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Molecular Phylogenetics and Evolution 34 (2005) 645–654
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Unraveling evolutionary lineages in the limbless fossorial skink genus *Acontias* (Sauria: Scincidae): are subspecies equivalent systematic units?

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Received 23 August 2004; revised 25 October 2004
Available online 1 January 2005

Abstract

Subspecies in the limbless, endemic African fossorial skink genus *Acontias* constitute ill-defined operational taxonomic units, consequently considerable systematic debate has lingered on the systematic diversity within *Acontias*. In the present study, the systematic affinities among acontine taxa are explored with the utility of partial sequence data from two mitochondrial gene loci (16S rRNA and cytochrome oxidase subunit 1 (COI)) for all taxa, while two additional loci (12S rRNA, cytochrome *b*) were used to investigate relationships within the *Acontias meleagris* complex. Phylogenetic results, derived from the combined analysis, revealed two monophyletic clades. Clade 1 is comprised of small-bodied skinks while clade 2 comprised the medium bodied skinks. Within clade 2 none of the traditionally recognized subspecies formed reciprocally monophyletic groups. Furthermore, constraining the topology and enforcing sister taxa relationships between the assumed subspecies, consistently recovered a topology that was statistically significant worse, indicating that the traditionally designated subspecies groupings probably represent invalid taxonomic units, thus clearly reflecting considerable discord with current taxonomy. The burrowing life style of these lizards has probably led to marked convergent evolution and constrained the development of diagnostic morphological characters among these species. Morphological similarities in color as well as scale architecture within *Acontias* are labile and highly homoplaseous and do not reflect the evolutionary history of the group. Taxonomic implications of these results are discussed.

Keywords: *Acontias*; Mitochondrial DNA sequencing; Subspecies boundaries; Systematics; Fossorial skinks; Evolutionary lineages

1. Introduction

Delineating operational taxonomic units is often plagued by difficulty where taxa occur in allopatry. However, where multiple diagnostic differences (for example, behavioral, ecological, genetic, reproductive, physiological, and morphological) are exclusive to allopatric populations that form well-supported reciprocally monophyletic groups, most taxonomists would concur that these groups probably represent distinct evolutionary lineages. Conversely, the designation of subspecies boundaries has traditionally been virtually arbitrary, and considers only poorly defined morphological differences among allopatric populations to be indicative of subspecies status (Harris and Sá-Sousa, 2002; Wiens et al., 1999).

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doi:10.1016/j.ympev.2004.11.011

Despite being ill defined, disorderly operational taxonomic units under critical scrutiny for the past 50 years (literature reviewed in Frost and Kluge, 1994; Frost et al., 1992), subspecies remain extensively used to the present day.

The existence of subspecies is particularly problematic because it leads to considerable taxonomic confusion. Consequently, subspecies may artificially inflate or deflate α diversity, thus confounding the designation and prioritization of taxa worthy of conservation (Agapow et al., 2004; Zink, 2004). Limited consensus exists as to what value, if any, should be assigned to the conservation of subspecies. Crandall et al. (2000) argues that subspecies might reflect adaptive variation, important to a species survival and evolutionary potential, irrespective of mtDNA monophyly. In contrast, both Moritz (2002) and Moors and Atkins (2003) argue that adaptive variation may evolve rapidly; these authors favor the conservation of reciprocally monophyletic units. Irrespective of which of these two contradictory perspectives are adopted, most evolutionary biologists would agree that confidently delineating systematic boundaries are crucial in underpinning patterns and processes that sculpt evolutionary diversity and endemicity and thus maximizing the protection of biological diversity.

Undoubtedly, numerous subspecies could be masquerading as distinct evolutionary lineages, while certain subspecies may simply represent phenotypically induced ecological differences among geographically widespread conspecific populations. Arguably, the utility of highly variable external characters such as, color, length, height, coupled with ill-defined character descriptions and paucity of taxonomic material available to earlier α taxonomists, has led to the proliferation of poorly defined taxonomic units such as subspecies. Convergent, polymorphic morphological characters provide the ideal scenario for the designation of numerous subspecific entities that probably do not correspond to evolutionary lineages. Limbless fossorial skink taxa, limited in their dispersal abilities coupled with specific niche requirements, may be particularly prone to the development of locally adapted morpho-types that may or may not harbor discrete evolutionary lineages. Limited molecular research has been undertaken on phylogenetic relationships among fossorial lizards. A classical example of poorly defined subspecies occurs in the virtually exclusively southern African lizard genus *Acontias* (Broadley and Greer, 1969), where significant debate has lingered over the precise number of taxa contained within *Acontias* (Branch, 1999; Broadley and Greer, 1969). For example, within the widely distributed *Acontias meleagris*, marked intraspecific variation is evident with two nominate subspecies *A. m. meleagris* and *A. m. orientalis*. The latter subspecies possesses a distinct morph called "lineicauda," of uncertain taxonomic status. In allopatry, these two taxa (*A. m. meleagris* and *A. m. orientalis*) can be easily distinguished based on the presence or absence of six well-defined stripes on the dorsal surface. Generally, in *A. m. meleagris* stripes are absent, and the tail is non-tapered, while in both *A. m. orientalis* and the "lineicauda" morph six stripes are present on the dorsal surface, with the "lineicauda" morph being smaller bodied and slender compared to *A. m. orientalis* (Branch, 1998). Interestingly, taxa within this complex appear to have very distinct distributions, with *A. m. meleagris* occurring along the xeric western and eastern Cape coast of South Africa, while *A. m. orientalis* and its morph have a more localized distribution and occur along the more mesic eastern Cape coasts of South Africa. However, where these taxa occur in close proximity, subspecies boundaries become diffuse and accurate diagnosis becomes virtually impossible. For example, Hewitt (1938) documented the presence of striped *A. meleagris* forms on the Cape Peninsula, further disputing the contemporary designated subspecies boundaries. Broadley and Greer (1969) recommended that a systematic study be undertaken on this group to clarify their affinities, as the *A. meleagris* group has been a repository for ill-defined taxa.

