Zootaxa 2966: 37–50 (2011)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

A molecular phylogeny of the Grunts (Perciformes: Haemulidae) inferred using mitochondrial and nuclear genes

MILLICENT D. SANCIANGCO[1], LUIZ A. ROCHA[2] & KENT E. CARPENTER[1]

[1]*Department of Biological Sciences, Old Dominion University, Mills Godwin Building, Norfolk, VA 23529 USA. E-mail: msanc004@odu.edu, kcarpent@odu.edu*

[2]*Marine Science Institute, University of Texas at Austin, 750 Channel View Dr., Port Aransas, TX 78373, USA. E-mail: rocha@mail.utexas.edu*

Abstract

We infer a phylogeny of haemulid genera using mitochondrial COI and Cyt *b* genes and nuclear RAG1, SH3PX3, and Plagl2 genes from 56 haemulid species representing 18 genera of the expanded haemulids (including the former inermiids) and ten outgroup species. Results from maximum parsimony, maximum likelihood, and Bayesian analyses show strong support for a monophyletic Haemulidae with the inclusion of *Emmelichthyops atlanticus*. The former inermiids did not form a clade indicating that the highly protrusible upper jaw specialization to planktivory evolved more than once within Haemulidae. The subfamilies Haemulinae and Plectorhinchinae, currently diagnosed by eight morphological characters, most notably the number of chin pores and the origin of the retractor dorsalis, are also recovered from these analyses with the Haemulinae sister to the Plectorhinchinae. *Plectorhinchus* is monophyletic only with the inclusion of *Diagramma*. Within the Haemulinae, *Pomadasys* and *Conodon* are polyphyletic. In addition, *Anisotremus* is monophyletic only with the inclusion of *Genyatremus* and *Conodon nobilis*, and *Haemulon* is monophyletic only with the inclusion of *Xenistius*. These results suggest that further morphological and molecular studies are needed to revise the limits of several haemulid genera.

Key words: Inermiidae, taxonomy, biogeography, partitioned dataset

Introduction

The family Haemulidae, or grunts, include 18 genera and about 145 species (Nelson 2006) in the ill-defined order Perciformes, suborder Percoidei (sensu Nelson 2006). Grunts are circumglobal and often prominent in both hard- and soft-bottom nearshore tropical, subtropical, and warm temperate waters (McKay 1984; McKay & Schneider 1995; McKay 2001; Lindeman & Toxey 2003). Most are carnivorous, feeding opportunistically on a wide variety of benthic invertebrates including crustaceans, polychaete worms, clams, and echinoids, while smaller species primarily feed on plankton (Konchina 1977; Ogden & Ehrlich 1977; Williams *et al.* 2004).

Johnson (1981) used a number of characters to define Haemulidae and its subfamilies, Haemulinae and Plectorhinchinae (Appendix 1). He proposed the superfamily Haemuloidea to include the mostly bottom feeding Haemulidae and the planktivorous Inermiidae. The latter family, commonly known as bonnetmouths, contains only two species that are reef-associated, typically small, and specialized for planktivory with highly protrusible jaws and fusiform bodies (McEachran & Fechhelm 2005; Lindeman 2006; Nelson 2006). Johnson (1981) found that the families Haemulidae and Inermiidae share a suspensorium similar to that of the lutjanoids in having little direct osseous articulation and a simple symplectic but having a unique projection on the margin of the metapterygoid, which projects posteriorly as a vertically oriented rounded flange that overlaps the medial side of the lower arm of the hyomandibular. This, in addition to other osteological characters such as the number of branchiostegals; number of openings in pars jugularis; presence of chin pores and scales on lacrimal, snout, and preopercular margin; absence of subocular shelf and trisegmental pterygiophores; and specializations in their infraorbitals, suspensorium, and procurrent spur provide morphological evidence for a monophyletic Haemuloidea.

Accepted by M. Craig: 18 May 2011; published: 14 Jul. 2011

The presence of enlarged sensory chin pores and the attachment of the sixth infraorbital to the skull in haemulids are characters that are uncommon among percoids (Johnson 1981). These enlarged pores are also present in the Lobotidae, Hapalogenyidae, Sciaenidae, and several other families. However, these families are easily recognized based on the presence of other anatomical and osteological characters diagnostic of the members of those families. Lobotidae and Hapalogenyidae, for example, have more than six chin pores, while Sciaenidae has only one or two anal fin spines compared to three anal spines in haemulids. The number, shape, and position of chin pores also help diagnose subfamilies and genera within Haemulidae. Plectorhinchines have four to six chin pores while haemulines, including the former inermiids, possess either two chin pores, a median chin groove, or both (Johnson 1981). While both haemulid subfamilies and some genera appear to be well defined, many haemulid genera are not well defined and diagnosed only with superficial characters. For example, the monotypic *Genyatremus* was originally erected to differentiate what is currently recognized as *Anisotremus interruptus* from other higher bodied species of *Anisotremus* (Gill 1861), and it appears to have been only incorrectly placed in another genus and recognized as *Genyatremus luteus* (Johnson 1981; Lindeman & Toxey 2003). *Orthopristis* (Girard 1858) was erected based on superficial characters that are not currently used to distinguish members of the genus such as the body configuration and fin meristics (McKay & Schneider 1995; Lindeman & Toxey 2003). *Boridia, Conodon, Microlepidotus, Xenichthys,* and *Xenistius* were all designated by monotypy (Eschmeyer 1990) without extensive morphological comparisons.

A number of recent studies that help define the limits of haemulid species and genera (Courtenay 1961; Konchina 1976; Iwatsuki *et al.* 1998; Miles 1953; Ren & Zhang 2007; Rocha *et al.* 2008), or provide basic regional systematic information (Konchina 1977; Roux 1981; McKay 1984; McKay & Schneider 1995; McKay 2001; Lindeman & Toxey 2003; Bernardi & Lape 2005) are available; however, none of these studies have attempted to infer a phylogeny of the family Haemulidae using either molecular or morphological methods. Johnson (1981) studied the morphology of a number of families thought to be closely related to his proposed haemuloids (Haemulidae and Inermiidae) and suggested two additional superfamilies, the Sparoidea (including Sparidae, Centracanthidae, Nemipteridae, and Lethrinidae) and Lutjanoidea (including Lutjanidae and Caesionidae), but he could not find evidence to suggest that any of these groups were directly related to one another. He was not confident in polarizing morphological characters of Haemuloidea and therefore chose not to propose a phylogeny.

Recent studies conducted on higher-level relationships of percomorphs and acanthomorphs have shown potential outgroups for haemulids on the basis of molecular characters including Dettai & Lecointre (2005; Syngnathidae, Uranoscopidae + Cheimarrichthyidae + Ammodytidae, Moronidae, Drepanidae, and Scaridae + Labridae); Smith & Craig (2007; Lutjanidae, Lethrinidae + Priacanthidae, Moronidae, and Lobotidae); Craig & Hastings (2007; Moronidae and Cirrhitidae); and Mahon (unpublished; Dinopercidae and Drepanidae + Acanthuridae + Ephippidae). In addition, the interrelationships of families within the putative Percoidei, the suborder to which Haemulidae belongs (Nelson 2006), are not well understood, hence making it more challenging to define the possible sister-groups of haemulids. *Hapalogenys* has been classified in the Haemulidae because of the presence of chin pores (Richardson 1844; Iwatsuki *et al.* 2000, Iwatsuki & Russell 2006), however, the phylogenetic placement of the Hapalogenyidae (Springer & Raasch 1995; Ren & Zhang 2007) within the haemulids has also been controversial (Johnson, 1984; Iwatsuki *et al.* 2000; Lindeman & Toxey 2003; Iwatsuki & Nakabo 2005).

The purpose of this study is to infer a genus-level phylogeny of haemulids, including a former inermiid species, *Emmelichthyops*, test the validity of the two subfamilies, and provide a basis to further test hypotheses of morphological character evolution and biogeography of the family Haemulidae. Here we use molecular data to help frame questions of generic placement within Haemulidae. The markers used for this study include the mitochondrial Cytochrome Oxidase I (COI) and Cytochrome *b* (Cyt *b*) and three nuclear markers, Recombination Activation Gene-1 (RAG1), SH3 and PX domain-containing 3-like protein (SH3PX3), and pleiomorphic adenoma protein-like 2 (Plagl2) genes. A phylogeny of haemulids from most genera was inferred from maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses of a combined total of 4731 base pairs.

