All the speakers listed on the International Symposia were counted. From a total of 1,220 affiliated to SBI, 739 are men (60.6%) and 481 are women (39,4%). The number of SBI members almost doubled in the last 10 years (from 682 to 1,220), being the affiliations of last decade composed 59.4% of men and 40.6% women. The proportion of women as invited speakers at EBI increased from 21.7% in 2007 to 31.7% in 2011 (year with the highest female participation) and then decreased to 21.7% in 2013, 27.9% in 2015, and 22.6% in 2017. It shows the female low representativeness is long-standing and persistent. The proportion of female speakers in the International Symposium was 19.8% in 1997, and of 13.7% in 2017, a decrease in representation of 6.1%. Therefore, female under-representation in this meeting is more than 30% smaller than the proportion of women in the SBI (39.4%). Those results led us to address important questions on the reasons of gender inequality in speakers and in the Brazilian Ichthyology proper, comparing to the society in general. Detailed data will be presented regarding publications, leadership roles, and financial support, as well as the Systematics area profile. Affirmative policies in measuring, monitoring, and advancing gender equality in the Academy and Scientific Meetings will be presented, as well as thoughts on the Brazilian Ichthyology scenario.



#### Descriptive osteology of *Rineloricaria latirostris* (Boulenger, 1900) (Siluriformes: Loricariidae: Loricariinae)

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Osteological analysis and description have achieved fundamental relevance to the current understanding of the phylogenetic relationships, reinforcing the importance of the osteological morphology. In relation to the anatomy, fishes represent one of the most complex group of vertebrates, revealing several challenges related to the description of their plastic profusion, mainly in the Neotropical freshwater fish fauna. Therefore, the study intends to provide descriptive analysis and illustration of the bone sets of Rineloricaria latirostris, seeking to diagnose relevant characters for future ontogenetics researches, considering the intraspecific variation and also help elucidate features for the genus. The species is endemic of the Upper Paraná River basin and belongs to the most diverse genus in the Loricariinae subfamily. The genus distributional reach goes from the north of Argentina to the south of Central America. For this study, five adult specimens were dissected (DZSJRP2770 - 78,01mm, 3; DZSJRP15811 - 97,08mm, 2; DZSJRP15811 – 79,02mm, ♀; DZSJRP8020 – 106,02mm, ♀; DZSJRP17729 – 101,05mm, ♂). Length of the specimens was taken point to point using digital caliper accurate to 0.1 mm. Specimens were cleared and stained (c&s) (e.g, Taylor & Van Dyke). Osteological characters were described and compared with literature data, using appropriate nomenclature (e.g., Schaeffer, Aquino & Schaefer and Fichberg). Characters shared by R. latitostris and others species of the genus were found, as neurocranium, triangular, slender in the mesethmoid region. Large and numerous rostral bristles in males, absent in females. Robust teeth shaped into "Z", bicuspidate, a wider and large cuspid in relation to another. Interhial linked to the hyomandibular, reaching the anteroposterior limit of this bone and overtaking it. Rectangular pelvic bone, slightly expanded, longer than wide, composed by basipterigium and lateropterigium, absence of medial sulcus in the mesetmoid and absence of the first ceratobranchial anterior process. The five specimens had a total of 29-31 vertebrae, suggesting an ontogenetic variation. A remarkable character is the ringlike structure present over the dorsal fin second pterygiophore, this structure is inserted into a hole at the unbranched ray base, i.e., R. latirostris has no spine responsible for the dorsal fin locking mechanism, corresponding to an evolutionary loss. None pronounced intraspecific variation was observed. Through the results obtained new taxonomic and phylogenetic research can be performed.

Key words: Systematics; Neotropical; Freshwater; Ichthyofauna; Catfishes Financial support: CAPES



#### Molecular phylogenetics and diversification of South American Darters (Characiformes: Crenuchidae)

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Characiformes comprises >2,000 species, including well-known ornamental and aquarium fishes such as tetras and piranhas, and exhibit a remarkable array of ecomorphological diversity. Despite their exceptional ecological diversity, most characiforms are limited to pelagic habitats. The characiform family Crenuchidae, known as South American darters, due to their superficial morphological similarity to the North American darters (Percidae: Etheostomatini), is intriguing because most of the 86 described species are predominantly benthic (>70 species). A morphological phylogeny of the family supports 12 monophyletic genera divided between two subfamilies, the Crenuchinae (2 genera) and the Characidiinae (10 genera). That phylogeny also suggests the high diversity of benthic species may be due to repeated transitions from pelagic to benthic habitats, a hypothesis that can be tested by estimating character transition rates and comparing the fit of models with equal or biased transition rates. Our study aimed to determine the phylogenetic relationships of crenuchids, estimate the frequency of benthic/pelagic habitat transitions and test for biased transition rates from the pelagic habitats to the benthos. Our comprehensive molecular phylogeny based on 4 nuclear and mitochondrial loci revealed widespread discordance with the current taxonomy of crenuchids because most recognized genera are not monophyletic, including the most speciose genus Characidium. We also uncovered instances of undescribed diversity and species complexes. These results provide the first comprehensive molecular phylogeny of crenuchids and indicate a re-evaluation of current systematics of the family is needed. Using ancestral character reconstruction, we found crenuchids are ancestrally pelagic with one transition to the benthos and at least five transitions back to the pelagic realm, which rejects the hypothesis that biased character transition rates explain the high diversity of benthic darters. Instead, we suggest South American darters may have undergone an adaptive radiation facilitated by a transition to the benthos. This work gives important evolutionary insight into this enigmatic family of freshwater fishes.

Key words: Systematics; Taxonomy; Habitat Transitions; Diversification



#### Multigene phylogeny supports the South American miniature catfish Trichomycterus hasemani group as a new genus of the Tridentinae (Siluriformes: Trichomycteridae)

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Trichomycteridae is a well-corroborated catfish family that comprises about 300 valid species distributed from Costa Rica to Patagonia and in both Andean sides. Eight subfamilies are currently recognized among Trichomycteridae: Trichomycterinae, Vandelliinae, Stegophilinae, Tridentinae, Glanapteryginae, Sarcoglanidinae, Trichogeninae and Copionodontinae. Miniaturization is a widespread phenomenon in Trichomycteridae, occurring in all subfamilies except Copionodontinae and Trichogeninae. Trichomycterinae accounts for the highest number of miniaturized species among the family and the positioning of some miniaturized species are usually in the centre of discussions about its phyletic status. The miniaturized Trichomycterus hasemani aroup comprises four valid species: T. hasemani, T. johnsoni, T. anhanga and T. wapixana. Its species are distributed across lowlands in the Amazon rainforest and Pantanal. Several authors discussed the positioning of the group within the Trichomycteridae and there is a general agreement that these species are more related to the Tridentinae than to the Trichomycterinae. Despite this consensus, nor its monophyly neither its relationships were ever tested within a phylogenetic framework. The proposal of this work is to develop the first phylogenetic analysis focused on testing the positioning and monophyly of the T. hasemani group. Bayesian Inference and Maximum Likelihood analyses of a molecular dataset comprising partial sequences of the mithocondrial genes 12S and 16S and of the nuclear genes H3, MYH6 and RAG2 (2983 bp) for 26 taxa highly supported the miniature catfish Trichomycterus hasemani group as monophyletic and sister to the Tridentinge, consequently recognized as a member of this subfamily. The T. hasemani group is similar to the remaining Tridentinae genera by the presence of a wide cranial fontanel, delimited by the mesethmoid, frontals, sphenotics and parietooccipital; presence of a short ventral process in the opercular bone and by the origin of the dorsal fin placed in a vertical through the anal-fin origin. The group is also supported by an exclusive combination of five character states and two character states unique among the Trichomycteridae.

Key words: Systematics; Nuclear genome; Mithocondrial genome; Miniaturization; Neotropical



#### Historical patterns of *Hypostomus ancistroides* colonization in an Upper Paraná River sub basin using mitochondrial data

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Hypostomus ancistroides is a Neotropical species widely distributed in Upper Paraná basin. However, until this moment, information about the patterns of geographical distribution of this species along this large hydrographic basin are still scarce. Besides, taking into consideration that H. ancistroides apparently shows a limited displacement ability, a better knowledge about the local patterns of distribution of this species in the hydrographic basins comprising the Upper Paraná basin is especially relevant. In this case, the obtained information could be helpful to get a more complete understanding of how the colonization process by this species is occurring, along of time, in the different drainages. Thus, in this study, we used mitochondrial markers to investigate the possible patterns of distribution and colonization of a sub-basin belonging to Upper Paraná. We amplified and sequenced the D-loop region of mitochondrial DNA from H. ancistroides sampled in six tributaries from Laranjinha River, which comprises one of the sub-basins belonging to Upper Paraná, located in the northern Paraná, southern Brazil. Each stream was sampled in three sites (low, medium and high stretches), totaling 18 sites sampled in the study. In the total, 508 specimens of H. ancistroides were collected and analyzed. To increase our analyses, sequences of H. ancistroides collected in the Laranjinha River were also included. For a sequence of 556 bp from D-loop region, we identified 20 different haplotypes. The most common haplotype (H2) was shared for specimens collected both in the river and in the streams (N = 422). Half of haplotypes found in streams were also found in specimens of the river, however the other ten haplotypes were unique to samples of streams. In general, the mismatch distribution analysis and neutrality tests suggest that basin occupation for this species had influences of a founder effect during a recent colonization process. Similar patterns to these described herein, suggesting genetic bottlenecks and population expansions for H. ancistroides, are already known for other rivers in the upper Paraná River basin. Besides, based on our findings, it is possible to suggest that the basin colonization took place from the mouth of the Laranjinha River to the headwaters. This idea it is corroborated by the greater diversity of haplotypes found near the mouth of the river, where six haplotypes were found only in streams near to the mouth of the river. Thus, these data allow a better understanding of some evolutionary aspects as well as the distribution of H. ancistroides along the basin of the Laranjinha River and they evidence the importance of both rivers and their small tributaries in the maintenance of the species diversity.