As another example, within the *Acontias percivali percivali* species complex three allopatric subspecies are present: *A. p. percivali*, is restricted to Voi in south east Kenya and the eastern arc mountains of Tanzania and is separated from two other subspecies by marked physical boundaries such as the eastern arch mountains and Great Rift Valley, and considerable geographic distance (Spawls et al., 2002). *Acontias p. occidentalis* occurs in the Limpopo Province (South Africa), in Zimbabwe and west to Namibia and southern Angola, while *A. p. tasmani* is restricted to the eastern Cape in South Africa where it is often found sympatrically with *A. m.orientalis* (Branch, 1998). Broadley and Greer (1969) noted on the basis of aberrant specimens that *A. p. tasmani* and *A. m. orientalis* may hybridize where they occur in sympatry. Corroborative evidence that *A. p. tasmani* and *A. m. orientalis* may be connected by gene flow is suggested by low sequence divergence values (<1% for COI) further questions the validity of these taxa, and casts doubt on subspecies status within the medium-bodied *Acontias* taxa (Daniels et al., 2002). In addition, these authors also demonstrated that the "lineicauda" morph appears to be distantly related to *A. m. orientalis* suggesting that a species complex may be present within *A. m. meleagris*. Limited sample sizes and a lack of key subspecies in the study undertaken by Daniels et al. (2002) precluded these authors from reaching a firm conclusion on the lineage boundaries within this group. In the present study, a comprehensive geographic sampling of *Acontias* taxa is undertaken in an attempt to resolve the discrepancies among the subspecies. Sequence data derived from four mitochondrial DNA gene loci (16S rRNA, 12S rRNA, cytochrome *b*, and cytochrome oxidase I) that evolve at

varying rates, are targeted to first, elucidate the phylogenetic and phylogeographic affinities within *Acontias*, second to re-assess the taxonomy within this group.

2. Materials and methods

2.1. Sample collection

Acontias samples were collected (as shown in Fig. 1) from localities throughout South Africa, while *A. p. percivali* was collected in Tanzania (the latter locality is not shown). A list of the 71 samples collected in the present study is provided in Table 1. Animals were sacrificed by freezing at −20 °C, followed by the dissection of liver and muscle tissue that was stored in 95% ethanol in a freezer. Voucher specimens were preserved in 10% buffered formalin, to facilitate future examination of specimens, prior to storage in 70% ethanol.

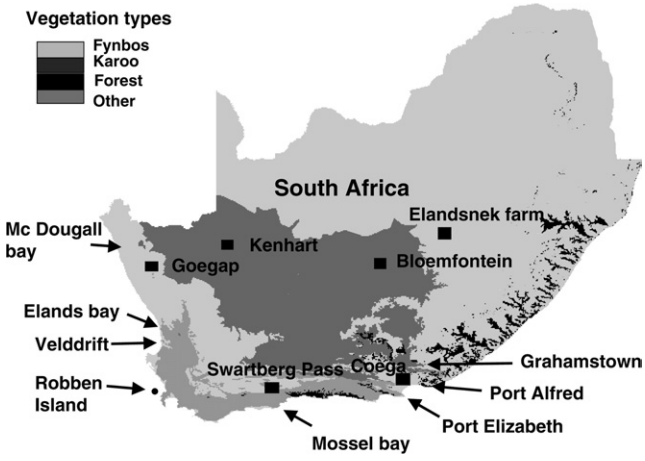

Fig. 1. A map showing *Acontias* sample localities throughout South Africa. The samples collected from the Dodoma region in Tanzania are not shown; localities are summarized in Table 1.

Table 1
List of the *Acontias* taxa sampled in the present study, with *N* denoting the total sample sizes per taxon

Taxon	Locality and province	*N*
A. gracilicauda gracilicauda	Bloemfontein, Orange Free State	1
A. gracilicauda gracilicauda	Elandsnek Farm, Orange Free State	2
A. litoralis	McDougall bay, Northern Cape	1
A. lineatus lineatus	Kenhart, Northern Cape	2
A. lineatus tristis	Goegap, Northern Cape	2
A. meleagris meleagris	Elands Bay, Western Cape	1
A. meleagris meleagris	Velddrif, Western Cape	10
A. meleagris meleagris	Robben Island, Western Cape	5
A. meleagris meleagris	Mossel Bay, Western Cape	8
A. meleagris orientalis	Grahamstown, Eastern Cape	10
A. m. o. lineicauda morph	Port Alfred, Eastern Cape	8
A. m. o. lineicauda morph	Port Elizabeth, Eastern Cape	8
A. percivali percivali	Dodoma region, Tanzania	5
A. percivali tasmani	Coega, Eastern Cape	7
A. percivali tasmani	Swartberg Pass, Western Cape	1

The *A. p. percivali* samples were deposited in the Transvaal Museum, Pretoria (Accession Nos. TM 85192–85196). The total number of individuals summarized here includes the 16 *Acontias* samples from the earlier study by Daniels et al. (2002).