Material and methods

Taxon sampling. Ten outgroup taxa were included from the families Nemipteridae (*Nemipterus marginatus*), Lethrinidae (*Lethrinus ornatus*), Lutjanidae (*Aphareus furca* and *Lutjanus fulviflamma*), Sparidae (*Sarpa salpa* and

Hapalogenyidae (*Hapalogenys aya*, *H. kishinouyei*, and *H. nigripinnis*). Lobotidae (*Lobotes pacificus* and *L. surinamensis*), another percoid family that possesses chin pores, was also included in the study. Among the ingroup taxa, 56 species belonging to 18 genera are included among the 144 species and 20 haemulid genera (Appendix 1). All genera of haemulids are represented except for the two monotypic genera *Parakuhlia* and *Xenocys*. Specimens were collected by trawling, hook and line, or spearfishing. Samples were also obtained from specimens from fish markets. Muscle tissue of the fish were dissected and preserved in 95% ethanol or DMSO solution (Seutin *et al.* 1990) and stored at -20°C until processed in the laboratory.

DNA isolation, amplification, and sequencing. Genomic DNA was extracted from approximately 20 mg of tissue following the DNeasy® Kit (Qiagen) protocol and Wizard® SV 96 Genomic DNA Purification System (Promega). Primers used to amplify the mitochondrial and nuclear genes are listed in Table 1. A total of 651 base pairs were amplified using the COI primers under the following conditions: initial denaturation at 95°C for one minute (to activate the Takara Ex Taq HotStart™ DNA polymerase, Takara Bio Inc.), followed by 30 cycles of 95°C for 30 seconds, 52°C for 30 seconds, and 72 °C for 45 seconds; followed by a five minute extension at 72°C. Cyt *b* yielded a total of 1140 base pairs, with amplification conditions similar to those of COI but with 32 cycles and annealing temperature of 52 °C for 45 seconds. For all the nuclear genes used, nested PCRs were employed to successfully amplify approximately 1431 base pairs of RAG1 gene, 705 base pairs of SH3PX3 gene, and 804 base pairs of Plagl2 gene from DNA extracts, with the following amplification settings: initial denaturation at 95 °C for one minute; 30 cycles of 95 °C for ten seconds, 56 °C to 63 °C for 45 seconds, and 72 °C for five minutes; with an additional final extension at 72 °C for five minutes. Amplification conditions for the second set of internal primers for three nuclear genes follow the same protocol as that of the first PCR, except with annealing temperature set to 63 °C for all three genes. A 0.2 μl of ExoSAP-IT® (USB Corporation) master mix (1:5 dilution of the enzyme) was added for every 1 μl of PCR product to purify the target gene, carried out at 37 °C for 30 minutes and 80 °C for 20 minutes.

TABLE 1. PCR primer sequences and annealing temperatures used to amplify the five markers used. 1st indicates the first round of nested PCR and 2nd for second round of nested PCR using the following primers for each gene.

Gene	Primers	Sequences	Tm (°C)	PCR	Reference
COI	CO1LBC_F	5' TCAACYAATCAYAAAGATATYGGCAC 3'	52	1st	Ward *et al.* 2005
	CO1HBC_R	5' ACTTCYGGGTGRCCRAARAATCA 3'		1st	Ward *et al.* 2005
Cyt *b*	Cytb_UniF	5' CGAACGTTGATATGAAAAACCATCGT 3'	52	1st	Orrell *et al.* 2002
	Cytb_UniR	5' ATCTTCGGTTTACAAGACCGGTG 3'		1st	Orrell *et al.* 2002
RAG1	2510F	5' TGGCCATCCGGGTMAACAC 3'	63	1st	Li & Orti 2007
	RAG1R1	5' CTGAGTCCTTGTGAGCTTCCATRAAYTT 3'		1st	López *et al.* 2004
	RAG1F1	5' CTGAGCTGCAGTCAGTACCATAAGATGT 3'	63	2nd	López *et al.* 2004
	RAG1R2	5' TGAGCCTCCATGAACTTCTGAAGRTAYTT 3'		2nd	López *et al.* 2004
SH3PX3	F35	5' AAAGYGARAACAAGGAGGAGAT 3'	56	1st	Pers. Comm. C. Li*
	R1373	5' AGCGACAGYTTGTCCARCAT 3'		1st	Pers. Comm. C. Li*
	F532	5' GACGTTCCCATGATGGCWAAAAT 3'	63	2nd	Li *et al.* 2007
	R1299	5' CATCTCYCCGATGTTCTCGTA 3'		2nd	Li *et al.* 2007
Plagl2	F9	5' CCACACACTCYCCACAGAA 3'	58	1st	Li *et al.* 2007
	R1430	5' TCGTACTGAGGCTRGAGCTGAA 3'		1st	Pers. Comm. C. Li*
	F51	5' AAAAGATGTTTCACCGMAAAGA 3'	63	2nd	Li *et al.* 2007
	R920	5' GGTATGAGGTAGATCCSAGCTG 3'		2nd	Li *et al.* 2007

Sequencing reactions were conducted in forward and reverse directions using primers for the second set of PCR. Sequences were assembled and edited in Sequencher version 4.10.1 (Gene Codes). The trimming criteria for sequences include trimming no more than 25% until the first 20 bases contain at least three bases with confidences below 20% for the five-prime end and trimming until the last 20 bases contain less than three bases with confidences below 20% for the three-prime end. Sequences were then trimmed according to a reference sequence for

each gene obtained from GenBank, including COI: FJ237890 *Pomadasys maculatus* (Zhang & Hanner 2007), Cyt *b*: EF512297 *Pomadasys maculatus* (Zhu *et al.* 2007), RAG1: EF095661 *Haemulon aurolineatum* (Chen *et al.* 2007), SH3PX3: EF033010 *Lutjanus mahogani* (Li *et al.* 2007); and Plagl2: EF033023 *Lutjanus mahogani* (Li *et al.* 2007). Multiple alignments of sequences were performed using ClustalX (Thompson *et al.* 1997) using default settings (Hall 2004).

Phylogenetic analysis. The concatenated data matrix of five genes was partitioned by gene and by codon position, producing 15 data blocks. Each of the data blocks was initially optimized independently under a GTR + Γ model implemented in MrBayes, with two million MCMC generations and seven chains (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003; Nylander *et al.* 2004). Following Li *et al.* (2008), the overall similarity among data blocks was evaluated on the basis of their estimated parameter values, counting five substitution rates, three base composition proportions, the gamma parameter (alpha), and the rate multiplier for each data block. Using a hierarchical cluster analysis in SAS, each data partition was used as an observation, with the ten independent parameters estimated from MrBayes as values for each observation. The resulting clustering dendrogram was then used as a guide tree to identify the two most similar data blocks for grouping two partitions and subsequently adding one data block at a time based on overall similarity from the guide tree until only one large data block remained. The AIC values and Bayes Factor have proven that partitioning following the guide tree always resulted in better partitioning scheme than randomly grouping two other partitions (Li *et al.* 2008). To evaluate the best partitioning scheme, the harmonic means for each MrBayes run was recorded to calculate and compare the harmonic means and Bayes Factor (BF = (−ln*Li*) − (−ln*L*best)). The optimal partitioning strategy is chosen based on the best ln score (top two among all partitioning schemes for comparison) and with the fewest number of parameters. If there is not much difference between the top two ln scores, the one with a fewer number of parameters estimated and has a fewer number of partition is preferred. The best strategy should also have a 2lnBayes factor of more than 10 between that scheme and the next (stepwise) partitioning scheme. A 2ln Bayes factor of ≥ 10 is strong evidence against the alternative hypothesis (Kass & Raftery 1995; Brandley *et al.* 2005; Li *et al.* 2008).