Key words: catfish, streams, *D-loop*, basin colonization Financial support: CAPES, CNPq, Fundação Araucária, UEL and UENP



#### Phylogenetic and biogeographic analyzes of the genus Jenynsia (Cyprinodontiformes: Anablepidae) based on morphological and molecular data

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Anablepidae is a Neotropical family with three genera: Oxyzygonectes and Anableps, found in brackish waters; and Jenynsia, with most species found in freshwater habitats. Oxyzygonectes is a monotypic genus endemic to Pacific drainages of Central America. Anableps comprises three species inhabiting coastal drainages, one in the Pacific coast of Central America and two in the Atlantic coast of Northern South America. Jenynsia is the genus with most species of Anablepidae, with 14 species found in small drainages of Paraná-Paraguay river basin and coastal drainages of Brazil, Uruguay and Argentina. Despite the well-known distribution, the biogeography of the family was never deeply explored. The morphological characters used in the study were based on previous papers focusing on the phylogenetic analyzes of Cyprinodontiformes and Anablepidae. For the molecular data, seven nuclear genes were analyzed. A combined matrix, with 175 morphological characters and 6074 nucleotides, was elaborated for all species of Anablepidae and with Tomeurus gracilis as outgroup, with a total of 19 taxa. The Bayesian analysis were executed, with four independent Markov Chain Monte Carlo (MCMC) performed with 2 million generations each. A burn-in of 25% was removed and the final tree was analyzed using the Bayesian Binary MCMC (BBM) analysis. Six areas were defined according to the distribution of the analyzed species: (A) coastal basins of northern South America; (B) Pacific coastal basins of Central America; (C) coastal basins of south-eastern South America; (D) Uruguay river basin; (E) Iguaçú river basin; (F) Paraguay river basin. A second BBM analysis was conducted to evaluate the number of transitions between brackish and freshwater environments. The phylogenetic analysis high supported Jenynsia and its both subgenera. The biogeographic analysis indicated that the most recent common ancestor of Jenynsia probably inhabited the coastal basins of south-eastern South America, two lineages of the subgenus Plesiojenynsia independently colonized the Uruguay and Iguacú river basins and two lineages of the subgenus Jenynsia dispersed through the Uruguay and Paraguay river basins, respectively. The analyses indicated that the most recent ancestor of Jenynsia was a brackish water species inhabiting the area near the La Plata river mouth. The transitions from brackish to freshwater environments and subsequent diversification of the genus Jenynsia was probably benefited by events of marine incursions in the La Plata river, wherein complex ecosystems were generated with different kinds of environments helping the transition from brackish to freshwater. We hypothesized that the transition to freshwater environments occurred twice in Jenynsia, once for each subgenus.

Key words: Event-based methods, Marine incursions, Neotropical, Systematics Financial support: FAPERJ (E-26/200.381/2016)



### Fish diversity from streams of the Laranjinha River basin, Upper Parana River

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The Brazilian freshwater ichthyofauna includes 20% of known freshwater species of the world. However, knowledge about the total diversity of this fauna is certainly still incomplete. The Parana River basin, the second largest drainage in South America, harbors a great portion of this diversity. For instance, presently about 370 fish species are known only in the Upper Parana River, many of which endemic to this portion of the basin. The fish fauna of the entire basin is under serious threats due to intense anthropogenic impacts. Streams are environments of considerable importance for the freshwater fish fauna of the Neotropical region, due to their small sizes that provide different conditions from those found in larger drainages. Studies aiming to identify the diversity of species of this type of environment are still limited. The present study presents a survey of the ichthyofauna of five tributaries from Laranjinha River basin, which belongs to the Upper Parana basin, located in northern Parana state. The five streams surveyed are: 1) Grande, 2) Barreiro 3) Alecrim, 4) São Francisco and 5) Água Grande. The collections were performed in three sites in each stream, one near the headwater (high), one in the middle, and one in the mouth (low), totalizing fifteen sampling sites from April 2015 to July 2016. Three sampling campaigns were carried out at each site. Specimens were collected with gill nets and mesh trays. All specimens collected were anesthetized with clove oil, fixed, identified and cataloged in the Museu de Zoologia da Universidade Estadual de Londrina (MZUEL). A total of 1644 specimens were collected, distributed among five orders, 13 families, 26 genera and 38 species of the Teleostei. The Siluriformes was the order with the highest number of species (n=18), with Hypostomus ancistroides (Ihering 1911) (37.8%), Imparfinis schubarti (Gomes 1956) (3.8%) and Hypostomus strigaticeps (Regan 1908) (3.6%) comprising the three species more frequently collected. Characiformes, with 13 species, was the second order most diverse in number of species. Bryconamericus iheringii (Boulenger 1887) (12%) and Astyanax paranae (Eigenmann 1914) (8.3%) were the two species more abundant of that order. The most frequent species present in all streams were Hypostomus ancistroides, Geophagus brasiliensis (Quoy & Gaimard 1824) and Rhamdia guelen (Quoy & Gaimard 1824). During the surveys, three nonnative species of Oreochromis, Poecilia and Crenicichla were also collected. The streams with the greatest abundance of specimens were the Grande (34.2%) and Barreiro (22%). The present findings reinforce the importance of the streams to the maintenance of the diversity of the ichthyofauna of the Upper Parana basin.

Key words: Freshwater; Neotropical, Conservation, Survey Financial support: CAPES, CNPq, Fundação Araucária, UEL and UENP



## Anatomical variations on the extrinsic eye musculature in the Lamniformes (Chondrichthyes: Elasmobranchii)

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The family Odontaspididae encompasses the genera Carcharias and Odontaspis and is currently considered paraphyletic in both morphological and molecular phylogenetic studies. Despite presenting different topologies, both hypotheses suggest that morphological characters within the family must be revised. The present study examined the extrinsic eye musculature in members of Odontaspididae not only to broaden but to recognize variations in this anatomical complex. Specimens from Odontaspididae (six Carcharias taurus, one Odontaspis noronhai) Lamnidae (Isurus oxyrinchus), Pseudocarcharidae (Pseudocarcharias kamoharai), Mitsukurinidae (Mitsukurina owstoni) and Carcharhinidae (two Rhizoprionodon lalandii) were comparatively analyzed. The examined shark specimens present six extrinsic eyes muscles following the general pattern observed in other elasmobranchs: rectus dorsalis (Rd), rectus lateralis (RI), rectus ventralis (Rv), rectus medialis (Rm), obliguus dorsalis (Od) and obliguus ventralis (Ov). The Od muscle was precisely identified by its innervation by the trochlear nerve (IV), from its origin in the encephalon to its insertion in the musculature. This procedure allowed recognizing variations in the origin of the Od from the general pattern observed in other elasmobranchs. We verified two different conditions in the origin of the Od according to its topography: origin on the nasal capsule dorsal to the origin of the Ov (present in Odontaspis noronhai, Alopidae, Pseudocarcharidae, Mitsukurinidae and apparently corresponding to the general pattern observed in other elasmobranchs) and origin on the nasal capsule ventral to the origin of the Ov (present in Carcharias taurus and Rhizoprionodon sp.). This preliminary result suggests that the ventral origin of the Od may have evolved independently in Carcharias taurus and Rhizoprionodon sp. and may be autapomorphic for these species. Isurus oxyrinchus (Lamnidae) presented a variation in the origin of the Ov contrary to the condition observed in Carcharias and Rhizoprionodon. In this species, the topography of the Od is similar to the observed in other elasmobranchs, however the origin of the of Ov is dorsal in relation to the Od. For a better comprehension of the phylogenetic meaning of the variations observed in the Od in Carcharias taurus and Rhizoprionodon sp. and in the Ov in Isurus oxyrinchus, additional representatives of the Lamniformes as well as other elasmobranchs must be comparatively analyzed.

Key words: Anatomy; obliquus dorsalis; obliquus ventralis; eye; elasmobranchs Financial support: Fapesp (processes 2003/03102-7; 2010/52677-6; 2012/22692-9)



## Identification of *Pimelodus maculatus* (Siluriformes: Pimelodidae) from different hydrographic basins using DNA barcoding

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Pimelodus maculatus is a migratory catfish with distribution in the Paraná and São Francisco River basins, in Argentina, Brazil, Paraguay, Bolivia and Uruguay. This species is usually identified based on morphometric, meristic and color pattern, but with the increase in the number of DNA barcoding sequences of P. maculatus deposited in the public databases, BOLD and GenBank, it is possible to test if these identifications are correct, as well as to evaluate the current geographic distribution of this species. The method of DNA barcoding uses a short (~650 bp) and standardized DNA fragment from the mitochondrial COI gene to identify species based on their differences. DNA barcoding can be helpful to species diagnosis because sequence divergences are ordinarily much lower within species than between closely related species. Eighteen samples from the Piquiri, Uruguay, Iguaçu and La Plata rivers were sequenced, and 48 haplotypes from five hydrographic basins (São Francisco, Upper Paraná, Lower Paraná, Paraíba do Sul, and Paraguay) were obtained from BOLD and GenBank. The aenetic distances among and within species were calculated using the Kimura-2-Parameter (K2P) distance model, and a neighbor-joining (NJ) dendrogram of K2P distances were generated using MEGA v 6.0. The species delimitation was initially carried out using 2% divergence as a cutoff value, as employed in others barcoding surveys. The 61 samples from Upper Paraná, Uruguay, Lower Paraná, Paraíba do Sul, and São Francisco rivers were grouped and identified as P. maculatus, with 0.54±0.12% of average genetic divergence between individuals. The remaining five haplotypes, from Paraguay and Lower Iguaçu rivers downstream the Iguaçu Falls differed from P. maculatus with a genetic divergence higher than 5%, indicating a possible problem in species identification. A wide variance in morphology and color pattern in the genus Pimelodus, could be responsible for this problem and is the cause of misidentifications on deposits of COI haplotypes. The occurrence of P. maculatus in three isolated basins (Upper Paraná, São Francisco, and Paraíba do Sul Rivers) deserves a more encompassing research. Maybe, historical (vicariant) or anthropogenic events (e.g. introductions of species or river transpositions) can be involved in the current distribution. Thus, the COI data bank analysis of P. maculatus have been useful to get more information about the species, providing correct identification, and allowing to fill data gaps and correct the mistakes.

#### Key words: Catfish; COI; Distribution

Financial support: CAPES, CNPq, Fundação Araucária, ICMBio-Parque Nacional do Iguaçu, UEL, Unioeste and UENP



#### Ontogeny of the pectoral spine in siluroid and loricarioid catfishes

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Catfishes (Siluriformes) are characterized by several skeletal autapomorphies including the modification of the anteriormost pectoral-fin ray into a lockable spine. This pectoral-fin spine exhibits remarkable variation in size, shape and ornamentation (including serrations, denticulations and the presence of odontodes) and appears to have evolved an association with venom glands independently multiple times within the order. As a result there have been numerous studies into different aspects of the spine in adult stages including how it locks into place, how it is involved in the production of sound in some taxa and how it grows through the addition of distal segments. Many researchers have also recognized the systematic importance of this unique character complex and have utilized it to distinguish both extant and fossil taxa as well as to provide evidence to support phylogenetic hypotheses within some groups of catfishes. Though well studied in adult stages, relatively little information is currently available on the early development of the catfish pectoral-fin spine, with these studies focusing only on siluroid taxa. Given its potential as a resource for taxonomic and phylogenetic studies, it is essential that the development of such a structure is understood. In order to accomplish this we document and compare the earliest stages of pectoral-fin spine formation in representative siluroid (Noturus gyrinus, Ictalurus punctatus, Silurus glanis and Akysis vespa) and loricarioid (Corydoras panda and Ancistrus sp.) catfishes and compare the growth of the spine in representative species of most siluriform families using a combination of clearing and double staining and serial sectioning. We show that despite great variation in adult morphology, formation of the pectoral-fin spine follows a common ontogenetic pathway with adult variation resulting from minor changes to the underlying framework of development. Additionally, we discuss the potential for utilizing pectoral-fin spine ontogeny in future morphological phylogenetic investigations of catfishes.