2.2. DNA sequencing and phylogenetic analysis

A detailed description of the DNA extraction protocol employed in the present study is outlined in Daniels et al. (2002). The primers 16Sa (5′-CGC CTG TTT ACT AAA AAC AT-3′) and 16Sb (5′-CCG GTC TGA ACT CAG ATC ACG T-3′) were used to amplify the 16S gene, while the primers COIa (5′-AGT ATA AGC GTC TGG GTA GTC-3′) and COIf (5′-CCT GCA GGA GGA GGA GAT CC-3′) were used to amplify the cytochrome oxidase one (COI) gene fragments to investigate the phylogenetic relationship among all the acontine samples. The primer sets, 16S rRNA and COI were obtained from Cunningham et al. (1992) and Palumbi et al. (1991), respectively. In addition, the systematic relationships within the *A. m. meleagris* species complex were investigated using sequence data derived from two additional mtDNA gene loci (12S rRNA and cytochrome *b*). For the 12S gene region the primers 12S A (5′-CTG GGA TTA GAT ACC CCA CTA-3′) and 12S B (5′-TGA GGA GGG TGA CGG GCG GT- 3′) while the primers for the cytochrome *b* (hereafter cyt *b*) region was WWF (5′-AAA YCA YCG TTG TWA TTC AAC TAC-3′) and cyt *b* R2 (5′-GGG TGR AAK GGR ATT TTA TC-3′) the 12S and cyt *b* primers were from Kocher et al. (1989) and Whiting et al. (2004), respectively.

For each PCR, a 25 μl reaction was performed that contained 14.9 μl of millipore water, 3 μl of 25 mM $MgCl_2$, 2.5 μl of 10× Mg^{2+} free buffer, 0.5 μl of a 10 mM dNTP solution, and 0.5 μl of the primer sets at 10 mM, 0.1 U of *Taq* polymerase, and 1–3 μl of template DNA. The PCR temperature regime for both gene fragments was 95 °C for 2 min; 95 °C for 30 s; 50 or 55 °C for 40 s; 72 °C for 1 min, and then 32–40 cycles for the last three steps, followed by a final extension of 10 min at 72 °C. PCR products were electrophoresed in a 1% regular agarose gel containing ethidium bromide for 30 min at 70 V. Products were visualized under UV light. PCR products were purified using a PCR purification kit (QIA gen) followed by gel purification using the kit QIA quick gel extraction kit. Purified PCR products were cycle sequenced using standard protocols (3 μl of the purified PCR product, 4 μl of the fluorescent-dye terminators with an ABI PRISM Dye Terminator Cycle Sequencing Reaction Kit, Perkin–Elmer, and 3 μl of a 10 μM primer solution for each primer pair). Unincorporated dideoxynucleotides were removed by gel filtration using Sephadex G-25 (Sigma). Sequencing was performed on an ABI 3700 automated machine.

2.3. Outgroup selection

An earlier phylogenetic study of the subfamily Acontinae by Daniels et al. (2002) demonstrated that the monotypic *Acontophiops lineatus* is the sister taxon of *Acontias*, consequently this monotypic genus was used as an outgroup. Daniels et al. (2002) also demonstrated that *Acontias gracilicauda gracilicauda* would be an appropriate outgroup for examining relationships within the *A. m. meleagris* complex, hence the former taxon was used as an outgroup.

2.4. Phylogenetic analysis

Each sample was sequenced in both directions, and aligned forward and reverse sequences were checked for base ambiguity in Sequence Navigator (Applied Biosystems) and a consensus sequence was created. The 16S rRNA sequences were aligned in CLUSTAL X (Thompson et al., 1997) using the default parameters of the program and further adjusted by eye where obvious mismatches were made by the computational alignment. The protein coding COI sequences were aligned manually. Both the 16S rRNA and the COI sequences from this study have been deposited in GenBank. The GenBank Accession Nos. AY683682–AY683735 and AY683736–AY683789 are for the 16S rRNA and COI gene regions, respectively. The samples sequenced in the present study were combined with the 16 *Acontias* samples previously sequenced by Daniels et al. (2002). In addition, sequences for 16S rRNA of two *Typhlosaurus* species (*T. lomii* and *T. vermis*, AY028894 and AY028895, respectively) deposited in an earlier study by Daniels et al. (2002) and a single species (*T. caecus*, AY217947) deposited by Whiting et al. (2004) were downloaded from GenBank and used to compare levels of uncorrected sequence divergence within the subfamily, as these related lineages should share similar substitution rates. For the 12S rRNA and the cytochrome *b* (cyt *b*) on the *A. m. meleagris* complex a subset of the original data set used in the larger phylogenetic analysis were considered as exploratory data analysis of the 16S rRNA, and COI revealed low levels of intraspecific variability. The 12S rRNA and cyt *b* sequences were deposited in GenBank under Accession Nos. are AY683643–AY683681 and AY683790–AY683828, respectively.