We used MP, ML, and Bayesian analyses to infer phylogeny. The minimal length trees were obtained using a heuristic search and 1000 replicates of random taxon addition with tree-bisection-reconnection (TBR) branch swapping algorithm, saving all trees per replicate. In addition to Bremer support (decay index, Sorensen & Franzosa 2007), relative internal branch support was estimated with bootstrap analysis with 1000 replicates, with TBR branch swapping and simple taxon addition. Tree statistics included the consistency index and retention index. MrModelTest2 (Nylander *et al.* 2004) was used to determine the best-fit model for each of the data partitions following the best partitioning scheme, with models scored in PAUP* version 4.0b10 (Swofford 2002). ML was performed using the partition version of the program Genetic Algorithm for Rapid Likelihood Inference (GARLI; Zwickl 2006), with internal branch support estimated with 100 bootstrap replicates for each of the independent search runs. The repeatability of results (recovering the same best scores and same topologies, with very similar log-likelihood scores, at least twice) across independent search replicates indicates the number of search replicates to be conducted. A total of eight independent search replicates were conducted for this study. Trees were collected and scored using Mesquite (Maddison & Maddison 2007). MrBayes was also used to estimate the evolutionary parameters using posterior probabilities (Ronquist & Huelsenbeck 2003). The Markov chain Monte Carlo parameters (MCMC) for the final partitioned dataset included 10 million generations with seven chains sampling every one thousand. Convergence was assessed using Tracer looking at the ESS value for each log-likelihood trace and plotting the posterior probability density for the mutation rate (Rambaut & Drummond 2007) and AWTY (Are We There Yet?) comparing split frequencies, looking at each independent trajectory, and checking for presence of or absence of splits throughout the chain for each one to make sure that the chains are sampling particularly well (Nylander *et al.* 2008). Resulting topologies for all analyses were viewed in Mesquite (Maddison & Maddison 2007) and bootstrap values from MP and ML mapped on the Bayesian topology.

Results

The characteristics of the five mitochondrial and nuclear genes are shown in Appendix 2. The concatenated dataset of five loci generated a total of 4731 characters for the 66 taxa included in this study. The dataset was partitioned by gene and by codon position yielding 15 block partitions (5 genes x 3 codon positions). Appendix 3 shows the ten parameters estimated in MrBayes. These parameters were then employed into a hierarchical cluster analysis in

SAS. The output from cluster analysis showing which data block should be grouped based on overall similarity using the ten parameters estimated in MrBayes is shown in Fig.1. The resulting dendrogram from SAS is read from left to right looking at the terminal branches, concatenating data blocks on the first node and then concatenating data blocks on the subsequent nodes, adding one data block at a time. Table 2 also shows how the 15 data blocks down to one data block (no partition) were clustered. Starting with 15 partitions (where all data blocks are treated as separate), the 14-partitioning scheme has (SH3PX3_3 and Plagl2_3) concatenated as one data block, plus the rest of data blocks (13 other data blocks, each treated as separate). The 13-partitioning strategy has (SH3PX3_3 and Plagl2_3) as one data block and (COI_3, Cytb_3) as another data block, plus the rest of data blocks (11). Data blocks were concatenated following the dendrogram until only one data block with no partition is left. Boxed text indicates the best partitioning schemes, with 11- and 15- data partitions, chosen by different model selection criteria in this study. Although the 15 data block partitioning scheme is the best partition based on the likelihood scores, it has 40 more parameters than the 11 data block partitioning scheme. Also, the difference between the 11- and 12-partitioning schemes has a value of 42.82, which is more than 10 and satisfies the conventional criterion for choosing the best strategy. Hence the 11-data block partitioning scheme was chosen as the best partitioning strategy (Brandley *et al.* 2005; Li *et al.* 2008) in this study (Table 3).

TABLE 2. Comparison of log likelihoods and Bayes factors among different partitioning schemes (from one to 15 partitions). Results show the total number of parameters; the harmonic mean of -log likelihood calculated using MrBayes; the Bayes factor calculated by comparing model i to the model with maximum likelihood, BF = (-ln*Li*)- (-ln*L*best); and the clustering of data blocks for each partitioning scheme based on the hierarchical cluster grouping. Boxed text indicates the best partitioning schemes chosen by different model selection criteria. Concatenated data blocks are enclosed in parentheses. S=SH3PX3; P=Plagl2; R=RAG1; C=COI; Cy=Cyt *b*. Numbers (1,2,3) after gene initials refer to codon positions 1, 2, and 3, respectively.

No. of partitions	No. of parameters	Ln	2LnBayes Factor	Data block partition
1	10	-58368.64	233.16	all together
2	20	-58252.06	4483.72	(S3P3R3Cy1P1R1S1R2S2Cy2C2C1C3Cy3) and P2
3	30	-56010.2	144.62	(S3P3R3Cy1P1R1S1R2S2Cy2C2C1)(C3Cy3) and P2
4	40	-55937.89	216.44	(S3P3R3Cy1P1R1S1R2S2Cy2C2)(C3Cy3) and the rest
5	50	-55829.67	466.68	(S3P3R3Cy1P1R1S1R2S2Cy2)(C3Cy3) and the rest
6	60	-55596.33	110.92	(S3P3R3Cy1P1R1S1)(C3Cy3)(R2, S2Cy2) and the rest
7	70	-55540.87	221.58	(S3P3R3Cy1P1)(C3Cy3)(R2S2Cy2)(R1S1) and the rest
8	80	-55430.08	138.16	(S3P3R3Cy1P1)(C3Cy3)(R2S2Cy2) and the rest
9	90	-55361	248.8	(S3P3R3Cy1P1)(C3Cy3)(R2S2) and the rest
10	100	-55236.6	418.44	(S3P3R3)(C3Cy3)(R2S2)(Cy1P1) and the rest
11	110	-55027.38	-145.08	(S3P3R3)(C3Cy3)(R2S2) and the rest
12	120	-55099.92	42.82	(S3P3R3)(C3Cy3) and the rest
13	130	-55078.51	-48.68	(S3P3)(C3Cy3) and the rest
14	140	-55102.85	256.78	(S3P3) and the rest
15	150	-54974.46		all separate

In the limited outgroup comparisons of this study, *Hapalogenys* is sister to *Lobotes*. In addition, the lutjanids are sister to haemulids. A monophyletic Haemulidae, including the former inermiids, is well supported in all analyses (with a Bremer support of 66, bootstrap value of 100 for MP and ML and a posterior probability of 1.0 in Bayesian analysis) (Fig. 2). The phylogenetic position of *Haemulon vittatum* (formerly in *Inermia*) first reported in Rocha *et al.* (2008) is confirmed. In addition, *Xenistius californiensis* is also nested within *Haemulon*. *Emmelichthyops* is sister to *Microlepidotus brevipinnis* and these, sister to *Isacia*. These three species are sister to *Orthopristis*.

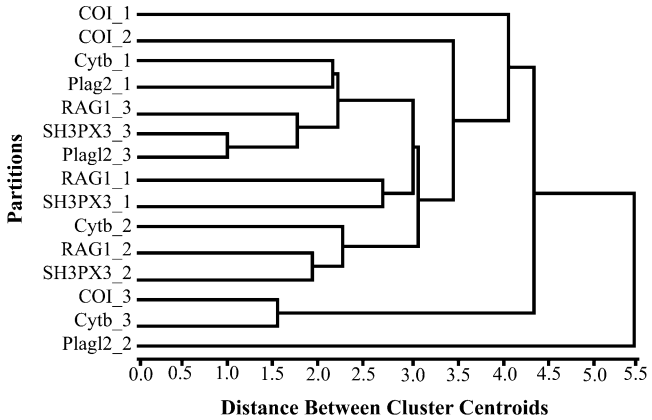

FIGURE 1. Clustering diagram showing overall similarity among 15 data blocks of the full data set (5 genes × 3 codon positions) using SAS. Each block is indicated at the tip of terminal branches by gene name and codon position. Each node shows clustering terminal branches (data set) based on hierarchical clustering algorithm using a Bayesian approach.

TABLE 3. Models selected by MrModelTest2.0 (Nylander 2004) under the AIC criterion for the optimal 11-partition scheme for Bayesian analysis, with –lnL values and number of parameters for each data block.