Keywords: Siluriformes, Development, Morphology



## Genetic diversity in *Rhamdia quelen* Quoy & Gaimard, 1824 (Siluriformes: Heptapteridae)

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The species of Rhamdia are distributed throughout the Neotropical region and, in Brazil, they are commonly known as jundiás. Several species have been described, added, synonymized or revalidated after the description of the genus, resulting in more than 100 names associated to Rhamdia. Regardless the large number of species, there is only one revision of the species of the genus published. This revision is based on characters of external and internal morphology and concluded that Rhamdia contained only 11 valid species among more than 100 available names. Currently, 27 species are recognized for the genus. The largest complexity and unresolved diversity among Rhamdia species is associated to Rhamdia quelen that has 47 nominal species as synonyms. Rhamdia quelen has a wide geographic distribution, from Mexico to Argentina, and it has been recognized as a large species complex. In an attempt to resolve the issues surrounding this complex, an analysis of the genetic diversity of populations related to the R. quelen was made. Therefore, samples throughout the area of distribution of R. quelen and four molecular markers were used: the genes COI and CytB of mtDNA and the genes RAG2 and MyH6 of nDNA. Phylogenetic relationships were inferred by Bayesian Inference using a gene tree and a species tree. The results indicate that this genus is a wellsupported monophyletic group and the specimens of Rhamdia are distributed in two large clades: Mesoamerican clade and South-American clade. The Mesoamerican clade is formed by R. guatemalensis and R. cinerascens. The South American clade is divided into three main groups: the Amazon clade, the Upper-Amazon clade and the Brazilian-Shield clade. The Brazilian Shield clade is composed of two major groups, the Continental Clade and the Atlantic coastal clade, being the last one divided in the highland Atlantic coastal clade and the lowland Atlantic coastal clade. Instead of a polymorphic species with a large distribution (Rhamdia quelen), we found evidence of the existence of multiple species, including the cooccurrence of two, three or even four lineages of Rhamdia in a single drainage. The recent description of two of these lineages as two new species (R. gabrielae and R. eurycephala) with genetic, morphological and geographical patterns consistently defined may indicate that all the lineages recovered herein are different species. Actually there are only five species of Rhamdia considered as valid and in use in rivers draining the eastern and western portions of the Brazilian-Shield (R. branneri, R. enfurnada, R. jequitinhonha, R. quelen, and R. voulezi) contra 14 lineages defined herein, indicating several species to be described or removed from the synonym of other species of Rhamdia, especially R. guelen.

Key word: Neotropical region, taxonomy, systematics, catfish



#### Towards disassembling the non-monophyletic catfish genus Trichomycterus (Siluriformes: Trichomycteridae)

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The Neotropical catfish genus Trichomycterus comprises around 180 valid species and its distribution spans the whole geographic range of the family in South and Central America. However, its non-monophyletic status has been broadly agreed and repeatedly corroborated. A recently published multilocus analysis of the family, with a comprehensive taxonomic sampling of trichomycterine representatives confirmed this situation, but at the same time has revealed a series of clades with some clear geographic circumscription (e.g. Trichomycterus sensu stricto found to be restricted to Southeastern Brazil). In the context of an extensive phylogenetic analysis of Trichomycteridae based in morphology, we studied almost 40 species of Trichomycterus coming from different drainages of Bolivia, Brazil, Chile, Colombia, Panama, Peru, and Venezuela, but mostly coming from trans-Andean and Andean systems, whose representation in the most encompassing available studies is largely missing. Among the Trichomycterus species incorporated into the analysis, there are some key species that have been proposed as members of putative Trichomycterus subclades (e.g. Trichomycterus brasiliensis species-complex). The results derived from this preliminary analysis revealed four monophyletic subsets, restricted to well-defined regions in northern South America (Colombia and Venezuela) and supported by several exclusive synapomorphies. In addition, a homoplastic nature of the single morphological synapomorphy so far proposed for Trichomycterinae is found. The aim of this ongoing initiative is to provide a morphological basis to define and diagnose the different clades obtained from molecular analyses, where a proper understanding of the phenotypic information is at best fragmentary and incomplete.

Key words: Trichomycterinae; Morphology; Phylogenetic analysis; South America



#### Dental morphology and sexual dimorphism of softnose skates genus *Psammobatis* Günther, 1870 (Chondrichthyes: Batomorphi: Arhynchobatidae) from the Atlantic coast of southern Brazil

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The genus Psammobatis Günther includes eight species: Psammobatis bergi Marini, P. extenta (Garman), P. lentiginosa McEachran, P. maculatus Hildebrand, P. normani McEachran, P. parvacauda McEachran, P. rudis Günther – type-species, and P. rutrum Jordan. Psammobatis bergi, P. extenta, P. lentiginosa, and P. rutrum are found in the Atlantic coast of Brazil being distributed from Rio de Janeiro to Rio Grande do Sul states. In Elasmobranchii the dental morphology associated with the sexual dimorphism or ontogenetic changes is a valuable tool to taxonomy, phylogenetic studies and to distinguish ontogenetic state in phylogenetically close species. This work aims to study the dental morphology of P. extenta, P. lentiginosa and P. rutrum. The specimens were captured between 40 m and 145 m depth in the coast of Rio Grande do Sul State and Rio de Janeiro State and are deposited at Fundação Universidade do Rio Grande and Universidade Estadual do Rio de Janeiro. The dental arches were detached from the body, carefully cleaned and photographed in scanning electron microscope. Partial results have shown adult females of the three species with pavement-like dentition, with flat, smooth, monocuspidate teeth (crusher type); the crown had a diamond shape, with the symphyseal teeth slightly larger than the others. The teeth of adult males of the three species, although also monocuspidate, have longer sharp cusp than females (grabber type). Additionally, males' teeth point inward in the symphysis region and sideward (with shorter but still pronounced cusp) in the distal region cross section view, the longest cusp in adult males of P. extenta showed a circular shape while males of P. rutrum and P. lentiginosa have an elliptical shape, with a slight groove in the middle of the cusp. Sub adult males and females, of P. extenta presented no difference related to dentition; the sub adults showed crusher type teeth as the adult females.

Keywords: taxonomy, SEM, fishes, teeth Financial support: CNPq



# Microsatellites mapping reveals distinct patterns of genomic compartmentalization in *Gymnotus* (Gymnotiformes: Gymnotidae) species

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Gymnotus is the most species-rich genus of Neotropical electric knife fish and exhibits a combined electrogenic-electroreceptive system useful for electrolocation and electrocommunication, providing this species as an interesting model for evolutionary and adaptation studies. Previous cytogenetic studies in this group evidenced that the interespecific diversification is followed by extensive karyotype changes, with diploid chromosome numbers ranging from 2n=34 in G. capanema (Milhomem et al. 2012) to 2n=54 chromosomes in G. inaequilabiatus, for example. Herein we described and compared the physical location of three different microsatellite motifs in three Gymnotus species belonging to the G. carapo clade. Our main purpose was to better understand the evolutionary dynamics of different microsatellite sequences in distinct species, which could allow insights into the genome organization and repetitive DNA evolution in fish. Three Gymnotus species were analyzed; G. sylvius, G. inaequilabiatus, G. pantanal. Oligonucleotide probes containing microsatellite sequences (CA)<sub>15</sub>, (GA)<sub>15</sub>, and (GAG)<sub>10</sub> were directly labeled with Cy3 during synthesis. The analyzed species exhibited the expected diploid chromosome numbers; 2n=40 for G. sylvius, 2=54 chromosomes for G. inaequilabiatus and 2n=39 on G. pantanal. All the mapped microsatellite motifs yielded visible signals after FISH, showing conspicuous and clustered hybridization patterns, with signals occurring closely together in a chromosome region yielding a band-like hybridization pattern. In this sense, the markings correspondence between homologues, as evidenced by the karyotypes, enable the correct pairing of homologue chromosomes. Thus, microsatellites might be very useful in the identification of homologues in fish species with homogeneous karyotypes. Genomic abundance of microsatellites provides an interesting opportunity to address issues like chromosome structure and evolution. In the present study, the chromosomal mapping of distinct di- and trinucleotides SSRs was carried out in three species and evidenced that, in general, di- and trinucleotides are very abundant on Gymnotus species. Specific patterns of microsatellite location could be observed, corroborating the hypothesis that microsatellite accumulation in eukaryotic genomes is nonrandom and closely related species show preferential accumulation of specific SSR repeats with a particular chromosomal distribution.

Key words: cytogenetics, FISH mapping, repetitive elements Financial support: CAPES, CNPq, Fapesp



## Molecular phylogeny of the Neotropical weakly-electric fishes of the order Gymnotiformes (Actinopterygii)

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The Neotropical knifefish order Gymnotiformes, comprises 200+ species divided into five families (Apteronotidae, Gymnotidae, Hypopomidae, Rhamphichthyidae, and Sternopyaidae). These fishes have a distribution that includes Central and South America, and inhabit a variety of freshwater habitats. Gymnotiforms are capable of producing and detecting species-specific electrical signals using specialized electric organs and electroreceptors. For these reasons, knifefishes are excellent models for studying biogeography, speciation, and the evolution of communication. However, these studies rely on a clear understanding of gymnotiform phylogeny. To date, attempts at resolving the internal relationships of the Gymnotiformes have yet to produce an unambiguous, well-supported species-level phylogeny. In order to resolve the phylogeny of Gymnotiformes, we used 197 species representatives from nearly all recognized clades and combined molecular data for seven nuclear and two mitochondrial genes (10,603 base pairs). We performed maximum likelihood and Bayesian analyses to produce phylogenies of the order. We found support for the monophyly of all families within Gymnotiformes with the exception of the Hypopomidae sensu Mago-Leccia, which was not monophyletic because the genera Steatogenys and Hypopygus were more closely related to Rhamphichthys and Gymnorhamphichthys than to other hypopomids. Also of note was the prevalence of paraphyly and polyphyly of the genera and recognized major clades found within the Apteronotidae. Sternarchogiton is polyphyletic as Sternarchogiton preto is sister to all other species of Navajini, potentially indicating a new separate lineage requiring reclassification. Porotergus and Apteronotus are also polyphyletic, indicating a need for some Apteronotus species to be reclassified as Porotergus. The genera Sternarchorhynchus and Adontosternarchus were strongly supported as monophyletic. We propose two new major clades within Apteronotidae based on our results: a monophyletic Leptorhynchini clade containing Apteronotus leptorhynchus and close relatives, and a monophyletic Platyurosternarchini clade composed of the two described species of Platyurosternarchus. Our study provides a robust phylogenetic framework for future evolutionary and ecological investigations of gymnotiform fishes.