Phylogenetic data analyses were executed in PAUP*4 version beta 8 (Swofford, 2002) using parsimony (MP) and maximum likelihood (ML) optimality criteria. For the MP analysis, trees were generated using the heuristic search option with TBR branch swapping using 1000 random taxon additions. For the MP analysis gaps were excluded as characters. For the ML analysis, the appropriate substitution model was calculated using MODELTEST version 3.06 (Posada and Crandall, 1998). The best-fit maximum likelihood score was chosen using the Akaike Information Criterion (AIC) (Akaike, 1973), since this reduced the amount of unnecessary parameters that contribute little to describing the data by penalizing more complex models (Bernham and Anderson, 2002; Nylander et al., 2004). Uncorrected sequence divergence values were calculated between samples. Phylogenetic confidence in the nodes recovered from parsimony was estimated by bootstrapping (Felsenstein, 1985), analyzing 1000 pseudoreplicates of data sets, while due to computational constraints only 100 pseudo-replicates were performed for ML. In this study, we regarded bootstrap values >75% as strongly supported. The partition-homogeneity test (Farris et al., 1995), as implemented in PAUP, was used to evaluate the congruence of the combined 16S rRNA and COI data sets, as well as for the four loci in the *A. m. meleagris* complex. The Shimodaira and Hasegawa (1999) test as implemented in PAUP*4 version beta 10, was used on the total-evidence tree (16S and COI) to test the traditionally defined subspecies boundaries. The sister taxon relationship between *A. p. percivali* and *A. p. tasmani* was enforced, as well as the assumed sister taxon relationships between *A. m. meleagris* and *A. m. orientalis*, while the sister taxon relationship between *A. m. orientalis* and its morph "lineicauda" was also enforced. In addition, the 12S rRNA and the cyt *b* data sets were exclusively used to examine relationships within the *A. meleagris* complex, these two gene regions were then combined with the reduced 16S rRNA and COI data (for which best fit models were recalculated—results not shown) to investigate phylogenetic relationships within the *A. m. meleagris* complex exclusively. Bayesian inferences were used to investigate optimal tree space using the program MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003). For each analysis four Markov chains were run, with each chain started from a random tree and three million generations generated, sampling from the chain occurred every 5000 tree, for each four gene fragments separately, and then the combined 16S rRNA and COI data for all *Acontias* species followed by the four mtDNA loci in the *A. m. meleagris* complex. For each gene fragment analyzed, MODELTEST was used to select the appropriate substitution model, while in the combined analyses data sets were partitioned according to these models, using unlinked paramaters. A 50% majority rule consensus tree was generated from the trees retained (after the burn-in trees were discarded), with posterior probabilities for each node estimated by the percentage of time the node was recovered. Posterior probabilities of <0.95 are regarded as poorly supported.

3. Results

Combined analysis of the two gene fragments (16S rRNA and COI mtDNA). A partition homogeneity test

revealed that the two gene regions, 16S rRNA and COI were congruent, thus allowing for the combination of the data sets (1108 bp). The substitution model selected for the ML analysis was TVM + I (the number of invariable sites) + Γ (gamma shape parameter), with the base frequencies A = 0.3352, C = 0.2373, G = 0.1780 and T = 0.2495, with the rate matrix as follow, R(a)[A–C] = 1.65, R(b)[A–G] = 5.01, R(c)[A–T] = 1.02, R(d)[C–G] = 1.12, R(e)[C–T] = 5.01, and R(f)[G–T] = 1.00, while I was 0.48 and Γ was 0.76. Evident from the ML topology are two well-supported clades, comprising the small-bodied (clade 1) and medium-bodied (clade 2) taxa, with 100% bootstrap support (Fig. 2; $-\ln L = 5172.82$; AIC = 10363.64). Within clade 1, sister taxon relationships between *A. l. tristis* and *A. litoralis* was recovered with 79% bootstrap support. Within the medium-bodied clade the sister taxon relationship between *A. p. percivali* and *A. g. gracilicauda* was recovered with 99% bootstrap support. The MP analyses, contained 252 parsimony informative characters, with a tree length of 576 steps, CI = 0.58 and RI = 0.90 with 1250 trees. The bootstrapped MP and posterior probably Bayesian topology