Partition	Data blocks	Model chosen by MrModeltest2.0	-lnL	No. of parameters
1	SH3PX3_3.Plagl2_3.RAG1_3	GTR+G	11765.6377	9
2	COI_3.Cytb_3	GTR+I+G	28687.7891	10
3	RAG1_2.SH3PX3_2	GTR+I+G	2035.7582	10
4	COI_1	GTR+I+G	946.9229	10
5	COI_2	F81	350.0114	3
6	Cytb_1	GTR+I+G	3815.7031	10
7	Cytb_2	GTR+I+G	1661.8229	10
8	RAG1_1	GTR+I+G	2196.0671	10
9	SH3PX3_1	JC+G	641.6643	1
10	Plagl2_1	HKY+G	563.8015	5
11	Plagl2_2	F81	460.4087	3

Two well-supported clades (Bremer support of 56) corresponding to the subfamilies Plectorhinchinae and Haemulinae were recovered in this study (Fig. 2). Within Plectorhinchinae, *Parapristipoma* is sister to a clade containing the members of the genus *Plectorhinchus*, with the inclusion of *Diagramma pictum*. In addition to the *Haemulon* plus *Xenistius* clade noted above, a number of putative haemuline genera appear to be para- and polyphyletic. Species of *Pomadasys* are recovered in three separate clades and the genus is polyphyletic. Within the haemuline assemblage, a clade (*Pomadasys* I) containing *Pomadasys perotaei*, *P. incisus*, and *O. olivaceus* is sister to the rest of the haemulines. Several *Pomadasys*, including *P. striatus*, *P. argyreus*, *P. maculatus*, *P. kaakan*, and *P. stridens* (*Pomadasys* II) plus *Brachydeuterus* were clustered in a separate clade, and is sister to the remaining haemulines. A clade containing additional species of *Pomadasys* (*Pomadasys* III), *Boridia*, *Conodon serrifer*, *Xenichthys*, and *Haemulopsis* and the clade containing species of *Orthopristis*, *Isacia*, *Emmelichthyops*, and *Microlepidotus* is sister to a clade containing *Anisotremus* and *Haemulon*. *Anisotremus* is monophyletic with the inclusion of *Conodon nobilis*. *Conodon*, therefore, is polyphyletic. *Genyatremus* is monophyletic, and the clade containing the three species included in this genus was also recovered by a recent morphological analysis (Tavera *et al.* 2011), albeit branch ordering within the clade is different.

Discussion

The interrelationships of haemulids. Previous molecular studies on higher-level percomorphs and acanthomorphs have shown possible outgroups for haemulids but did not provide morphological evidence to support their relationship. The outgroup sampling for this study is not exhaustive and obviates definitive statements about sister taxa of the Haemulidae. However, our results do confirm recent conclusions that *Hapalogenys* is not a member of the Haemulidae (Springer & Raasch 1995; Ren & Zhang 2007). The presence of short barbels or furlike papillae on the chins of hapalogenyids and antrorse spine before the first dorsal fin spine separate them from the haemulids. There is also some support (a clade supported by a decay index of 4, 100% bootstrap for MP and ML and a posterior probability of 1.0 for Bayesian analysis) that *Lobotes* may be sister to *Hapalogenys* (Fig. 2) based on the molecular data and some morphological characters such as the rounded shape of the caudal fin, absence of distinct canines on palatine and vomer, and the presence of more than six sensory pores on the chin. The possession of sensory chin pores, however, does not appear to be a synapomorphy for haemulids plus *Hapalogenys* and *Lobotes*, since our analysis recovers lutjanids as sister to haemulids. More comprehensive taxon sampling of perciform fishes is required to further test this relationship.

The intrarelationships within haemulids. The monophyly of Haemulidae is only well supported if the former inermiid*s* are included. The placement of this species within Haemulidae is not surprising given the many synapomorphies that are shared among them. Johnson (1981) presented a list of shared meristic and osteological characters between "inermiids" and haemulids and also noted the differences between them, most notably the highly protrusible jaws of *Haemulon vittatum* (formerly *Inermia vittata*) and *Emmelichthyops atlanticus*. He noted that the neurocranium bears little resemblance to the typical haemuloid type, which gives way to its modification for the reception of the extremely long ascending processs of the premaxillary, which is a specialization for planktivory. He believed that this degree of morphological and ecological divergence to other haemulids warrants familial recognition. Rocha *et al.* (2008) recovered *Inermia vittata* nested within *Haemulon* and proposed that *Inermia* should be recognized as *Haemulon vittatum* based on both cladistic pattern and genetic sequence divergence. They further hypothesized that the disparity in external morphology between *Haemulon* and *Inermia* can be attributed to the morphological specializations brought about by rapid ecological shifts. The specialization to plankton feeding is also seen in other haemulines, such as in some species of *Anisotremus*, *Orthopristis*, *Pomadasys*, *Haemulon*, and *Xenistius*, although these genera do not possess a highly specialized jaw similar to that of *Haemulon vittatum* and *Emmelichthyops*. Similarly, *Emmelichthyops* appears to have adapted to planktivory. However, unlike *Haemulon vittatum* (which is nested deep within the well-supported genus *Haemulon*), *Emmelichthyops* is on a long branch within a poorly supported clade (low bootstrap, posterior probability, and Bremer support) that includes *Isacia*, *Microlepidotus*, and *Orthopristis* (Fig. 2). A more precise phylogenetic placement for this species will require exhaustive sampling in the *Orthopristis-Haemulopsis* clade and rigorous morphological comparisons. This study supports the hypothesis by Rocha *et al.* (2008) of the placement of *Haemulon vittatum* and also now provides molecular evidence for the placement of *Emmelichthyops* in Haemulidae. It is important to note that the placement of these two species in the subfamily Haemulinae is also supported by the following morphological characters: two chin pores and low vertebral, pleural, and epipleural rib counts. Therefore, we recommend that the family Inermiidae should no longer be treated as valid.