Keywords: Systematics, Genetics, Central and South America, Freshwater Financial Support: University of Toronto, University of Central Florida, The Academy of Natural Sciences of Drexel University, Pontificia Universidad Javeriana, NSERC, NSF



#### DNA Barcode on the identification of fish fauna from Upper Piraí Basin, Rio Claro, RJ, Brazil

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Identification of freshwater fishes from Neotropical region is historically difficult due to reasons ranging from the great number of undescribed taxa to existence of recently diverged cryptic species. DNA barcoding methodology is an efficient tool to identify the megadiverse Neotropical ichthyofauna. In the present work, we aimed to identify the fish species from the upper Piraí river drainage using DNA barcodes. The Piraí river was a major right bank tributary of the Paraíba do Sul, which has been artificially diverted to the coastal Guandu river system in Southeastern Brazil. DNA sequences of the Folmer region of the Cytochrome Oxidase c subunit I (COI) were produced using standard barcoding methods and highthroughput Sanger sequencing of both DNA strands. The sequences were aligned with the software Geneious v9.1 and a neighbor-joining (NJ) tree of Kimura-two-parameter (K2P) distances was created to represent the divergence pattern among species with MEGA v7.0.26. Complete sequences were obtained for 65 specimens collected in the study area. Using the 2% divergence threshold heuristic cut-off value for species delimitation, we recognized 25 species belonging to 18 genera, 12 families and 5 orders. The inter-specific genetic divergence values ranged from 4.3% to 30,3%, and only three species showed intra-specific genetic divergence on COI region, with values ranging from 0.1% to 0.2%. A comparison between our sequences sequences and morphology-based identifications and data available on Genbank demonstrated 80 % congruence of taxonomic identifications. Within the five species with incongruent identifications, four belong to the genus Trichomycterus. Two of these may represent undescribed cryptic species, one represents a novel DNA barcode for a described species (T.nigroauratus) and another indicates either morphological misidentification in the published database. Our sequences of Phalloceros harpagos also do not match sequences of this species available on Genbank, corroborating the hypothesis from previous studies suggesting that this taxon is part of a species complex. In this study, the COI sequence from Geophagus brasiliensis is identical to sequences of congeneric samples, unidentified at species level, introduced in Australia, showing that they are probably the same species. The present work in progress confirms the efficiency of DNA barcode as a tool to discriminate fish species from Upper Piraí basin, contributing to the knowledge of the ichthyofauna from Neotropical region. Finally, combination of molecular data generated in this study with morphological analyses help clarify taxonomic problems in previous studies.

Keywords: DNA Barcode; Upper Piraí; Taxonomy; Neotropical fishes Financial support: CNPq; CAPES; FAPERJ; SAMN; TNC



#### Phylogenetic position and molecular delimitation of an undescribed species of *Characidium* (Characiformes: Crenuchidae) from coastal streams of Espírito Santo and Bahia, Brazil

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Characidium comprises a diverse Crenuchidae genus with eighteen species described along coastal drainages in eastern Brazilian rivers. Among these, seven species are known from the northeastern Mata Atlântica: Characidium bahiense, C. bimaculatum, C. clistenesi, C. deludens, C. helmeri, C. samurai, and C. timbuiense. Although recent studies were dedicated to species diversity of northeastern coastal rivers, several areas are still in need of further investigation. An undescribed species of Characidium was previously reported from coastal streams extending between the Jucuruçu drainage in southern Bahia and the Itapemirim drainage in southern Espírito Santo. It belongs to a diverse clade of species with a naked area in the isthmus. A diagnosis of the species among other members of this group, however, has proven elusive, as most external characters are plesiomorphic and widespread among congeners, and fin pigmentation is ontogenetically variable, with caudal bars only visible in some large specimens (>32 mm SL). In order to investigate the limits of the species and its phylogenetic position, DNA sequences of the COI gene were produced using standard barcoding methods and highthroughput Sanger sequencing. The resulting sequence is more than 4% divergent in relation to the closest relative, which is well beyond the 2% level of divergence usually accepted as evidence of reproductive isolation among species. A phylogenetic analysis using maximum likelihood methods corroborated the hypothesis of membership on the diverse clade of species with a naked isthmus. Within this group the species is included within the monophyletic clade that also comprises C. alipioi and at least two additional cryptic species from the Paraíba do Sul drainage. The pigmentation pattern of the caudal fin in large specimens and the large distance (when compared with specimens of the C. grajahuense group) between the anal pore and the anal fin is consistent with the hypothesis of a close relationship with C. alipioi. The plesiomorphic pattern of pigmentation (similar to that prevailing in the C. zebra species complex), however, stands out among members of the clade of coastal species with a naked isthmus. The ontogenetically delayed appearance of the dark bars in the caudal fin suggests that the lighter pigmentation of the body may be a paedomorphic process, possibly correlated with the occurrence of the species in areas of low altitude, such as the tableland areas of southern Bahia.

Key words: Molecular Taxonomy; Systematics; Freshwater; Northeastern Mata Atlântica Financial support: CNPq (proc. 301038/2017-1 to EM; proc. 302248/2016-1 to LMSS; proc. 476822/2012-2, 440566/2015-0 to PAB; proc. 350672/2011-3 to LFSI), CAPES (PAB, ACGS, LFSI), FAPES (proc. 53132203/2011, 68856598/2014 to LFSI), FAPES (MML), FAPERJ (PAB)

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#### Phylogeny of Loricariidae (Siluriformes): New mitochondrial genomic data underscores the need to reevaluate the monophyly of the Hypotopomatinae

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The Loricariidae is the fifth most species-rich family among all vertebrates. Several molecular studies based on a limited number of DNA fragments have produced conflicting hypotheses of phylogenetic relationships. Here we used new mitochondrial genomic data obtained through direct DNA next-generation sequencing (NGS) methods to complement a previous study based on mitogenomes assembled using RNA-Sequence data. Paired-end genomic sequences of eight species of various subfamilies of the Loricariidae were produced using Illumina HiSeg 2500 equipment. Complete coding and partial non-coding regions of the mitogenome were reconstructed using denovo and reference-based methods in the Geneioussoftware. Sequences from 15 protein- and rRNA-coding genes were added to the matrix of a recently published phylogenetic study, thus comprising a total of 41 loricarioid taxa and 14,328 nucleotides. A phylogenetic hypothesis was constructed using the maximum likelihood method using the GTR+G+I model in the Mega 7,0 software. Clade robustness was evaluated based on 1,000-replicate bootstrap values. The previous RNA-based phylogenetic hypothesis was largely corroborated in the new analysis. The newly sequenced species were positioned according to morphology-based expectations with 100% bootstrap support, except for two species of Pareiorhina. The resulting cladogram exposes long lasting problems affecting reconciliation of morphological and molecular hypotheses. Pareiorhina rudolphi and P. brachyrhyncha do not form a monophyletic group, with the latter included within Neoplecostomus. This is not surprising because associated bootstrap values are less than 100%, but similar results have previously been obtained by other independent studies. Another recurring issue concerns the position of Pareiorhaphis garbei. In our previous mitogenomic study, this neoplecostomin species had been related to the othothyrin Schizolecis guntheri. Our new data includes two species of Otothyris that form a clade with Schizolecis as expected, and P. garbei forms a monophyletic aroup with the Otothyris + Schizolecis clade. Despite full bootstrap support, such close relationship between a species of Pareiorhaphis is highly unexpected based on morphologic data. However, similar results had been independently obtained by other authors using molecular data. Our new data also includes an additional species of Hisonotus. The inclusion of this additional species corroborates our previous hypothesis that the Otothyrini is not monophyletic. These results suggest that recurrent discrepancies between morphological and molecular data are not statistical artifacts but a real phenomenon that requires further investigation.

Key words: Phylogenomics; Biodiversity; Next-Generation Sequencing Financial support: CAPES, CNPq (proc. 476822/2012-2, 440566/2015-0), FAPERJ, USAID (PGA-2000003446 and PGA-2000004790)



## Review of the freshwater pipefish genus *Pseudophallus* Herald (Teleostei: Syngnathiformes: Syngnathidae)

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The Synanathidae includes different species of pipefishes, seahorses and seadragons, with a great array of characteristics that make this group easily recognizable such as their unique body armor, edentulous jaws, absence of pelvic fins and intermuscular bones, gill opening reduced to a pore on the opercular membrane and, even more remarkably, by the fact that the males carry the eggs in a brood pouch. We present herein a taxonomic review of the genus Pseudophallus, originally described to include two freshwater pipefish species from the Pacific coastal drainages of the neotropics: P. starskii and P. elcapitanensis with the later addition of P mindii, a widespread species from the Atlantic coastal drainages. Pseudophalus mindii was recognized as a single species ranging from the Brazilian state of São Paulo to Belize in Central America, including the Caribbean islands, until 1974, when P. brasiliensis was described, based on specimens collected in Rio Tocantins, with lower meristic values than the Central American specimens and the ones from the rest of the Brazilian coast. In addition, Brazilian specimens of Pseudophallus presented a decreasing clinal variation in a South to North direction in some meristic values. In 1982, P. brasiliensis was synonymized with P. mindii due to the fact that specimens collected in other localities presented meristic values similar to those collected in Rio Tocantins, indicating that the lower counts of the latter species were putatively due to an intraspecific variation along the distribution of P. mindii. Until recently, the restricted number of specimens available in ichthyological collections hindered a proper approach to this taxonomic issue, and to a clear understanding of these clinal variations. We examined 443 specimens, including type material of all species of Pseudophallus from their entire distribution along the Atlantic and Pacific drainages. Five species are recognized in this work: P. elcapitanensis and P. starksii from the Pacific drainages, P. mindii and P. brasiliensis from the Atlantic drainages and a new species, endemic to Lago Izabal, in Guatemala. Diagnostic features of the species include mainly meristic characters, such as number of body rings and number of fin rays, which showed a particular pattern of variation for each species. The Atlantic species, P. mindi is redefined herein to include specimens with higher values and narrow range of variation compared to the other species and is restricted to the northern coastal drainages of Venezuela and Central America including the Caribbean. P. brasiliensis presents a broader variation in counts with a distinct clinal pattern along its latitudinal range, and it occurs along coastal drainages from São Paulo (Brazil) up north to the Orinoco Delta in Venezuela. The new species from Lago Izabal in Guatemala, also has narrow ranges of counts but with lower values, and is distinct from surrounding populations of P. mindii. Both species from the Pacific coastal drainages were corroborated herein: P. starskii occurring from Ecuador to Mexico and P. elcapitanensis distributed from Panama to Costa Rica.