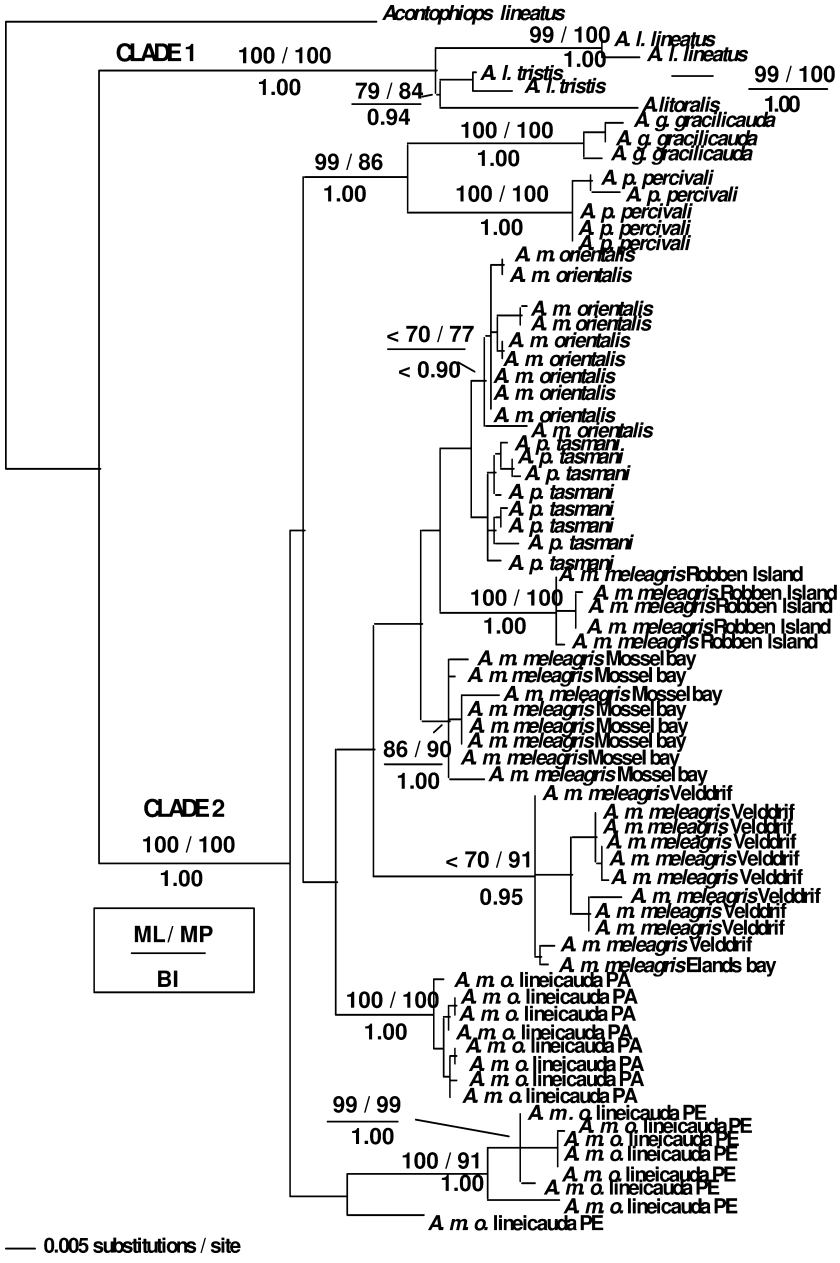

Fig. 2. A ML topology ($-\ln L = 5172.82$) derived from the combined analysis of the two gene fragments (16S rRNA and COI) TVM + I + Γ model, for all *Acontias* taxa sequenced. Values above the nodes for each clade represent the bootstrap value obtained from ML and MP, respectively, while the posterior probabilities obtained from the Bayesian inferences (BI) are shown below the node. The abbreviation PA represents the Port Alfred population, while PE represents the Port Elizabeth population of the "lineicauda" morph.

recovered and supported the same nodes present on the ML topology (Fig. 2).

Enforcing the monophyly of the subspecies, using the SH test always recovered a topology that was statistically worse than the best tree. For example, when the sister taxa relationship between all the *A. m. meleagris* populations (Velddrif, Robben Island, Mossel Bay, and Elands Bay) and *A. m. orientalis* was enforced (comparing the unconstrained tree to the null hypothesis) ($-\ln L_0 - \ln L_1 = \Delta - \ln L$), a statistically worse relationship was recovered (5127–5166 = 38; $P < 0.005$). Similarly, when *A. m. orientalis* is forced to be the sister taxon of the "lineicauda" morph (5127–5265 = 137; $P < 0.001$) and *A. p. percivali* was forced to be the sister taxon of *A. p. tasmani* (5127–5215 = 87; $P < 0.001$) statistically worse relationships were recovered in both instances.

Relationships derived from the combined analysis of all four gene regions (16S rRNA, 12S rRNA, COI, and cyt *b*) for the *A. m. meleagris* complex.

Partition homogeneity results revealed that the data sets could be combined into a single data matrix that comprised a total of 2044 bp. The substitution model selected for the ML analysis was TVM + I + Γ, with the base frequencies A = 0.3234, C = 0.2545, G = 0.1789, and T = 0.2432, with the rate matrix as follow, R(a) [A–C] = 1.25, R(b)[A–G] = 4.67, R(c)[A–T] = 0.65, R(d) [C–G] = 1.21, R(e)[C–T] = 4.67, and R(f)[G–T] = 1.00, while I was 0.64 and Γ was 0.86. The ML topology (Fig. 3) ($-\ln L = 6584.56$; AIC = 13187.12) recovered a well-supported clade (with 94% bootstrap support) comprised of Robben Island (*A. m. meleagris*), *A. p. tasmani*, *A. m. orientalis*, and Mossel Bay (*A. m. meleagris*), with the sister taxa relationships between *A. p. tasmani* and *A. m. orientalis* having 95% bootstrap support. Interestingly, the two "lineicauda" populations formed two distinct groups (with 100% bootstrap support), similarly the remaining *A. m. meleagris* population from Velddrif formed a well supported (with 98% bootstrap support) but distinct clade. The terminal relationships were always well supported. The MP analysis revealed 292 parsimony informative characters, with CI = 0.61, RI = 0.91, while the tree length was 562 steps and 200 equally parsimonious trees were recovered. The bootstrapped MP and posterior probably Bayesian topology recovered and supported the same nodes present on the ML topology (Fig. 3).