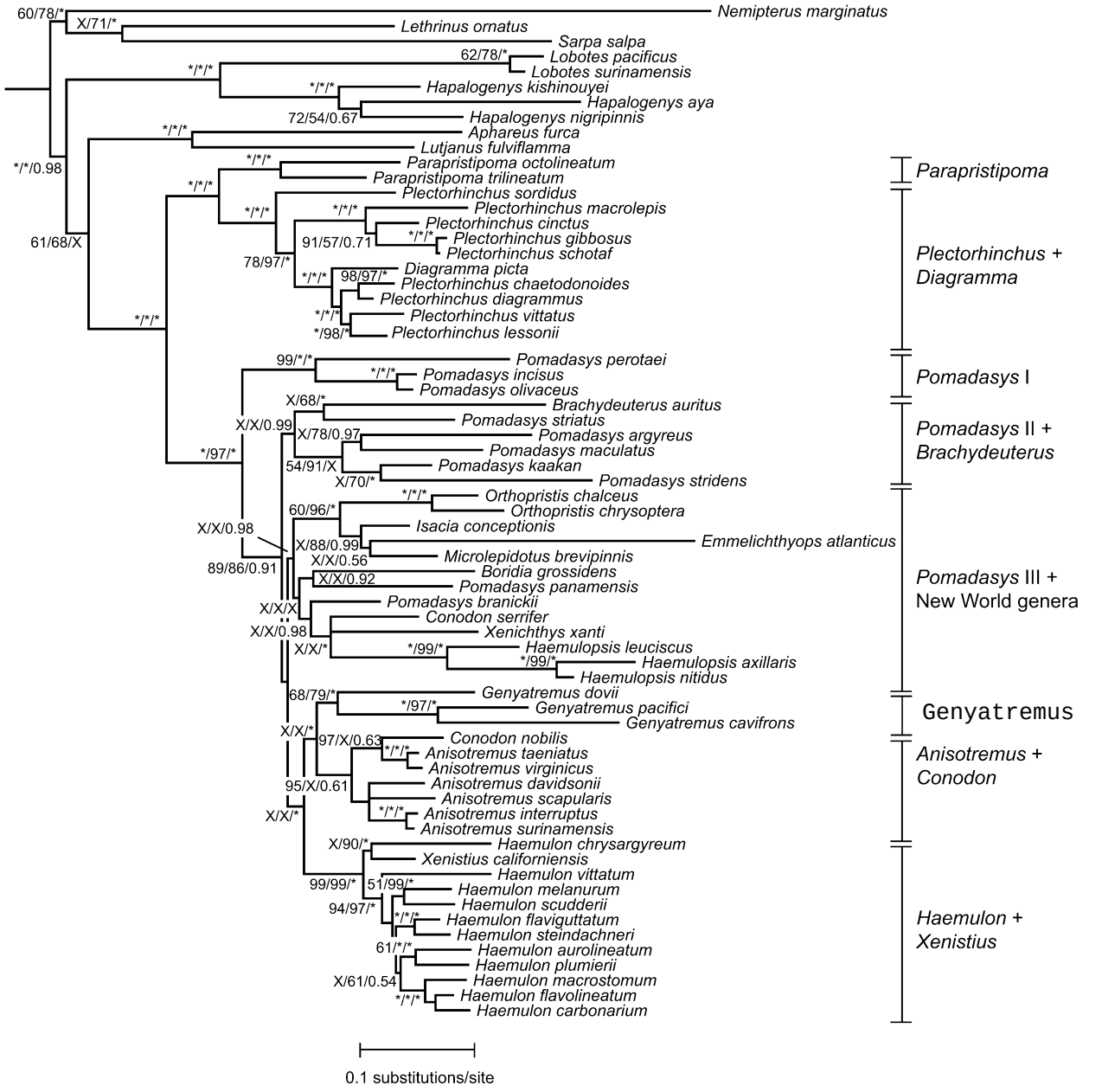

FIGURE 2. The tree represents a 50% majority rule consensus of the Bayesian topology (numbers represent the posterior probability of the clades), with bootstrap values from MP and ML mapped onto the topology. MP, ML, and Bayesian analyses produced similar topologies (MP: TL = 12,869, consistency index CI = 0.2372, retention index RI = 0.4450; ML: Ln Likelihood = -54309.4503) with differences mostly on nodes with low bootstrap support. The numbers on branches are MP and ML bootstrap values and posterior probabilities from Bayesian analysis, respectively. Asterisks indicate a bootstrap value of 100% for MP and ML and 1.0 for Bayesian analysis. Nodes with less than 50% bootstrap value are marked with an X if the clade had less than 50% support in any of the MP, ML, or Bayesian analyses.

The morphological basis for Haemulinae and Plectorhinchinae (Johnson 1981) is also corroborated by our molecular analyses. The Plectorhinchinae recovered here includes well-supported clades (Bremer support of at least 12 and high bootstrap and posterior probability) for all species of *Parapristipoma* and *Plectorhinchus*. However, the paraphyletic *Plectorhinchus* includes *Diagramma*. These two genera are very similar in appearance externally and differ mostly in dorsal-fin ray counts, scale counts, and shape of the swimbladder (Smith 1962; McKay 2001). Final disposition of species within the clade containing all *Plectorhinchus*, including *Diagramma*, should await a more exhaustive sampling of these species and re-examination of morphological characters. It is interesting

to note that the colorful Indo-Pacific coral reef *Plectorhinchus* + *Diagramma* form a clade within a clade that includes mostly drab species, including the only member of this group found in the Atlantic.

The clades recovered within the Haemulinae call into question the monophyly of a number of genera (Fig. 2). *Pomadasys* is polyphyletic and found in three separate clades that correspond roughly to different biogeographic regions. Haemulinae clade I is composed of *Pomadasys* found in the eastern Atlantic (although one is also found in the Indian Ocean). Clade II is composed of *Pomadasys* from the Indo-West Pacific and the eastern Atlantic *Brachydeuterus*. Clade III includes only species found in the Americas (New World): two eastern Pacific *Pomadasys* plus eastern Pacific/western Atlantic *Orthopristis*, eastern Pacific *Isacia*, *Haemulopsis*, *Xenichthys*, *Microlepidotus* and *Conodon*, and the western Atlantic *Boridia*. If new morphological information corroborates the polyphyly of *Pomadasys*, this and the other genera in these basal haemuline clades will need to be reclassified. The distinct or nearly distinct geographic distribution of these clades suggests interesting biogeographical relationships that warrant further study.

Two haemulid clades are confined to the New World and are composed primarily of *Haemulon* and *Anisotremus*. As noted above, the *Haemulon* clade is paraphyletic with the inclusion of *Xenistius californiensis*. Jordan & Gilbert (1882) diagnosed *X. californiensis* using several meristic and anatomical characters such as having an oblong body; a moderate, very oblique terminal mouth, with the lower jaw strongly protruding; soft parts of vertical fins densely scaled; the two dorsal fins are almost separate; caudal fin forked; and most notably, having the soft dorsal fin shorter than the spinous dorsal fin and composed of 11 or 12 rays and anal fins also short, with second and third anal spines high. These characters are also diagnostic of the members of the genus *Haemulon* (Courtenay 1961). The recognition of *Xenistius* under *Haemulon* is supported by our independent and combined analyses of five genes (MP, ML, and Bayesian) and we conclude that *X. californiensis* should be treated as *Haemulon californiensis*.

Similarly, the limits of genera within the '*Anisotremus*' clade also need to be redefined. *Anisotremus* was erected without morphological justification (Gill 1861) by monotypy (Eschmeyer 1990) and subsequently recognized to encompass other high-bodied haemulids with black bars (McKay & Schneider 1995; Lindeman & Toxey 2003). The molecular analysis appears to support this ill-defined genus with the inclusion of *Conodon nobilis*. Here we follow the taxonomic suggestions of Tavera *et al.* (2011) and classify the former *Anisotremus dovii* and *A. pacifici* in the genus *Genyatremus*. We also use the name *Genyatremus cavifrons* to refer to the species historically identified as *G. luteus*, as suggested by Tavera *et al.* (2011). The molecular and morphological evidence indicates that further comprehensive examination of osteological and other morphological characters of the members of this clade may result in a revision of generic assignments. The monophyly of *Conodon* is also rejected in this study. *Conodon nobilis*, inhabiting the western Atlantic, is clustered within the *Anisotremus* clade as noted above while *C. serrifer* is clustered together in a clade with eastern Pacific species, including *Xenichthys xanti*, *Haemulopsis leuciscus*, *H. axillaris*, and *H. nitidus*. Aiming to avoid future reversals, we defer taxonomic rearrangement of these genera to a future study with better taxon sampling and a more detailed morphological analysis.

The current study presents the first nearly comprehensive phylogenetic hypothesis of haemulid genera. The monophyly of the family and subfamilies and distinct clades within the subfamilies are well supported in all analyses (Bremer support of 56, bootstrap values above 95% and posterior probability of 1.0). This phylogeny calls into question the validity of some haemulid genera and leaves a number of other questions unanswered. The placement of *Xenocys* and *Parakuhlia* within the Haemulidae remains unresolved until specimens become available. However, morphology indicates that their subfamilial designation is Haemulinae. Defining the limits and relationships of the questionable genera will require detailed morphological examination to test and refine the current phylogenetic hypothesis. The molecular data largely corroborate the morphological data that define the family, subfamilies, and some genera. It also appears that the specialization to "extreme" planktivory evolved separately in some haemulines. A closer examination of the feeding apparatus of the "inermiids" may uncover fundamental differences that support alternative sister species relationships. Detailed morphological examinations are warranted given the results of this study, as are more tests that may help shed light on the biogeographical history of the Haemulidae.

Acknowledgements

We thank Y. Iwatsuki, P. Hastings, H.J. Walker, B. Collette, A. Bentley, E. Wiley, R. Robertson, and A. Carvalho-Filho for providing tissue samples. Some samples are gifts from the Natural History Museum & Biodiversity

Research Center, University of Kansas as approved by A. Bentley and E. Wiley. We also thank C. Li and D. Zwickl for helping with the analyses. We thank the two anonymous reviewers, W.L Smith, and K. Lindeman, who provided suggestions to improve this manuscript. Some samples were processed and sequenced at the Laboratory of Analytical Biology (Smithsonian Institute). The Master's thesis on the phylogeny of the Haemulidae was funded by the Fulbright Philippine Agriculture Scholarship Program to M. Sanciangco. L. Rocha was funded by the University of Texas Marine Science Institute. Travel support to K. Carpenter for incidental collection of specimens came from the International Union for Conservation of Nature/Conservation International, Global Marine Species Assessment project supported by Tom Haas and the New Hampshire Charitable Foundation. Most of this work was supported by the NSF Euteleost Tree of Life Grant (DEB: 0732894 to K. Carpenter, with M. Sanciangco, GRA).