Keywords: Clinal variation, peripheral freshwater fishes, coastal drainages Financial support: FAPESP (Proc. Number 2013/19979-7 and 2015/08024-1)



#### Broad survey of mitochondrial DNA and genomic RAD data: species boundaries, introgression, and phylogeny of Odontesthes silverside fishes (Teleostei: Atheriniformes)

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Odontesthes silversides (pejerreyes) inhabit coastal marine and freshwater habitats in temperate South America. With patterns of rapid speciation and gene flow, their relationships have been difficult to disentangle. We collected a large survey of mitochondrial cytochrome b haplotypes spanning the range of this genus. Mitochondrial DNA does not distinguish species in the O. argentinensis species aroup, between O. mauleanum and O. brevianalis, nor between marine species O. regia and O. aracilis. Interestinaly, this dataset suggests mitochondrial capture of a marine haplotype by some populations of O. mauleanum and O. brevianalis. We further explored relationships and species boundaries in this genus with a large ddRAD dataset. Genome-wide data clearly distinguishes between O. argentinensis, O. humensis, and O. bonariensis, though does not fully separate O. perugiae and O. ledae into monophyletic groups. It also places the Patagonian pejerrey O. hatcheri sister to the O. argentinensis species group, contrary to previous findings. This ddRAD data, like the mitochondrial dataset, does not separate O. regia or O. gracilis into clearly monophyletic groups, suggesting that they are the same species. And, despite mitochondrial capture of a marine haplotype, populations of freshwater Odontesthes in southern Chile have a very low signal of nuclear introgression. Over all, genome-wide RAD data clearly resolve relationships and distinguish species where classical mitochondrial markers have failed.

Key words: Atherinopsidae, Species Delimitation, RADseq, Phylogenomics, Gene Flow



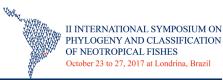
## A new species of *Mastiglanis* Bockmann, 1994 (Siluriformes: Heptapteridae) from Rio Tocantins basin, northern Brazil

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The heptapterid genus Mastiglanis was established by Bockmann in 1994 to include the sanddwelling M. asopos. This psammophilous species has a translucent body in live and is observed in streams with silica sandy bottoms. A remarkable foraging tactic of "drift-trap" is employed by M. asopos only at night; during the daytime it remains buried in superficial part of the sand. The distribution of this genus was reported initially to the Rio Capim and some major tributaries of the Amazon River, as the Negro, Solimões and Tapajós Rivers. The distribution range of this genus has increased significantly in recent years, to include Madeira, Xingu, Tocantins-Araguaia, Branco, Orinoco, Essequibo River basins and coastal drainages of the Guyana Region. Despite this, the aenus Mastialanis remains monotypic. An onaoina taxonomic revision of this aenus is beina carried out so that a large number of specimens from various hydrographic basins is under analysis. The detailed study of individuals of Mastiglanis from tributaries of the Rio Tocantins resulted in the discovery of an undescribed species of this genus. Mastiglanis sp. n. is promptly separated from M. asopos by the dense concentration of dark brown chromatophores in the dorsal half of trunk (vs. dorsal half of trunk with scattered melanophores in M. asopos). Additionally, the new species can be differentiated from its congener by the presence of an evident blotch in the caudal-fin base (vs. absent in M. asopos); presence of dark brown chromatophores along the anal-fin base, forming a stripe (vs. absent in M. asopos); greater posterior internarial width (10.0-14.7% of HL vs. 8.2-9.7% in M. asopos); and longer snout (snout length 36.5-41.6% of HL vs. 23.4-33.6% in M. asopos). The new species is widely distributed in the upper Rio Tocantins basin. The discovery of this new species indicates that Mastiglanis asopos may actually be a complex of species, which is also corroborated by the discrimination of other putatively undescribed species in collection specimens currently identified as M. asopos, which will be also commented on in this work.

Key words: Heptapterid; Psammophily; Taxonomy Financial support: CNPq



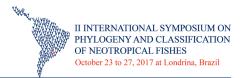
## Phylogeny of the genus *Gymnotus* Linnaeus (Gymnotiformes: Gymnotidae) emphasizing species of eastern Brazil

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Gymnotus is a widely spread genus, occurring from southern Mexico to northern Argentina. Species of Gymnotus have been placed into three species groups based on a morphological phylogeny, G. cylindricus, G. pantherinus and G. carapo species groups. In the past few years, new phylogenetic hypotheses, based mainly on molecular data, have pointed two possible scenarios: the inexistence of these groups as monophyletic entities or the necessity of revision of the morphological characters supporting each group. Nevertheless, all these hypotheses focused Amazonian taxa, including few species from eastern Brazil. The late area is covered by the Atlantic Forest Hotspot biodiversity and comprises numerous isolated drainages, sheltering a considerable species diversity and, thus, being of great value to understanding of phylogeny and biogeography. We here performed a molecular analysis aiming the inclusion of new information generated by the analysis of 13 taxa (seven new species) from eastern Brazil. The matrix was based on four markers (one mitochondrial and three nuclear) totalizing 3704 bp. Bayesian and maximum parsimony analyses were conducted. Our preliminary results indicated that the G. pantherinus species group is paraphyletic, being recovered as three distinct well supported lineages, one bearing eastern species and the others with amazonic species. Due to the low support, the relationship among those three lineages of G. pantherinus species aroup and the clade composed by G. carapo and G. cylindricus species aroups is still not clear. The G. cylindricus species group is corroborated as the sister group of the G. carapo species group. Opposing to the most recent hypothesis, monophyly of G. carapo species group was recovered, including two well supported sister lineages. However, unlikely to G. pantherinus species group, relationships among these species are very complex and do not follow a regional grouping pattern, as some species from eastern Brazil appear to be closely related to amazonic ones.

Key words: Bayesian Analysis; molecular; Atlantic Forest; Electric-fishes Financial support: CNPq (Process 153329/2015-7)



## Caudal musculature in Carcharhiniformes (Elasmobranchii: Chondrichthyes), with remarks of the phylogenetic signal present within

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The musculature of the caudal region was dissected in carcharhiniform sharks to give a better understanding of the evolution patterns contained within this anatomical complex. Although many studies have already been made on this order, composed by many well-known representatives, the trunk musculature, especially the muscles associated with the tail, have not been explored to their full potential. Shirai, on his work in 1992, revealed that this complex could be a source of information among the squalean groups. Since this work, not many works of comparative anatomy with a phylogenetic background were made, resulting in a gap of knowledge in galeomorph sharks. On this work, we dissected six of the eight families recognized by Compagno (1988) and representatives of other chondrichthyan groups. They were then described and compared to evaluate possible convergences of genera and/or families and, therefore, possible interrelationships. The results showed that Carcharhinidae, Sphyrnidae and Hemigaleidae have many convergent muscular patterns, reinforcing the idea they are related with each other. Carcharhinids showed two main patterns on the axial musculature, one exclusive to the family and another shared with sphyrnids and hemigaleids, indicating a possible transition between a plesiomorphic state to an apomorphic state. The only non-axial muscle present in the caudal region is the flexor caudalis, which has a singular distribution. It has a flexed shape on carcharhinids and in no other galeomorph group seen. The only other group with this disposition is Squatiniformes, but with a mirrored appearance (the apex of the "V" shaped fibers is anterior in squatinids and posterior in carcharhinids). Leptocharias was found to be closely related to this crown-group among carcharhiniforms, having a similar pattern to the one present in sphyrnids and hemigaleids. Scyliorhinidae and Triakidae appeared to have a common pattern as well, hence indicating a possible close relationship of these two families. Among the triakids, Hemitriakis japonica seemed to be slightly more like the crown-group than other members of the family. It could be due to its size and its ecology, which are similar to the crown-aroup than other triakids.

Key words: Comparative anatomy; sharks Financial support: FAPESP (process 2014/13023-1)



#### Morphological variation of *Metynnis lippincottianus* (Cope) (Characiformes: Serrasalmidae)

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Metynnis lippincottianus is a small-sized Serralmidae that rarely exceeds 140 mm of standard length. This species has a very high range of number of lateral line scales and a great variance of body shape and color pattern (marked by dark maculae on flank), characteristics that difficult its identification, causing confusion among males and females, young and adults, which were in some cases described as different species. Currently, there are seven nominal species considered junior synonyms of M. lippincottianus (M. dungerni, M. goeldii, M. heinrothi, M. orbicularis, M. roosevelti, M. seitzi, and M. snethlageae). Within Metynnis, M. lippincottianus is the most widespread species, occupying the river basins of the whole cis-Andean region of South America, except from Paraguay River basin. Despite this great distribution, in a study developed by the second author, specimens from different locations did not present molecular variance on mitochondrial DNA, cytochrome c oxidase subunit I (COI), except for two individuals from Catalão lake, Iranduba, Amazonas State, that along with one specimen from Madeira river, Porto Velho, Rondônia State, formed a distinct clade, recovered as sister aroup of M. mola. Therefore, the main goal of this study was to investigate morphological variation between M. lippincottianus populations, especially those from localities where individuals presented this high genetic variation. The morphological analysis involved 23 measurements and 15 counts taken on left side of specimens whenever possible. Approximately 90 specimens from different river basins were examined, including Amazonas, Guaporé, Madeira, Paraná, São Francisco, Solimões, Tocantins, Tracateua, Trombetas and Xingu. The greatest number of specimens were from Madeira and Solimões Rivers. The main result was the recognition of an uncommon characteristic within Metynnis, which is a morphological variation of the pelvic fin, consisting in the ramification of the first ray, therefore all six pelvic-fin rays are branched, whereas the usual morphology for the genus is the presence of one unbranched ray and five branched rays (i,5). This feature was observed only in specimens from three lots from Catalão lake (including the voucher of specimen that presented genetic variation), one lot from Solimões River, in Manacapuru, and one from Guaporé River. This pelvic fin variation had never been mentioned for M. lippincottianus before, neither cited in the original description of the species and of its synonymies nor by posterior reviews. However, Metynnis roosevelti, was described for Manaus, very close to Catalão lake, the locality where were found the greatest number of individuals with all pelvic-fin rays branched. In the light of the results and analysis of this study, it is concluded that there is both molecular and morphological evidence suggesting the presence of an undescribed species or also that one of M. lippincottianus synonyms, most likely M. roosevelti, is in fact a valid species. Investigation of type specimens of M. roosevelti is already in progress, and this is another example of studies allying molecular and morphological tools to reveal differences not detected before.