4. Discussion

Phylogenetic results derived from the combined sequence topology (for 16S rRNA and COI mtDNA) yielded a well-resolved phylogeny that demonstrated the presence of two main clades among the acontine taxa sampled (Fig. 2). Morphologically, these two clades are easily distinguishable, with the small-bodied skinks (clade 1) possessing a snout vent length (SVL) between 119 and 148 mm, with a mid-body scale row count between 12 and 14. The medium-bodied skinks (clade 2) have a SVL between 197 and 490 mm, with a mid-body scale row count between 14 and 20 (Broadley and Greer, 1969). The small-bodied taxa usually have three supraciliary scales, while the remaining taxa generally have four. In *A. lineatus* there are one or two supraoculars while in *A. litoralis* a single supraocular is present. In addition, in the *A. lineatus* group, Broadley and Greer (1969) state that "the third subocular tends to be displaced to a position posterior to the rear corner of the eyelid" particularly in taxa characterized by four upper labials such as *A. lineatus* and *A. litoralis*. Furthermore, the snout among species in clade 1 is depressed with sharp horizontal edges, while it is round among species in clade 2. Finally, the tail among taxa in clade 1 is "depressed distally and flattened below, ending in a point" as pointed out by Broadley and Greer (1969). Biogeographically, the taxa in clade 1 (small-bodied skinks) is restricted to the dry Succulent Karoo biome on the west coast of South Africa, while clade 2 (medium bodies skinks) has a wider distribution throughout southern Africa. The single character that unites *Acontias* taxa is the movable eyelid, a character that has been shown to represent the plesiomorphic condition in these skinks (Daniels et al., 2002). Considering these morphometric, morphological and geographic differences, the marked sequence divergence between the two clades, together with the fact that two non-monophyletic clades were recovered from the molecular data (Fig. 2) suggest that *Acontias* may probably be comprised of two distinct genera instead of one, represented by the small (clade 1) and medium-bodied taxa (clade 2), respectively. More alarmingly, none of the traditionally recognized subspecies within *Acontias* formed reciprocally monophyletic evolutionary lineages, thus seriously question the validity of the currently designated subspecies units (Fig. 2). Clearly a taxonomic revision of *Acontias* is required.

Morphologically, the relationships within the *A. m. meleagris* complex are confounded by the absence of well-defined characters. For example, *A. m. orientalis* can be distinguished from *A. p. tasmani* in possessing six distinct dorsal stripes, but the taxon, *A. m. meleagris* also possesses distinctly striped forms at Paarl, Hermanus, and Devils Peak (Fitzsimon, 1943; Hewitt, 1938; Daniels pers. obs). Conversely, Broadley and Greer (1969) reported that certain populations of *A. m. orientalis* occasionally contain uniform, unstriped specimens that are indistinguishable from *A. m. meleagris*. Broadley and Greer (1969) further observed that variation in *A. meleagris* is mosaic and not clinal. Coastal populations of *A. m. meleagris* (such as Robben Island, Velddrif, and Mossel Bay), for example, are characterized by melanistic individuals in which the dorsal surface may be uniformly dark, suggesting that melanism may have evolved inde-