References

Bernardi, G., Alva–Campbell, Y., Gasparini, J. & Floeter, S. (2008) Molecular ecology, speciation, and evolution of the reef fish genus *Anisotremus*. *Molecular Phylogenetics and Evolution*, 48, 929–935.

Bernardi, G. & Lape, J. (2005) Tempo and mode of speciation in the Baja California disjunct fish species *Anisotremus davidsonii*. *Molecular Ecology*, 14, 4085–4096.

Brandley, M.C., Schmitz, A. & Reeder, T.W. (2005) Partitioned Bayesian Analyses, Partition Choice, and the Phylogenetic Relationships of Scincid Lizards. *Systematic Biology*, 54, 373–390.

Courtenay, W.R.J. (1961) Western Atlantic fishes of the genus *Haemulon* (Pomadasyidae): Systematic status and juvenile pigmentation. *Bulletin of Marine Science*, 66–149.

Chen, D., Burleigh, J.G. & Fernández-Baca, D. (2007) Spectral Partitioning of Phylogenetic Data Sets Based on Compatibility. *Systematic Biology*, 56, 623–632.

Craig, M.T. & Hastings, P.A. (2007) A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini. *Ichthyological Research*, 54, 1–17.

Dettai, A. & Lecointre, G. (2005) Further support for the clades obtained by multiple molecular phylogenies in the acanthomorph bush. *C. R. Biologies*, 328, 674–689.

Eschmeyer, W.N. (1990) *Catalog of the Genera of Recent fishes*: Special Publication, California Academy of Sciences, VI, 697 pp.

Gill, T.N. (1861) Catalogue of the fishes of the eastern coast of North America, from Greenland to Georgia. *Proceedings of the Academy of Natural Sciences of Philadelphia, 13 (Suppl.)*, 1–63.

Girard, C.F. (1858) Notes upon various new genera and new species of fishes, in the museum of the Smithsonian Institution, and collected in connection with the United States and Mexican boundary survey: Major William Emory, Commissioner. . *Proceedings of the Academy of Natural Sciences of Philadelphia*, 10, 167–171.

Hall, B.G. (2004) Phylogenetic trees made easy: A how–to–manual (Vol. Second ed.). Sinauer Associates, Inc. 221 pp.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17, 754–755.

Iwatsuki, Y., Kohno, H. & Ida, H. (1998) Redescription of a poorly–known haemulid fish, *Pomadasys unimaculatus* Tian, 1982, from Malaysia and comparison with *P. maculatus* (Bloch 1797) (Perciformes: Haemulidae). *Ichthyological Research*, 45, 195–200.

Iwatsuki, Y. & Nakabo, T. (2005) Redescription of *Hapalogenys nigripinnis* (Schlegel *in* Temminck & Schlegel 1843), a senior synonym of *H. nitens* Richardson, 1844, and a new species from Japan. *Copeia*, 4, 854–867.

Iwatsuki, Y. & Russell, B.C. (2006) Revision of the genus *Hapalogenys* (Teleostei: Perciformes) with two new species from the Indo–West Pacific. *Memoirs of Museum Victoria*, 63, 29–46.

Iwatsuki, Y., Satapoomin, U. & Amaoka, K. (2000) New Species: *Hapalogenys merguiensis* (Teleostei; Perciformes) from Andaman Sea. *Copeia*, 1, 129–139.

Johnson, G.D. (1981) The Limits and Relationships of Lutjanidae and Associated Families. *In*: Cox, C.S., Vincent, E.S., Fleminger, A. & Rosenblatt, R.H. (Eds), *Bulletin of the Scripps Institution of Oceanography.* University of California Press, Berkeley, pp. 1–112.

Johnson, G.D. (1984) Percoidei: development and relationships. *In*: Moser, H.G. *et al.* (Eds), *American Society of Ichthyologists and Herpetologists Special Publication* no. 1, pp. 464–498.

Jordan, D.S. & Bollman, C.H. (1890) Descriptions of new species collected at the Galapagos Islands and along the coast of the United States and Colombia. *Proceedings of the United States National Museum*, 12, 149–183.

Jordan, D.S. & Gilbert, C.H. (1882) Synopsis of the fishes of North America. *Bulletin of the United States National Museum*, 16, 1–1018.

Kass, R.E. & Raftery, A.E. (1995) Bayes Factors. *Journal of the American Statistical Association*, 90, 773–795.

Konchina, Y.V. (1976) The systematics and distribution of the grunts family (Pomadasyidae). *Japanese Journal of Ichthyology*, 16, 883–900.

Konchina, Y.V. (1977) Some data on the biology of grunts (Family Pomadasyidae). *Japanese Journal of Ichthyology*, 17, 548–558.

Li, C., Lu, G. & Ortí, G. (2008) Optimal Data Partitioning and a Test Case for Ray–Finned Fishes (Actinopterygii) Based on