### Diversity of attachment organs in characiforms (Teleostei: Ostariophysi)

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After hatching from their eggs larvae, numerous teleosts have the ability to attach to various substrates during the first few days of their lives. Attachment is achieved with the aid of specialized cells or organs that are only present and functional during a short period of the larval phase, often between hatching and free-swimming while the yolk sac is resorbed. Larval attachment organs are generally difficult to study due to their restricted temporal presence and have therefore been reported only for a comparatively small number of bony fishes. We describe here the structure of attachment organs in more than a dozen species of characiforms from five different families (Lebiasinidae, Characidae, Alestidae, Erythrinidae, Ctenoluciidae) based on scanning electron microscopy (SEM) of larvae after hatching. Our SEM studies were complemented by histological and transmission electron microscopic investigation of attachment organs in selected species, as well as PAS (periodic acid Schiff) staining of organs. The three types of attachment organs (I. scattered individual attachment cells, II. multicellular glands without a central lumen, and III. multicellular glands with a central lumen) identified by IIg are present in characiforms. We highlight the diversity of attachment organ structure, which is still largely unexplored, and introduce a new character system providing potentially phylogenetically interesting information that could aid in further resolving characiform intrarelationships.

Key Words: Taxonomy; Systematics; Neotropical Otophysi; Morphology



#### Phylogenetic inferences of gross brain morphology in Siluriformes (Teleostei: Ostariophysi)

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Although there is large amount of literature about the nervous system of fish, only 1% of the synapomorphies for teleosts have a neuroanatomic origin. The objective of this work is to describe neuroanatomical patterns in Siluriformes, as a survey of new characters that can elucidate phylogenetic issues for the group. A total of 309 specimens from 35 families were examined. The information was collected through dissection of the neurocranium and direct observation of the brain. Most of the changes in brains occurred in the rhombencephalon, with the lobus facialis displaying almost exclusively shape for each of the families and subfamilies sampled. Diplomystes presented the exclusive features a torus longitudinalis exposed and swollen, and dorsolateral position of the olfactory organ. It shares some characteristics with the Loricarioidea: slender lobus vagi; lobus facialis lateral and without subdivisions; lobus vestibulolateralis at anterior position; corpus cerebelli high, and with semicircular posterior margin extending to anterior margin of lobus vagi. The shape of the lamellae of the olfactory organ is unique for the clade Nematogenyidae + Trichomycteridae. The shape and position of the lobus facialis and the shape of posterior margin of the corpus cerebelli suggest unique character states for Trichomycterinae. Stegophiliinae could be divided into two groups, based on specialization in vision and smell, and this is supported by data on feed habits present in the literature. There are evidences for the basal position of Neoplecostominae in Loricariidae and possibly a clade containing Astroblepus, Lithogenes and elements of Neoplecostominae, based on the morphology of corpus cerebelli and lobus facialis. The brains of the Kronichthys, Parotocinclus and Rineloricaria are very different from the general pattern of their respective subfamilies, which signs the complexity present in Loricariidae. The position and shape of bulbus olfatorius and olfactory organ as well as the increase in the number of lamellae indicates specialization of smell sense and may be related to feed habits in Stegophiliinae, Cetopsinae and Delturus. An assemblage composed by Cetopsinae and Helogeninae was corroborated mainly by the morphology of tectum mesencephali and olfactory organ. Plotosids Plotosus and Tandanus showed an increase in volume of the lobus vagi and exclusive features in relation to other Siluriformes in the morphology and position of the olfactory organ. In Ariidae, specimens of Bagreinae presented an elliptical, asymmetric olfactory organ, with dorsal flaps in lateral position and with short base; in Ariinae, the organ shows a triangular, symmetrical shape, the posterior flaps being gradually higher than the anterior and longer base. The morphology of the corpus cerebelli and lobus vestibulolateralis, lobus facialis and bulbus olfactorius, and the number and shape of the lamellae of the olfactory organ indicate large groups in the Siluriformes. Such patterns reveal that the central nervous system holds important phylogenetic information to be explored.



#### Gross brain morphology of some Peruvian Astroblepidae (Teleostei: Ostariophysi: Siluriformes) and its phylogenetic implications on sistergroup Loricariidae

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Astroblepidae is a diverse genus with a restricted distribution to the northern portion of the Andes on both cis- and trans-Andean regions, inhabits streams between 1000 to 3500 meters above sea level. There are 81 valid species described, and many possible new species not yet described recognized in museum collections. This group is possibly the most in need of study among the Loricarioidei, there is currently no accepted phylogenetic framework for it. Although the current consensus as the sister-group of the species-rich Loricariidae, previous hypotheses considered astroblepids as a member of the latter, or composing a clade with Lithogenes, sister-group to other Loricariidae. Despite the vast amount of neuroanatomical information for fishes, the phylogenetic implications of such data remain sparse. This study provides the gross brain morphology comparison among 20 astroblepid species, including several undescribed, and also representatives of 75 species included in all Loricariidae subfamilies. The oval shape of olfactory organ, its lamellae dorsal flaps laterally positioned and the bulbus olfactorius positioned between olfactory organ and telencephalon, are Astroblepids exclusive features within Loricarioidei. The relationship with Lithogeninae and Neoplecostominae is supported by: the lobus facialis conical shape, laterally positioned, and without callosities; the semicircular shape of posterior margin of the corpus cerebelli extending beyond the posterior margin of the lobus vagi; the tectum mesencephali dorsal to trunchus cerebri. Delturinae have the nervus tractus olfactorius three times thicker than the nervus opticus and shares with Hypostominae and Ancistrinae the shape of the lobus vagi partially pentagonal; the shape of corpus cerebelli semicircular in lateral view; and the position of bulbus olfactorius fused to the olfactory organ. Hypoptopomatinae exhibits most of its structures less developed among all Loricariidae, and present cases of sexual dimorphism. In Loricariinae, the Loricariichthys group is supported by the swelling of the lobus vagi and lobus inferior hypothalami; and position of the lobus facialis in relation to corpus cerebelli. However, Rineloricaria shows the swollen lobus vagi, as Farlowella (versus slightly swollen in Harttia and very swollen in Loricaria and Loricariichthys group), and the lobus facialis at a less inclined angle to the parasagittal axis, different from approximately 45 degrees that is present in Farlowella. Farlowelling and Harttiing are also corroborated by neuroanatomical data. Those morphological features were present in all specimens dissected and indicate that the brain has valuable information to enhance phylogenetic studies.

Key words: Andean catfish, Neuroanatomy, Loricarioidei, Phylogeny, Evolution



#### Chromosomal analysis in Amazonian species of Parauchenipterus (Siluriformes: Auchenipteridae): B chromosomes in sympatric species

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Auchenipteridae belongs to Siluriformes, represented by a group of small to medium catfish, with 24 genera and approximately 120 species. It is divided into two subfamilies: Centromochlinae and Auchenipterinae. Parauchenipterus is considered by some authors as synonymous of Trachelyopterus due to the morphological similarity present in the species of these genera, however, another authors consider Parauchenipterus as valid. Basic and molecular cytogenetic techniques were performed in two sympatric populations of Parauchenipterus: P. galeatus and P. porosus from Catalão Lake, Amazon river basin. The staining by Giemsa identified the diploid number of 58 chromosomes and the presence of 1 to 3 B chromosomes for both species, which presented numerical variation intra and interindividual. The population of P. galeatus presented a karyotypic formula with 20m+12sm+18st+8a, while P. porosus presented 22m+16sm+10st+10a. The variations related to karyotypic formula may be originated from nonrobertsonian rearrangements, such as translocations and/or pericentric inversions. C-Banding showed heterochromatic bands in the terminal regions of most chromosomes of A complement with pale markings, common pattern in Auchenipteridae, and the B chromosomes were totally heterochromatic. Of the 13 analyzed specimens of P. galeatus, 6 (5  $\bigcirc$  and 1  $\bigcirc$ ) presented small and metacentric B chromosomes. Of the 14 P. porosus analyzed specimens, 8 (5  $\varphi$  and 3  $\beta$ ) presented B chromosomes. It was detected in two females of P. porosus variant B chromosomes (B2), in addition to the small metacentric one (B1) that are more frequent in the population. The variant B chromosomes are largest than the more frequent B chromosomes, moreover are smaller than the chromosomes of A complement. AgNORs were marked in only one terminal subtelocentric pair at the short arm in each species (pair 20 in P. galeatus and pair 23 in P. porosus), which is a common feature in Auchenipteridae. Fluorescence in situ hybridization with 18S rDNA probes confirmed the AgNORs in both species. FISH with 5S rDNA probe detected sites at the short arm of pair 12 and at the long arm of pair 16 in P. galeatus, both submetacentric. In P. porosus, the 5S rDNA sites were detected at the short arm of pair 14 and in the long arm of pair 16, both also submetacentrics, results that are similar to other previously studied populations of Parauchenipterus. The similar cytogenetic results to other populations of this genus previously studied added to B chromosomes of same morphology support close phylogenetic relationships among these taxons. B chromosomes totally heterochromatic, small and metacentric are similar between the sympatric species analyzed and the P. galeatus population of the São Francisco River Basin. However, the intrapopulational frequency of B chromosomes is higher at the Amazon populations compared to the São Francisco river population, which has a higher interindividual frequency. This population of P. porosus is the second species of Auchenipteridae with B chromosomes, which has a different morphology and size of the other populations, suggesting a distinct origin between these two morphologies in addition to an old origin in the group due the presence in different taxa.



## Basic and molecular cytogenetic analysis in three species of Auchenipteridae (Siluriformes)

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Among Siluriformes, Auchenipteridae represents an endemic fish group from Neotropical region and is divided in two subfamily, Auchenipterinae and Centromochlinae. Chromosomal analysis in this family cover only a few genera: Ageneiosus, Auchenipterus, Glanidium, Parauchenipterus and Tatia. Taxonomy of Parauchenipterus is historically confusing and has problem since its description, some authors considered this taxon synonymy of Trachelyopterus and others considered as a valid genus. Thus, three species, Parauchenipterus galeatus and Trachelyopterus coriaceus from the river Araguaia basin which are sympatric, and Parauchenipterus striatulus from the river Doce basin were chromosomally analyzed. Through Giemsa staining technique, 2n=58 chromosomes was identified for the three populations, and karyotypes formulas were composed of 20m+18sm+18st+8a for P. galeatus and T. coriaceus, and 18m+20sm+10st+10a for P. striatulus. The differences found in the karyotype formula must be related to rearrangement such as pericentric inversions and/or translocations. Heterochromatin was preferably distributed in terminal regions of most chromosomes in all analyzed species, which is a common character in Auchenipteridae. The silver nitrate staining revealed simple NORs allocated in the terminal region of the short arm of subtelocentric pair 23 for T. coriaceus and P. striatulus and subtelocentric pair 24 for P. galeatus. The 18S rDNA-FISH (fluorescence in situ hybridization) confirmed the results revealed by silver nitrate staining in the three species; the quantity and localization of NORs is common when compared with another species of clade (Parauchenipterus+Trachelyopterus), however, this marker vary so localization among others species of Auchenipteridae. The 5S rDNA-FISH probe evidenced interstitial fluorescent signals for all the species. These sequences were allocated on the short arm of metacentric pair 3 in P. galeatus, T. coriaceus had two pairs with these sequences, short arm of the metacentric pair 3 and long arm of the submetacentric pair 17. Indeed, this quantity (2 pairs) and interstitial localization has already been registered in P. galeatus, however, both in P. galeatus previous studied are submetacentric pairs. On the other hand, P. striatulus had three pairs carrying 5S rDNA, on the short arm of the submetacentric pairs 10 and 13, and on the long arm of the submetacentric pair 15. The results demonstrated similarity between the species analyzed and with to other populations of Parauchenipterus previously studied, which confirms the phylogenetic proximity between the genera, though, the presence of just one pair with 5S rDNA marker in P. galeatus is a difference that possibly indicates the existence of a new taxonomic unit related to Araguaia river basin.