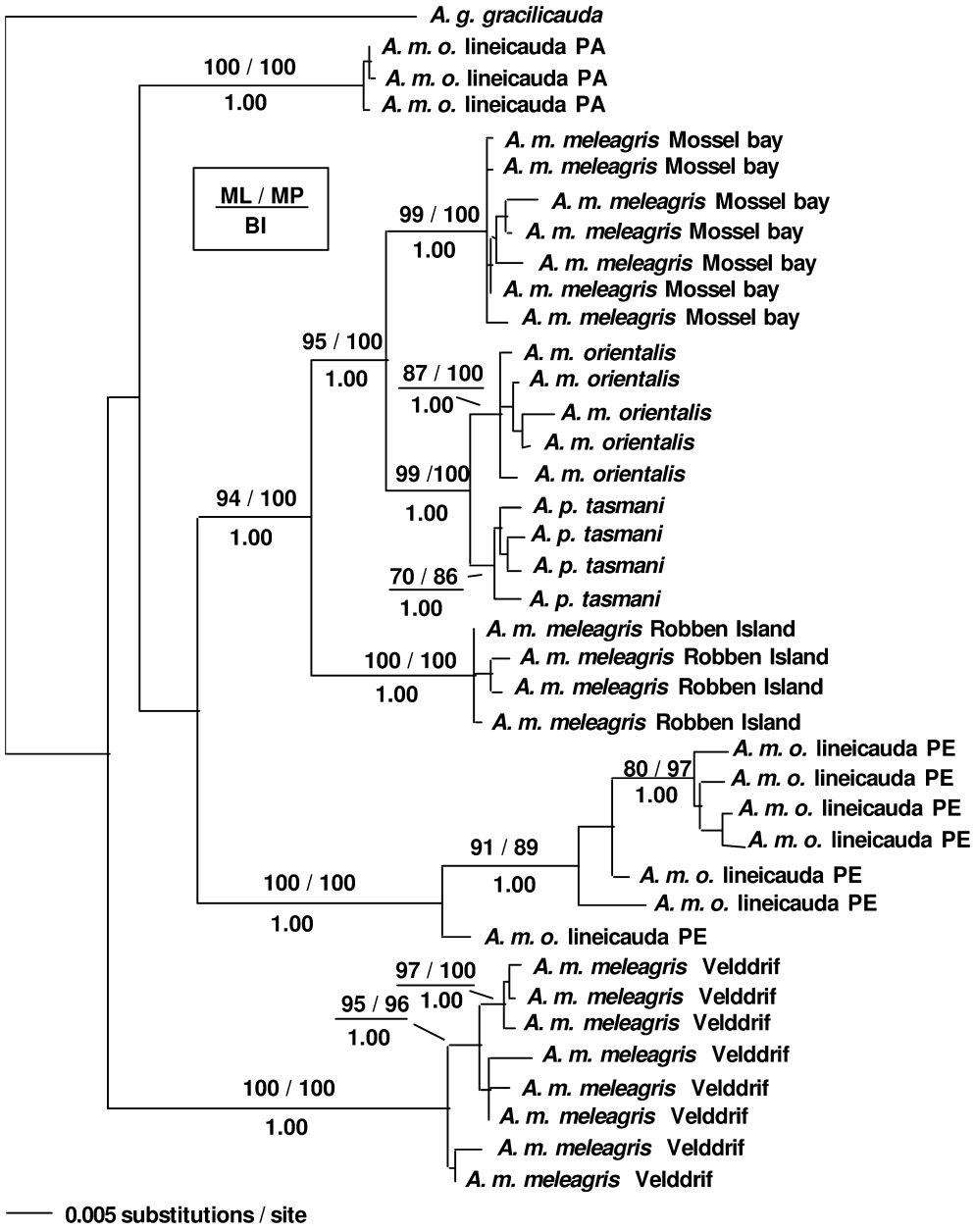

Fig. 3. A ML topology ($-\ln L = 6584.56$) derived from the combined analysis of the four mitochondrial gene loci (12S rRNA, 16S rRNA, COI, and cyt *b*) for the *A. m. meleagris* complex derived from the TVM + I + Γ model. Values above the nodes for each clade represent the bootstrap value obtained from ML and MP, respectively, while the posterior probabilities obtained from the Bayesian inferences (BI) are shown below the node. The abbreviation PA represents the Port Alfred population, while PE represents the Port Elizabeth population of the "lineicauda" morph.

pendently several times in response to selection for enhanced thermoregulatory adaptations to colder climatic conditions. Numerous melanistic lizards occur along the southern African west coast, and are thought to be relicts of colder paleo-climatic conditions (Mouton and Van Wyk, 1990, 1995). Burbrink et al. (2000) reported a similar pattern of color variation among populations of the American rat snake, *Elaphe obsolete* in North America. Color as well as the absence or presence of stripes are clearly labile characters. These results suggest that reliance on external morphological characters exclusively in designating evolutionary lineages in *Acontias* is flawed. Similarly, Kearney and Stuart (2004) recently reported that among the limbless fossorial worm lizards (Amphisbaenians), marked levels of convergence are present that obscure phylogenetic relationships.

Marked genetic distance of 3% among *A. m. meleagris* populations for 16S RNA separates the Robben Island population from the geographically closer Velddrif population. Surprisingly, although collected along the Cape west coast, the Robben Island population has its closes phylogenetic affinity with the east coast populations (*A. m. orientalis*, *A. p. tasmani* and the Mossel Bay taxon).

Such, geographically incoherent results have been attributed to transmarine dispersals in geckos and other lizards (Carranza et al., 2000; Harris and Sá-Sousa, 2002). However, *Acontias* are limbless, probably rendering them incapable of rafting and no evidence exists that the presence of the taxon on the island is the result of anthropomorphic influences. Geologically, Robben Island is a continental relic and the *A. m. meleagris* population on the island have probably been isolated by marine transgressions that occurred throughout the Miocene and Pliocene. This is an interesting scenario, and suggests that the *A. m. orientalis* group (the sister taxon to the Robben Island population) may historically have had a broad distribution in the western Cape. Interspecific sequence divergence values are low, for example, 1% between *A. p. tasmani* and *A. m. orientalis* for 16S rRNA (with a shared haplotype for this gene region), while the divergence between the *A. m. orientalis* and the "lineicauda" morph is 3%. A comparison of sequence divergence values for 16S rRNA among three of the ten *Typhlosaurus* species (one of the three genera that constitutes the subfamily Acontinae) ranged from 1.36% between *T. caecus* and *T.lomii*, to 3.84% between *T. vermis* and *T. caecus*. Interspecific sequence divergence values for *Acontias* are low, but very similar in magnitude to those between the *Typhlosaurus* species. Interestingly, the sequence divergence for COI between *A. p. percivali* and *A. g. gracilicauda* is >6%, while the intraspecific variation for the latter taxon is 1.1%, noticeably this value is similar to the sequence divergence (of <1%) between *A. m. orientalis* and *A. p. tasmani*. These results suggest that the latter taxa are capable of genetic exchange, and probably do not constitute distinct operational taxonomic units. Alternatively these results may simply reflect the retention of an ancestral haplotype, prior to divergence. Rapidly evolving nuclear markers, including microsatellites and nuclear sequence data coupled with more intensive geographic sampling strategy are required to investigate the degree of genetic differentiation within the *A. m. meleagris* complex. A study by Harris (2002) suggests that interspecific cytochrome *b* sequence divergence for congeneric reptile taxa is generally in the range of 13.6%. Divergences within *Acontias* are considerably lower, for example the maximum sequence divergence between *A. g. gracilicauda* and *A. m. meleagris* was 6.1%. The exclusive use of sequence divergence from a single gene region to establish a metric benchmark to designate evolutionary units across unrelated groups ignores the inherent stochasticity of coalescent process.