Ten Nuclear Loci. *Systematic Biology*, 57, 519–539.
Li, C. & Ortí, G. (2007) Molecular phylogeny of Clupeiformes (Actinopterygii) inferred from nuclear and mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 44, 386–398.
Li, C., Ortí, G., Zhang, G. & Lu, G. (2007) A practical approach to phylogenomics: the phylogeny of ray–finned fish (Actinopterygii) as a case study. *BMC Evolutionary Biology*, 7, 44.
Lindeman, K.C. (2006) CHAPTER 141 – INERMIIDAE: Bonnetmouths. *In:* Richards, W. J. (Ed), Early Stages of Atlantic Fishes: An Identification Guide for the Western Central North Atlantic, Two Volume Set. Taylor & Francis, CRC Press, USA, Boca Raton, FL., 1341pp.
Lindeman, K.C. & Toxey, C. (2003) Haemulidae – Grunts. *In*: Carpenter, K.E. (Ed), The living marine resources of the Western Central Atlantic. *FAO species identification guide for fishery purposes and American Society of Ichthyologists and Herpetologists Special Publication* No. 5, FAO, Rome, Italy, pp. 1375–2127.
López, J.A., Chen, W.–J. & Ortí, G. (2004) Esociform Phylogeny. *Copeia*, 3, 449–564.
Maddison, W.P. & Maddison, D.R. (2007) Mesquite: a modular system for evolutionary analysis. Version 2.71
Mahon, A.R. (2007) Molecular phylogenetics of perciform fishes using the nuclear recombination activating gene 1. *PhD Dissertation*, Norfolk, Virginia, USA, p. 303.
McEachran, J.D. & Fechhelm, J.D. (2005) *Fishes of the Gulf of Mexico: Scorpaeniformes to Tetraodontiformes* (Vol. 2). University of Texas Press, Austin, 228 pp.
McKay, R.J. (1984) Haemulidae. *In*: Fischer, W. & Bianchi, G. (Eds), FAO species identification sheets for fishery purposes. Western Indian Ocean (Fishing Area 51), p. pag. var.
McKay, R.J. (2001) Haemulidae (= Pomadasyidae). Grunts (also sweetlips, rubberlips, hotlips, and velvetchins). *In*: Carpenter, K.E. & Niem, V. (Eds), FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific, FAO, Rome, Italy, p. 589.
McKay, R.J. & Schneider, M. (1995) Haemulidae. Burros, corocoros, chulas, gallinazos, roncos. *In:* Fischer, W., Krupp, F., Schneider, W., Sommer, C., Carpenter, K.E. & Niem, V. (Eds), *Guia FAO para Identification de especies para lo fines de la pesca. Pacifico Centro–Oriental*. Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 1136–1173.
Miles, C. (1953) A New Pomadasid Fish from the Colombian Caribbean. *Journal of the Linnean Society of London*, 42, 273–275.
Nelson, J. (2006) Fishes of the world (Fourth ed.). John Wiley & Sons, Inc., Hoboken, New Jersey, 601 pp.
Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P. & Nieves–Aldrey, J.L. (2004) Bayesian Phylogenetic Analysis of Combined Data. *Systematic Biology*, 53, 47–67.
Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics*, 24, 581–583.
Ogden, J.C. & Ehrlich, P.R. (1977) The behavior of heterotypic resting schools of juvenile grunts (Pomadasyidae). *Marine Biology*, 42, 273–280.
Orrell, T.M., Carpenter, K.E., Musick, J.A. & Graves, J.E. (2002) Phylogenetic and Biogeographic Analysis of the Sparidae (Perciformes: Percoidei) from Cytochrome *b* Sequences. *Copeia*, 3, 618–631.
Rambaut, A. & Drummond, A. (2007) Tracer v1.4. Available from http://beast.bio.ed.ac.uk/Tracer (accessed 5 June 2010).
Ren, G. & Zhang, Q. (2007) Phylogeny of haemulid with discussion on systematic position of the genus *Hapalogenys*. *Acta Zootaxonomica Sinica*, 32, 835–841.
Richardson, J. (1844) Description of a genus of Chinese fish. *Annals and Magazine of Natural History*, 13, 461–462.
Rocha, L.A., Lindeman, K.C., Rocha, C.R. & Lessios, H.A. (2008) Historical biogeography and speciation in the reef fish genus *Haemulon* (Teleostei: Haemulidae). *Molecular Phylogenetics and Evolution*, 48, 918–928.
Ronquist, F. & Huelsenbeck, J. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
Roux, C. (1981) Pomadasyidae. *In:* Fischer, W., Bianchi, G. & Scott, W.B (Eds), FAO species identification sheets for fishery purposes. Eastern Central Atlantic; fishing areas 34, 47 (in part). Department of Fisheries and Oceans Canada and FAO. Vol. 3. Bony fishes (Malacanthidae to Scombridae) FAO, Rome, Italy, p. pag. var.
Seutin, G., White, B.N. & Boag, P.T. (1990) Preservation of avian blood and tissue samples for DNA analysis. *Canadian Journal of Zoology*, 69, 82–90.
Smith, J.L.B. (1962) Fishes of the Family Gaterinidae of the Western Indian Ocean and the Red Sea with a Resume of all known Indo Pacific Species. *Ichthyological Bulletin*, 25, 469–502.
Smith, W.L. & Craig, M. (2007) Casting the Percomorph Net Widely: The Importance of Broad Taxonomic Sampling in the Search for the Placement of Serranid and Percid Fishes. *Copeia*, 1, 35–55.
Sorenson, M.D. & Franzosa, E.A. (2007) TreeRot, version 3. Boston University, Boston, MA.
Springer, V.G. & Raasch, M.S. (1995) Fishes, angling, and finfish fisheries on stamps of the world. American Topical Association. Handbook 129. Tucson, Arizona, 110 pp.
Swofford, D. (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.
Tavera, J.J., Pizarro, A.A., de la Cruz–Agüero, J. & Balart, E.F. (2011) Phylogeny and reclassification of the species of two neotropical grunt genera, *Anisotremus* and *Genyatremus* (Perciformes: Haemulidae), based on morphological evidence. *Journal of Zoological Systematics and Evolutionary Research*. DOI: 10.1111/j.1439-0469.2011.00622.x.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 24, 4876–4882.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. & Hebert, P.D.N. (2005) Barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London B*, 360, 1847–1857.

Wiley, E.O. & Johnson, G.D. (2010) A teleost classification based on monophyletic groups. *In:* Nelson, J.S., Schultze, J.S. & Wilson, M.V.H. (Eds), *Origin and Phylogenetic Interrelationships of Teleosts*. Verlag Dr. Friedrich Pfeil, München, Germany, pp. 123–182.

Williams, A., Begg, G., Garrett, R., Larson, H. & Griffiths, S. (2004) Coastal Fishes. *In*: National Oceans Office. Description of key species groups in the Northern Planning Area. National Oceans Office, Hobart, Australia, p. 320.

Zhang, J. & Hanner, R. (2008) DNA Barcoding of fishes in the South China Sea. *Unpublished*.

Zhu, S.H., Zheng, W.J. & Zou, J.X. (2007) Molecular phylogenetic relationship of Carangidae based on sequence of complete mitochondrial cytochrome *b* gene. *Zoology*, 53, 641–650.

Zwickl, D.J. (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. The University of Texas at Austin, Austin, Texas.

APPENDIX 1. List of species and the accession number of haemulid specimens (56), including outgroups (10).

Species	Accession Number (Voucher)	GenBank Accession Numbers				
		COI	Cyt *b*	RAG1	SH3PX3	Plagl2
Ingroup						
Subfamily Plectorhinchinae						
Diagramma picta	ODU 3219	HQ676758	HQ676699	HQ676637	HQ667185	HQ667252
Parapristipoma octolineatum	ODU 3220	HQ676781	HQ676726	HQ676666	HQ667214	HQ667281
Parapristipoma trilineatum	ODU 3221	HQ676782	HQ676727	HQ676667	HQ667215	HQ667282
Plectorhinchus chaetodonoides	SAIAB 78103	HQ676783	HQ676728	HQ676668	HQ667216	HQ667283
Plectorhinchus cinctus	Photo voucher	HQ676784	HQ676729	HQ676669	HQ667217	HQ667284
Plectorhinchus diagrammus	SAIAB 77791	HQ676785	HQ676730	HQ676670	HQ667218	HQ667285
Plectorhinchus gibbosus	SAIAB 77941	HQ676786	HQ676731	HQ676671	HQ667219	HQ667286
Plectorhinchus lessonii	ODU 3225	HQ676787	HQ676732	HQ676672	HQ667220	HQ667287
Plectorhinchus macrolepis	ODU 3226	HQ676788	HQ676733	EU167861.1	HQ667221	HQ667288
Plectorhinchus schotaf	ODU 3228	HQ676790	HQ676735	HQ676674	HQ667223	HQ667290
Plectorhinchus sordidus	ODU 3229	HQ676791	HQ676736	HQ676675	HQ667224	HQ667291
Plectorhinchus vittatus	SAIAB 78102	HQ676789	HQ676734	HQ676673	HQ667222	HQ667289
Subfamily Haemulinae						
Anisotremus davidsonii	SIO-04-181	HQ676749	HQ676689	HQ676626	HQ667172	HQ667239
Anisotremus interruptus	ODU 3232	EU697525.1	HQ676690	HQ676628	HQ667174	HQ667241
Anisotremus scapularis	ODU 3234	HQ676751	HQ676692	HQ676630	HQ667176	HQ667243
Anisotremus surinamensis	KU 30405	HQ676752	EU697500.1	HQ676631	HQ667177	HQ667244
Anisotremus taeniatus	ODU 3235	EU697527.1	HQ676693	HQ676632	HQ667178	HQ667245
Anisotremus virginicus	USNM 343868	FJ582849.1	EU694336.1	EU167810.1	HQ667179	HQ667246
Boridia grossidens	ODU 3237	HQ676754	HQ676695	HQ676634	HQ667181	HQ667248
Brachydeuterus auritus	ODU 3238	HQ676755	HQ676696	EU167811.1	HQ667182	HQ667249
Conodon nobilis	KU 30150	HQ676756	HQ676697	HQ676635	HQ667183	HQ667250
Conodon serrifer	ODU 3239	HQ676757	HQ676698	HQ676636	HQ667184	HQ667251

Continued ...