Key-words: rDNA-FISH; Sympatry; Citotaxonomy, Parauchenipterus, Trachelyopterus Financial support: CNPq, Fundação Araucária



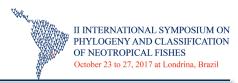
# Description of the gonopodial tip of *Phalloceros titthos* Lucinda, 2008 (Cyprinodontiformes: Poeciliidae) and extension of the species distribution area

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Phalloceros is a monophyletic genus of poeciliids distributed in freshwaters of southeastern South America, with coastal stream occurrences distributed between the southern portion of the Brazilian state of Bahia and Uruguay. For over a century Phalloceros was considered a monotypic genus until 2008 when a review of morphological characters led to the description of 21 additional species. However, due to the lack of adult males of Phalloceros titthos available for that study, the gonopodial tip of this species was not described. Phalloceros titthos is diagnosed solely by the presence of papillae in the mandibular symphysis of adult females. The description of Phalloceros titthos was based on specimens from a single location in the Paranaguá Bay and listed an additional sample from a stream near Guaratuba in Paraná State of Brazil. In the present study we describe the tip of the gonopodium of Phalloceros titthos, and update its distribution area. The descriptive nomenclature follows Lucinda & Reis. Our gonopodial description is based on the examination of 68 adult males (those with a fully developed gonopodium), from five lots cataloged in the Ichthyological Collection of Museu Nacional, Universidade Federal do Rio de Janeiro. Species delimitation is based on DNA sequences of the Folmer region of the COI mitochondrial gene. The specimens were identified based on their association with females that bear symphyseal papillae and general agreement with Lucinda's description (based on juvenile males). Location coordinates were obtained with GPS. According to our results, adult males of Phalloceros titthos have small, simple hooks (vs. sickle-like) in both gonopodial appendices. The distal half of appendix is narrower than the proximal half. No symphyseal papillae were found in juveniles and adult males. In adult females the quantity and size of the symphyseal papillae are variable, and may be absent in some specimens, including pregnant individuals. The distribution of this species is expanded to additional river drainages, with new occurrences in the Ribeira de Jauape and Morato river basins in the state of Paraná, and the Iguaçu and Itapocu river basins in the state of Santa Catarina. Except for the presence of symphyseal papilla in adult females, the external morphology of P. titthos, including the tip of the gonopodium, is guite similar to that of P. harpagos. However, DNA barcode data of specimens collected at the type locality of P. harpagos demonstrate that the specimens of P. titthos from Paraná and Santa Catarina are not closely related to topotypes of P. harpagos. Comparisons of DNA Barcode sequences of pregnant adult females of P. titthos with and without symphyseal papillae corroborate the hypothesis that the presence of symphyseal papilla is variable in adult females of this species. Variability in the presence of synphyseal papillae in P. titthos and the presence of this species in several river drainages, including the Iguacu drainage, suggest the possibility that specimens of the former might have been misidentified as P. harpagos in the literature, and may have contributed to the prevailing hypothesis that P. harpagos is a species complex.

Key words: Systematics; Taxonomy; DNA barcoding; Species delimitation; Livebearers Financial support: CAPES, CNPq (proc. 564940/2010-0, 476822/2012-2), FAPERJ E-26/111.404/2012, E-26/200.697/2014)



# A new species of the rare genus *Myroconger* (Anguilliformes: Myrocongridae) from Brazilian waters, western South Atlantic

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The family Myrocongridae includes a single genus, Myroconger, and five valid species of rare eels commonly known as red eels that inhabits the epi- and mesobenthonic zones at depths from 50 to 640 m. The family Myrocongridae is diagnosed by the following features: basibranchial absent; lateral line restricted to a few pores, only one to seven pores at anterior end of canal; position of posterior nostril high on head, before uppermost part of the eye; and the presence of pectoral fins. Some studies considered Myrocongridae as the sister group of the families Chlopsidae and Muraenidae, although more recently molecular analysis treated Heretenchelyidae as basal to a clade composed of Myrocongridae and Muraenidae. Myroconger compressus Gill (1890) is described from the Saint Helena Island in Central South Atlantic, and known from other parts of the eastern South Atlantic off the western coast of Africa and other oceanic islands: Cape Verde and Saint Thome and Prince. Four species were also discovered to date in the Indian and Pacific Oceans: M. gracilis Castle, 1991, from the Kyushu-Palau Ridge, in the western North Pacific; M. prolixus Castle & Béarez 1995, from off New Caledonia and Norfolk ridge, in the western South Pacific, M. niarodentatus Castle & Béarez 1995, described from a specimen acquired in a fish Marked in Manabí, Ecuador, eastern South Pacific; and M. seychellensis Karmovskaya, 2006, from the Seychelles islands, Indian Ocean. In the western Atlantic, the only record of the genus was based on a single specimen not identified to species level, collected during the REVIZEE Program. For this work, that specimen was reexamined and properly compared with additional material obtained from other museums and revealed to be an undescribed species. The aim of this contribution is to describe the new species of Myroconger. The new species can be diagnosed from M. nigrodentatus by absence of dark brown internal pigment on the tips of the maxillary teeth, and vomerian teeth are not smaller than the intermaxillary teeth (vs. presence of dark brown pigment on the tips of the maxillary teeth, and vomerian teeth smaller than intermaxillary teeth). The new species could be distinguished from M. compressus, M. gracilis and M. seychellensis by vomerine teeth uniserial (vs. biserial) and from M. prolixus by having 5 lateral line pores and total vertebrae 125 (vs. 11 lateral line pores and 147 vertebrae). So far, the species is only known from the single specimen, taken from the tropical western South Atlantic in Aracati bank, off Ceará State, Brazil, 253 meters depth.

Keywords: Taxonomy, marine fishes, eels Financial Support: CAPES



#### Molecular phylogenetics of Neotropical Beloniformes (needlefishes and allies): A current appraisal of phylogenetic and biogeographic patterns

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The order Beloniformes includes approximately 250 species distributed primarily in tropical waters. While many species are marine, a sizeable component of species diversity occurs in freshwaters. In the Neotropics, there are ten freshwater needlefish (Belonidae) species, including four species of Potamorraphis, two species of Belonion, two species of Pseudotylosurus, and two species of Strongylura (S. fluviatilis and S. hubbsi). There are also two species of freshwater halfbeaks (Hemiramphidae) in the Neotropics: Hyporhamphus brederi and H. mexicanus. It is generally accepted that the Neotropical freshwater beloniforms are the products of multiple evolutionary transitions between marine and freshwater lineages. However, determining the polarity and biogeographic basis of marine/freshwater transitions is dependent on denselysampled and robust species level phylogenetic hypotheses. Here, I provide a new multi-gene phylogenetic hypothesis for beloniform fishes, and an evolutionary reconstruction of marine/ freshwater habitat transitions. Optimization of habitat on the total evidence tree, combined with paleogeographic data, suggests that six independent transitions into freshwater have occured—two in Central America, and four in South America. I consider the role that marine incursions in South America may have played as a driver of freshwater habitat evolution and diversification in the beloniform lineages occupying Atlantic-draining rivers. Finally, I present new phylogeographic datasets for the main clades of freshwater needlefishes (Potamorrhaphis clade and Pseudotylosurus clade) that occupy the large Atlantic drainages of South America, including the Amazon, Orinoco, and de la Plata. Overall patterns of population structure reflect the ecological and life history characteristics of these clades. Potamorraphis individuals occupy smaller streams, floodplains, and lakes, and exhibit geographically based population structure at small spatial scales. Pseudotylorusus individuals occur in larger rivers and exhibit minimal population structure at large spatial scales. Patterns of relationships among Potamorrhaphis lineages provide evidence to infer connectivity of river basins, particularly the Amazon/ Orinoco. Evidence from this clade of needlefishes indicates that rivers of the Guianas region played a key role in connecting the Amazon and Orinoco basins. Phylogeographic patterns also suggest the existence of several morphologically cryptic species of Potamorrhaphis.

Key words: Systematics; Phylogeography; Paleogeography; Freshwater; Marine Financial support: NSERC Discovery Program, Canada



# Molecular phylogenetics of *Gymnotus* electric fishes, a model system for understanding behavioral and molecular evolution

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The Neotropical electric fish genus Gymnotus includes 40+ species distributed from southern Mexico to Argenting. The clade includes species distributed on both the Pacific and Atlantic sides of the Andes, and reaches its apex of diversity in the Central Amazon. Gymnotus are nocturnal predators of invertebrates and fishes, and are characteristic inhabitants of lentic systems such as swamps, floodplains, and low-aradient streams. Linnaeus described the first species of Gymnotus (G. carapo) from Suriname in 1758, but most species diversity in the genus has only been elucidated during the past three decades. The recency of most taxonomic discovery in the group means that species-level phylogenetic reconstructions are in their infancy. Here, we present the most recent multi-gene phylogeny for the genus Gymnotus, and provide comparisons to earlier morphological and molecular phylogenetic hypotheses. We observe concordance in many elements of these trees, indicating convergence towards a stable set of species relationships. We then provide examples of ongoing work, which uses Gymnotus phylogeny as a model system for understanding basic ecological and evolutionary questions. Adult Gymnotus individuals exhibit species specific electric organ discharges (EODs) which are used for both reproductive and navigational purposes. However, these EODs many be conspicuous to electroreceptive predators, hence natural selection is expected to drive the evolution of crypsis in these signals. We examine this idea using a phylogenetic and biogeographic framework, whereby we test whether Gymnotus that co-occur with electroreceptive predators have evolved more cryptic EODs compared to Gymnotus species that occur in regions with fewer electroreceptive predators. To investigate the molecular basis of evolutionary changes in communication signals, we explore rates and patterns of molecular evolution in genes associated with different sensory modalities. Since Gymnotus uses electroreception as a sensory system, with concomitantly reduced emphasis on vision, we hypothesize that a gene related to electric signal production (sodium channel 1.4a; Nav1.4a) will exhibit elevated rates of positive Darwinian selection in comparison with a nuclear gene (rhodopsin) associated with vision, and with nuclear and mitochondrial housekeeping genes. We use phylogenetically informed statistical approaches to test for signatures of selective forces in patterns of molecular sequence variation.