The question now arises whether these genetically distinct groups are evolutionary distinct lineages, or merely represent marked intraspecific divergence. Multiple diagnostic differences are generally required for the designation of unique evolutionary lineages. Defining species purely on mitochondrial sequence divergence is problematic as it is arguably only indicative of female gene flow (Sites and Crandall, 1997). Wiens and Penkrot (2002) argue, however, that because mtDNA coalesces four times faster than nuclear genes, it should be useful in detecting recently evolved species. These authors also formalized a set of criteria for detecting evolutionary lineages using mtDNA. First, ingroup lineages must be exclusive of the outgroup. Second, lineages within the species complex must be geographically exclusive of each other. Molecular results support the presence of a single species complex among *A. m. meleagris* taxa (encompassing Mossel Bay, Robben Island, *A. p. tasmani*, and *A. m. orientalis*. Furthermore, these results suggest that both *A. m. orientalis* and *A. p. tasmani* are invalid taxonomic designations, and should be regarded as junior synonyms of *A. m. meleagris*. Exclusivity in mtDNA haplotypes among most localities within the *A. m. meleagris* complex suggests that large-scale gene flow is not a major cohesive force, or that the maternally inherited marker suggest a high degree of phylopatry. Considering the low vagility and microhabitat specificity in this group, and the marked geographic distances between populations, it is hardly surprising that intraspecific variation is marked since the rapid fixation of mutational differences caused by genetic drift between small populations may occur frequently thus promoting divergence. Similar results have been reported by Sinclair et al. (2004) for the night lizard genus *Xantusia*.

Taxa that belong to the same *Acontias* subspecies cluster "inappropriately" in the combined sequence topology, thus reflecting the inaccuracies associated with the current classification scheme. Numerous species definitions exist however, among the most widely used are certainly the biological (Dobzhansky, 1970; Mayr, 1963), phylogenetic (Cracraft, 1989) and cohesion species concepts (Templeton, 1989, 2001). The biological species concept argues for the absence of gene flow between groups, however, this may simply reflect genetic divergence (genetic isolation) that does not preclude reproductive compatibility, while the phylogenetic species concept argues for the presence of distinct diagnosable characters, which is determined by the presence or absence of gene flow. The cohesion species concept argues that populations of a taxon should be comprised of a single evolutionary lineage that is defined as a field of gene recombination or ecological interchange ability. However, defining the taxa purely on sequence data is cautioned, and additional sources of evidence are required in support of this provisional conclusion. Arguably, the small sample sizes within the *A. m. meleagris* complex used in the present study would preclude a firm taxonomic decision, however, considering that samples were collected from virtually the entire distribution range this argument is nullified as the overall pattern of variation in this group is unlikely to change dramatically.

In making taxonomic recommendation, we subscribe to the view that the evolutionary lineages should

constitute monophyletic grouping, while non-monophyletic and paraphyletic group derived from multiple data sets requires taxonomic revisions to reflect the most recent findings. Absence of well-defined species-specific morphological characters in *Acontias* can possibly be attributed to convergent morphological evolution, induced by the fossorial life style of these lizards and reflecting the differential rate of character evolution between the morphological and molecular data. Extreme caution should be exercised in using poorly defined external morphological characters in highly polymorphic groups. Our results further suggest abandonment of the use of subspecies within *Acontias* as these represent artificial units that do not correspond to evolutionary lineages. Polymorphic morphological characters, although representative of variation should not be conserved in the absence of well-defined reciprocally monophyletic evolutionary units derived from multiple loci. Trinomials should be investigated to reject or affirm their systematic uniqueness, followed by a firm systematic amendment to current taxonomic practises, and subspecies should not be used as units for conservation where their status remains based on dubious features. The phylogenetic analysis presented here has been useful to disentangle intraspecific geographic variation from taxonomic artefact within *Acontias* and suggests a reduction in the current number of recognized *Acontias* taxa.

Acknowledgments

The University of Stellenbosch, National Research Foundation (South Africa) and the National Science Foundation (USA) are thanked for providing funding for the sequencing part of this project. In addition, the University of the Free State (Qwaqwa campus) provided logistical support. A grant from the National Research Foundation to Neil Heideman provided financial support for fieldwork. Dr. M. Cunningham is thanked for collecting the sample of *Acontias percivali tasmani*. Similarly, Shaun Davis and Estelle Esterhuizen are thanked for arranging and allowing the collection on Robben Island. Professors J. Sites, A. Bauer, Drs. M. Perez-Losada, and A. Whiting are thanked for constructive critical comment on earlier drafts of this manuscript. Two anonymous reviewers as well as the associate editor, Professor A. Caccone is thanked for constructive comments that helped to improve the quality of the manuscript.

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