... Continued

Species	No. (Voucher)	COI	Cyt b	RAG1	SH3PX3	Plag12
Emmelichthyops atlanticus	ODU 3265	HQ676759	HQ676700	HQ676638	HQ667186	HQ667253
Genyatremus cavifrons	ODU 3240	HQ676760	HQ676701	HQ676639	HQ667187	HQ667254
Genyatremus dovii	ODU 3231	HQ684719	EU694296.1	HQ676627	HQ667173	HQ667240
Genyatremus pacifici	ODU 3233	HQ676750	HQ676691	HQ676629	HQ667175	HQ667242
Haemulon aurolineatum	USNM 349060	HQ676761	HQ676702	HQ676640	HQ667188	HQ667255
Haemulon carbonarium	UF 119735	EU697531.1	EU697504.1	HQ676647	HQ667195	HQ667262
Haemulon chrysargyreum	USNM 349061	EU697532.1	HQ676703	HQ676641	HQ667189	HQ667256
Haemulon flaviguttatum	ODU 3242	EU697533.1	HQ676704	HQ676642	HQ667190	HQ667257
Haemulon flavolineatum	USNM 327584	EU697534.1	EU697507.1	HQ676643	HQ667191	HQ667258
Haemulon macrostomum	Photo voucher	HQ676762	HQ676705	HQ676644	HQ667192	HQ667259
Haemulon melanurum	ODU 3244	HQ676763	HQ676706	HQ676645	HQ667193	HQ667260
Haemulon plumierii	USNM 327585	EU697540.1	HQ676707	HQ676646	HQ667194	HQ667261
Haemulon scudderii	ODU 3246	EU697542.1	HQ676708	HQ676648	HQ667196	HQ667263
Haemulon steindachneri	ODU 3247	HQ676764	HQ676709	HQ676649	HQ667197	HQ667264
Haemulon vittatum	USNM 349224	HQ676771	HQ676716	HQ676656	HQ667204	HQ667271
Haemulopsis axillaris	ODU 3248	HQ676765	HQ676710	HQ676650	HQ667198	HQ667265
Haemulopsis leuciscus	ODU 3249	HQ676766	HQ676711	HQ676651	HQ667199	HQ667266
Haemulopsis nitidus	ODU 3250	HQ676767	HQ676712	HQ676652	HQ667200	HQ667267
Isacia conceptionis	ODU 3251	HQ676772	HQ676717	HQ676657	HQ667205	HQ667272
Microlepidotus brevipinnis	ODU 3252	HQ676777	HQ676722	HQ676662	HQ667210	HQ667277
Orthopristis chalceus	ODU 3253	HQ676779	HQ676724	HQ676664	HQ667212	HQ667279
Orthopristis chrysoptera	KU 27052	HQ676780	HQ676725	HQ676665	HQ667213	HQ667280
Pomadasys argyreus	ODU 3254	HQ676793	HQ676738	HQ676677	HQ667226	HQ667293
Pomadasys branickii	ODU 3255	HQ676794	HQ676739	HQ676678	HQ667227	HQ667294
Pomadasys incisus	ODU 3256	HQ676795	EF439221.1	HQ676679	HQ667228	HQ667295
Pomadasys kaakan	ODU 3257	HQ676796	HQ676740	HQ676680	HQ667229	HQ667296
Pomadasys maculatus	ODU 3074	HQ676797	AF240748.1	HQ676681	HQ667230	HQ667297
Pomadasys olivaceus	Photo voucher	HQ676798	HQ676741	EU182626.1	HQ667231	HQ667298
Pomadasys panamensis	ODU 3259	HQ676799	HQ676742	HQ676682	HQ667232	HQ667299
Pomadasys perotaei	ODU 3260	HQ676800	HQ676743	HQ676683	HQ667233	HQ667300
Pomadasys striatus	SAIAB 65239	HQ676801	HQ676744	HQ676684	HQ667234	HQ667301
Pomadasys stridens	ODU 3262	HQ676802	HQ676745	HQ676685	HQ667235	HQ667302
Xenichthys xanti	SIO62-706-44A	HQ676804	HQ676747	HQ676687	HQ667237	HQ667304
Xenistius californiensis	SIO64-830-44A	HQ676805	HQ676748	HQ676688	HQ667238	HQ667305
Outgroups						
Family Hapalogenyidae						
Hapalogenys aya	MUFS 23038	HQ676768	HQ676713	HQ676653	HQ667201	HQ667268
Hapalogenys kishinouyei	MUFS 23603	HQ676769	HQ676714	HQ676654	HQ667202	HQ667269
Hapalogenys nigripinnis	ODU 3264	HQ676770	HQ676715	HQ676655	HQ667203	HQ667270
Family Lethrinidae						

Continued ...

... Continued

Species	No. (Voucher)	COI	Cyt b	RAG1	SH3PX3	Plag12
Lethrinus ornatus	ODU 3266	HQ676773	HQ676718	HQ676658	HQ667206	HQ667273
Family Lobotidae						
Lobotes pacificus	SIO-98-170	HQ676774	HQ676719	HQ676659	HQ667207	HQ667274
Lobotes surinamensis	MUFS 23031	HQ676775	HQ676720	HQ676660	HQ667208	HQ667275
Family Lutjanidae						
Aphareus furca	ODU 3267	HQ676753	HQ676694	HQ676633	HQ667180	HQ667247
Lutjanus fulviflamma	ODU 3268	HQ676776	HQ676721	HQ676661	HQ667209	HQ667276
Family Nemipteridae						
Nemipterus marginatus	ODU 3104	HQ676778	HQ676723	HQ676663	HQ667211	HQ667278
Family Sparidae						
Sarpa salpa	SAIAB T29	HQ676803	HQ676746	HQ676686	HQ667236	HQ667303

* KU - University of Kansas Natural History Museum & Biodiversity Research Center; MUFS - Miyazaki University, Division of Fisheries Sciences, Miyazaki, Japan; NMFS - National Marine Fisheries Services; ODU - Old Dominion University, Norfolk, VA; SIO - Scripps Institution of Oceanography, University of California San Diego, CA; UF-University of Florida; USNM - United States National Museum, Smithsonian, Washington, D.C.

APPENDIX 2. Characteristics of the five markers amplified for haemulids. PI: parsimony-informative sites; CI: consistency index on the maximum parsimony tree.

Gene	Length (bp)	No. of constant sites	No. of PI sites	CI
COI	651	373	245	0.1317
Cyt *b*	1140	491	533	0.1698
RAG1	1431	870	385	0.4934
SH3PX3	705	499	144	0.3924
Plagl2	804	618	112	0.4989

APPENDIX 3. The ten independent parameters of 15 data partitions estimated in MrBayes. Data shows five substitution rates, three base composition proportions, the gamma parameter (alpha), and the rate multiplier for each data block.

Partitions	Substitution rates					Base frequencies			Alpha	Multiplier
	AC	AG	AT	CG	CT	A	C	G		
COI_1	0.008784	0.037748	0.010972	0.001026	0.917738	0.255555	0.299778	0.287757	0.152826	0.737942
COI_2	0.066943	0.199762	0.055384	0.372957	0.258476	0.152251	0.29242	0.14699	0.050595	3.897206
COI_3	0.031136	0.598278	0.024153	0.036301	0.262291	0.260951	0.347737	0.105675	1.704318	3.903533
Cytb_1	0.030897	0.258985	0.127909	0.038668	0.469524	0.248913	0.288502	0.260487	0.264226	0.461773
Cytb_2	0.063066	0.111883	0.079146	0.305716	0.390546	0.201883	0.234125	0.14732	0.242983	0.116774
Cytb_3	0.017662	0.540203	0.028811	0.043114	0.29457	0.300538	0.409259	0.076177	1.596014	5.545343
RAG1_1	0.246797	0.28678	0.155622	0.060578	0.178759	0.292375	0.19692	0.324992	0.276308	0.071722
RAG1_2	0.076017	0.351014	0.044473	0.202863	0.289293	0.319457	0.219731	0.19136	0.055902	0.033625
RAG1_3	0.084378	0.378065	0.062036	0.055922	0.377017	0.199733	0.270637	0.280215	1.081466	0.312716
SH3PX3_1	0.185005	0.06549	0.117652	0.134514	0.429116	0.285949	0.273238	0.260557	0.069021	0.0314
SH3PX3_2	0.038666	0.1394	0.025615	0.264729	0.455583	0.371979	0.208033	0.149063	0.104452	0.216042
SH3PX3_3	0.079503	0.36064	0.081655	0.022731	0.399024	0.124889	0.357135	0.349235	0.814905	0.396283
Plagl2_1	0.122813	0.247637	0.165761	0.071473	0.354064	0.2451	0.366692	0.221895	0.143035	0.020862
Plagl2_2	0.193565	0.238787	0.017314	0.402834	0.080442	0.377243	0.260475	0.173244	50.15845	0.510825
Pagl2_3	0.068246	0.455123	0.098299	0.019147	0.316163	0.125713	0.326448	0.32908	0.836552	0.215836