Key words: Systematics; Freshwater; Neotropical; Communication; Electric signals Financial support: NSERC Discovery Program, NSF DEB



# Cytogenetic markers applied for understanding the diversity and phylogenetic relationships in three genera of thorny catfishes (Siluriformes-Doradidae) with taxonomic problems: Anadoras, Astrodoras and Amblydoras

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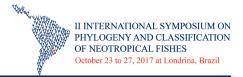
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Doradidae thorny catfishes is a monophyletic family which comprises 95 species endemic of South America freshwater systems. According to molecular and morphological phylogenetic analyses, this group is composed by three subfamily: Wertheimerinae, Astrodoradinae and Doradinae. Although it is a monophyletic group, the phylogenetic relationships of some genera remain uncertain, such as Anadoras, Astrodoras and Amblydoras. Thus, different species of these three genera was cytogenetically analyzed herein, aiming to understand the real diversity as well as to suggest new phylogenetic relationships. Anadoras is composed by two valid species: A. weddellii and A. grypus, here both were analyzed and had 2n=56 chromosomes with high divergence in distribution of rDNA. Anadoras sp. "araguaia" also had 2n=56 and shared the same karyotypic formula and rDNA pattern described for A. weddellii, but some differences in heterochromatin distribution. The molecular and morphological phylogenies were discordant regarding the relationship among species of Anadoras, therefore the cytogenetic markers reveal the same grouping obtained by morphological analysis with A. grypus as a sister group of the clade formed by A. weddellii + Anadoras sp. "araguaia". Astrodoras is a monotypic genus with wide distribution in rio Amazonas basin, cytogenetic analysis reveals differences between populations from Barcelos (rio Solimões) and Anavilhanas (rio Negro). Both exhibited 2n=52 chromosomes and the same pattern of rDNA, however the distribution and amount of heterochromatin blocks are distinct, probably due to cryptic diversification. Amblydoras is the most complex genus of Astrodoradinae; herein, we analyzed two populations of Amblydoras aff. affinis both had 2n=46 chromosomes and the same rDNA pattern, but high divergence in distribution of heterochromatin. Amblydoras hancockii also had 2n=46, and particular rDNA and heterochromatin patterns. The last phylogenetic analysis proposed the synomization of six species in A. affinis, including A. hancockii. Our data suggest the revalidation of A. hancockii and a taxonomic revision of A. affinis complex. The present study demonstrates the importance of karyotypes for understanding the diversity and phylogenetic relationships in groups with taxonomic problems. The next step will be to include DNA barcode and other molecular markers, this integrative approach may provide important subsidies for the hypotheses raised here.

Keywords: Karyotypes; Citotaxonomy; Astrodoradinae; cryptic species; DNA barcode Financial support: CAPES/ FUNDAÇÃO ARAUCARIA



### GenoPesq: the use of NGS methods for species identification in fisheries

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Fisheries is one of the most important economic activities in Brazil. The sustainability of the exploitation of natural fishery resources relies on solid data in order to guarantee the preservation of the potential for future exploitation, maintenance of genetic variation and consumer rights. Some of the major issues in this economic sector are the unauthorized exploitation of threatened populations and species, and the deliberate mislabelling of commercial products. Sequencebased forensic identification methods allow the detection of fraud in a reproducible and scalable manner and is the major focus of the main international programs concerning these issues. Furthermore, the application of these techniques also has large potential in related fields such as artificial selection and breeding programs. The development of new sequencing methods, collectively known as Next Generation Sequencing (NGS) allow for the collection of molecular sequence data with a great increase in efficiency, not only leading to a much larger volume of data (typically orders of magnitude larger than traditional sequencing methods), but also increasing reproducibility and the possibility of processing a large number of samples simultaneously. NGS methods can be applied to a great number of samples by a single individual with sufficient technical expertise decreasing the amount of variation among samples and facilitating the standardization of procedures. Sequence-base identification of fisheries is currently being done by several research groups in Brazil however, NGS methods are still not part of this repertoire. The objectives of this project are to promote the use of NGS methods by implementing and adapting a high-throughput platform for establishing a comprehensive molecular database and performing species identification in fisheries. Here, we complile and manually curate a large collection of marine water fish DNA sequences and develop a targeted-sequencing approach that allows for the rapid and efficient expansion of the database through the capture of hundreds of homologous loci in a wide taxonomic range. This project will be based on a newly build Center for the Molecular Identification of Fisheries at the Federal University of Rio de Janeiro.

Keywords: biodiversity, targeted capture



### The genetic basis of repeated phenotypic evolution in cichlid fishes

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The genetic basis of adaptive radiations and the mechanisms that influence the rate of convergent evolution have long been debated in the field of evolutionary genetics. The occurrence of high levels of phenotypic convergence in groups of organisms that underwent recent adaptive radiations has led to suggestions that convergence can result from internal factors, such as developmental biases. Cichlid fishes are the most species-rich family of vertebrates and exhibit an extraordinary level of evolutionary convergence, including a specialized morphology consisting of elongated heads and hypertrophied lips that has evolved several times independently. We used genetic crosses between "lippy" and "non-lippy species", RAD-sequencing and foraging performance assays to a) validate the ecological relevance of the morphological adaptations and to; b) map the loci responsible for this variation as well as for sex-determination. The mapped genomic regions for adaptive morphology show evidence of strong natural selection and affect the individual's foraging performance. The similar phenotypes that evolved in both lineages differ markedly in their genetic architecture. While in the cross between African species the traits have a highly polygenic basis, most of the morphological variation maps to a single locus in the Nicaraguan cichlid cross. The QTL that we detected in both crosses do not overlap, suggesting that repeated evolution in this case was not facilitated by biases in genetic variation. The same was observed regarding sex-determination, where both lineages evolved genetic sex-determining messages on non-homologous genomic regions. By uniquely combining genetic mapping and foraging performance trials, we uncovered that - as is already known for sex determination in fishes and was also shown here - convergent evolution of adaptive morphologies can occur through many genetic routes thus not requiring a particular type of genetic architecture.

Key words: adaptive radiation, linkage mapping, parallelism and convergence

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## Morphology and osteology of the cranial sexual dimorphism in the Amazonian electric knifefish Compsaraia samueli

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Gymnotiformes is a diverse group of Neotropical freshwater fishes that are difficult to collect in large numbers during the breeding season due to floods. In some dimorphic species, males and females can only be distinguished when sexually mature. In the family Apteronotidae, sexual dimorphism of snout lengths has arisen independently several times. In 2002, we obtained access to an amazing lot that contains 130 individuals of the sexually dimorphic Compsaraia samueli collected during the breeding season in a remote stream in the Rio Negro drainage by Dr. Labbish Chao and his students. Not only does this large lot contain a wide range of sizes in both sexes, but the majority of these individuals were in advanced stages of maturity. Females are easily identified by large eggs in the abdominal cavity, and the testes of males are well developed. In this species, male and female facial measurements are drastically different after a certain size. When they are smaller than this size it becomes difficult to tell them apart, even from other species. Our goal in this study is to describe sexual differences in detail and provide the basis for future identifications. Males show strong interlacing of dentary and anguloarticular; this is less pronounced in females. Males have a linear lower jaw, where females curve down. The corona meckelian is larger in males than in females. In our conclusion, we will correct some aspects of the description of this species, and discuss the impact of these types of morphological data on the study of apteronotid fishes.

Key words: Gymnotiformes; Sexual Dimorphism; Morphology Financial support: University of Massachusetts Collections and Virginia Institute of Marine Science



#### Deciphering the evolutionary relationship between Gymnogeophagus labiatus and G. lacustris (Cichlidae)

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Cichlidae is one of the most diverse vertebrate families, containing more than 1,702 valid species, 567 of which are grouped in the Cichlinae subfamily. Gymnogeophagus Mirada Ribeiro, 1918 harbor 17 valid species whose diagnostic synapomorphies are the presence of a spine on the first dorsal pterygiophore and lack of supraneurals, which are unique among Neotropical cichlids. Two species occur in the coastal plain of Rio Grande do Sul (CPRS), in Southern Brazil: G. labiatus, which occupy rocky bottom rivers in the Patos Lagoon (PLB) and Tramandaí River (TRB) basins, and G. lacustris, which occupy sandy bottom coastal lagoons in the Tramandaí River drainage, being endemic to this ecoregion. Recently, a DNA Barcode study using the mtDNA gene cytochrome oxidase I (COI) failed to separate these two species in TRB, but suggested genetic differences between G. labiatus from TRB and PLB. Because the CPRS is a recent geological environment, a plausible hypothesis is that G. lacustris diverged from G. labiatus in TRB very recently, such that COI, a relatively conserved mtDNA marker, is unable to separate between these two species. In this study, we aim to characterize the genetic structure in the G. labiatus/G. lacustris species pair using the mtDNA control region (D-loop), a fast-evolving genetic marker. We have analyzed 48 individuals from the UFRGS ichthyology collection, of which 19 individuals were identified as G. lacustris and 29 as G. labiatus. DNA was extracted using the CTAB method, and the D-loop fragment was amplified by PCR, purified enzymatically and subjected to Sanaer sequencina. All chromatoarams were checked using the Genious R10 software, and the resulting sequences were aligned using the Clustal W module in the Bioedit 7.2.5 software. The different haplotypes were identified using DnaSP 5.10, and analyzed in the program Network 5.0, which builds an evolutionary network using the median-joining method. Finally, we used Arlequin 3.5 to quantify the genetic structure among hydrographic basins using AMOVA and pairwise FST. Our data showed that G. labiatus and G. lacustris from TRB have three closely related haplotypes, two of which are shared between these species, while the other was found in a single G. lacustris individual. On the other hand, G. labiatus haplotypes from PLB showed two set of very divergent haplotypes, one of which was restricted to the Camaguã sub-basin (CSB), which was then considered as a different population in the genetic structure analysis. The AMOVA showed that 85.8% of the total genetic variation occurred among populations, with pairwise FST values ranging from 0.785 to 0.989 (all P<0.0005). Overall, our results corroborate the close evolutionary relationship between these species in TRB, and suggest that while further analyses are necessary to confirm G. lacustris as a valid species, the individuals from CSB may constitute an independent evolutionary lineage.

Key words: Evolutionary Relationship; Gymnogeophagus; D-loop; Neotropical Financial support: CAPES; FAURGS

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