
Taxonomic subdivisions within the fossorial skink subfamily Acontinae (Squamata: Scincidae) reconsidered: a multilocus perspective

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Despite recent molecular systematic studies on the fossorial southern African skink subfamily Acontinae, evolutionary relationships among the three genera remain unresolved and disputed. Among these, the most recent study suggests that both *Typhlosaurus* and *Acontias* are paraphyletic, contrasting earlier results that suggest the presence of two divergent clades within *Acontias*. Here we further investigate the evolutionary relationships in the limbless fossorial southern African subfamily Acontinae with partial sequenced data derived from four mitochondrial loci (16S rRNA, 12S rRNA, cytochrome oxidase I and cytochrome *b*), as well as two nuclear protein coding loci (*c-mos* and *RAG-1*), in an attempt to clarify evolutionary relationships. Phylogenetic results derived from combined data analyses (comprising all six loci and totalling ~3.1 kb) using maximum parsimony, maximum likelihood and Bayesian inferences converged on the same topology. The resulting phylogeny showed *Typhlosaurus* as monophyletic, while the monotypic genus *Acontophiops* was nested intermediate to two reciprocally monophyletic *Acontias* clades. These two *Acontias* clades can be distinguished on the basis of a number of morphological, morphometric and biogeographical characters, underscoring the presence of two distinct groups. In the present study, we propose the following taxonomic changes based on the multilocus phylogeny. We retain the genus name *Acontias* for the medium- and large-bodied skinks in clade 2 comprising all taxa in the *Acontias meleagris* complex as well as *Acontias plumbeus*, *Acontias gracilicauda gracilicauda*, *Acontias breviceps*, *Acontias percivali percivali* and *Acontias percivali occidentalis*. We designate a new genus *Microacontias* gen. nov. for the reciprocally monophyletic taxa in clade 1 comprised of all the small-bodied taxa that include *Microacontias litoralis*, *Microacontias lineatus lineatus*, *Microacontias lineatus grayi* and *Microacontias lineatus tristis*. We examine the evolution of characters used in the taxonomy of the Acontinae and suggest that symplesiomorphic morphological characters among fossorial taxa have been an impediment to understanding the evolution of this subfamily. This study underscores the importance of the application of multiple molecular markers (both nuclear and mitochondrial) in determining the taxonomic diversity among fossorial skinks and emphasizes the application of phylogenetics in defining synapomorphic (shared derived) features.

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Introduction

Fossorial skink systematics has remained relatively recalcitrant to modern systematic endeavours. However, renewed recent focus from a molecular systematic perspective suggests that

pervasive morphological convergent evolution in facia-cranial features, colour patterns, scale architectural characters and body elongation associated with a burrowing mode of life are highly homoplasious (Daniels *et al.* 2002, 2005; Whiting

et al. 2004; Schmitz et al. 2005). Consequently, taxonomic divisions among the fossorial skinks have been susceptible to erroneous conclusions since a number of the genera hitherto defined have been shown to be paraphyletic in molecular studies (Daniels et al. 2002, 2005; Schmitz et al. 2005).

The endemic southern African fossorial subfamily Acontinae (comprising the three genera *Typhlosaurus*, *Acontias* and *Acontophiops*) constitutes a basal, relictual skink lineage (Whiting et al. 2003; Townsend et al. 2004). Daniels et al. (2002, 2005) demonstrated that *Acontias* is comprised of two genetically highly divergent clades, both of which are reciprocally monophyletic based on two mitochondrial markers [16S rRNA and cytochrome oxidase subunit I (COI)]. Furthermore, these two genetically divergent groups (simply defined as clades 1 and 2 previously) can additionally be distinguished on the basis of a suite of independent morphological characters potentially alluding to the presence of two distinct genera within *Acontias*. Nevertheless, these authors withheld formal nomenclatural designations, in the absence of nuclear sequence data and limited taxonomic sampling. A recent study by Brandley et al. (2005) suggests that both *Acontias* and *Typhlosaurus* are paraphyletic, with *Acontophiops lineatus* nested within *Acontias*. This contrasts with the earlier work by Daniels et al. (2002) and further fuels the taxonomic debate about relationships within the Acontinae. On morphological grounds, the inclusion of *Acontophiops lineatus* within *Acontias* as suggested by Brandley et al. (2005) appears improbable because the former genus is defined by a number of morphological synapomorphies. Clearly there is considerable controversy surrounding the current placement and generic divisions within the Acontinae. In this regard, the application of multiple molecular markers may be particularly useful for disentangling evolutionary relationships within the subfamily.

While there is generally a large degree of phylogenetic congruence between mitochondrial (mtDNA) and nuclear data (nDNA), the exclusive reliance of evolutionary inferences derived from a single, effectively linked locus such as the mitochondrial genome (that is maternally inherited without recombination) coupled with poor taxon sampling is potentially flawed (Zhang & Hewitt 2003). It was demonstrated that the rapid rate of nucleotide substitution in the mitochondrial genome, the presence of pseudo-genes and high levels of homoplasy might lead to spurious evolutionary inferences (Wiens & Hollingsworth 2000; Engstrom et al. 2004). Homoplasious characters may not have a negative impact on our ability to accurately estimate topological relationships per se, and it has been suggested that these characters may actually be informative (Källersjö et al. 1998, 1999). In contrast, nuclear markers are renowned for a slower, more conserved evolutionary rate, making them less prone to homoplasy in the long term, but less variable in the short

term, particularly among taxa that have undergone recent cladogenic events (Bricks & Edwards 2002; Zhang & Hewitt 2003). In addition, nuclear markers are known to undergo recombination, exhibit intragenomic polymorphism, may be under selection and possess heterozygotes, have gene-specific mutational rates and history and may be notoriously difficult to amplify and sequence (Zhang & Hewitt 2003). The analysis of multiple unlinked loci derived from both mitochondrial and nuclear genes offer the most powerful attempt at accurately inferring evolutionary affinities.

In the present study, we use sequence data from four mitochondrial loci [16S rRNA, 12S rRNA and two protein-coding genes, COI and cytochrome *b* (*cyt b*)], as well as two nuclear loci that include the proto-oncogene *c-mos* that codes for the protein that arrests oocyte maturation and the recombination activation gene 1 (*RAG-1*) that mediates genomic rearrangements and is central to immunity in vertebrates. These loci have been successfully used to recover evolutionary relationships in a wide range of skink taxa, since they evolve at different mutation rates (Whiting et al. 2003, 2004; Townsend et al. 2004). The objectives of the present study are first to examine the placement of the three genera within the subfamily Acontinae and test the contrasting evolutionary relationships of both *Acontias* and *Typhlosaurus* suggested by Daniels et al. (2002, 2005) and Brandley et al. (2005); second, to explore the taxonomic distinctiveness of the two clades identified earlier (Daniels et al. 2005) within *Acontias*; and third, to use the phylogeny to critically evaluate the utility of the morphological characters that were used to designate the three existing genera. In this regard, we include three *Typhlosaurus* species, the monotypic genus *Acontophiops lineatus*, as well as seven of the eight *Acontias* species (excluding only *Acontias poecilus* a point endemic for which, despite exhaustive field collection efforts, no samples could be obtained). Formal nomenclatural changes based on the combined sequence data phylogeny are proposed, and a new genus is defined. These results are crucial in determining the true taxonomic diversity of the southern African herpetofauna, an area that is generally characterized by exceptional endemism, since the effective documentation of biodiversity is central to conservation.

Materials and methods

Sample collection

Samples of all three genera were collected from localities throughout southern Africa and Tanzania. We used a subset of the samples in earlier studies by Daniels et al. (2002, 2005) (Table 1) and included five additional taxa, *Acontias plumbeus*, *Acontias percivali occidentalis*, *Acontias breviceps*, *Acontias lineatus grayi* and *Typhlosaurus caecus*. Where possible, multiple representatives for a taxon were included in the analysis. Animals were killed by freezing at -20°C , followed by the dissection of liver and muscle tissues that were stored in 95% ethanol in

Table 1 A list of the Acontinae taxa sampled in the present study with their museum accession numbers. The Bloemfontein National History Museum is abbreviated as NMB, the Transvaal Museum is TM, while Aaron Bauer's collection number is AMB and the Los Angeles County Museum, USA is LACM.

Taxon	Locality, country	Museum accession numbers
<i>Typhlosaurus caecus</i>	Hopefield, WC, SA	NMB R8612
<i>Typhlosaurus lomii</i>	Alexander bay, NC, SA	NMB R8620
<i>Typhlosaurus vermis</i>	Mc Dougall Bay, NC, SA	NMB R8591
<i>Acontophiops lineatus</i>	Cheerio Farm, MP, SA	NMB R8608
<i>Acontias litoralis</i>	Port Nolloth, NC, SA	NMB R8593
<i>Acontias lineatus grayi</i> 1	Elandsbay, NC, SA	NMB R8593
<i>Acontias lineatus grayi</i> 2	Elandsbay, NC, SA	NMB R8594
<i>Acontias lineatus lineatus</i> 1	Kenhart, NC, SA	NMB R7554
<i>Acontias lineatus lineatus</i> 2	Soutdoring bos, NC, SA	NMB R7555
<i>Acontias lineatus tristis</i> 1	Goegap, NC, SA	NMB R7551
<i>Acontias lineatus tristis</i> 2	Port Nolloth, NC, SA	NMB R7552
<i>Acontias meleagris meleagris</i> 1	Robben Island, WC, SA	NMB R8595
<i>Acontias meleagris meleagris</i> 2	Veldrift, WC, SA	NMB R8595
<i>Acontias meleagris orientalis</i> 1	Grahamstown, EC, SA	NMB R8601
<i>Acontias meleagris orientalis</i> 2	Grahamstown, EC, SA	NMB R8602
<i>Acontias m. o. lineicauda</i> 1	Port Alfred, EC, SA	NMB R8502
<i>Acontias m. o. lineicauda</i> 2	Port Alfred, EC, SA	NMB R8503
<i>Acontias m. o. lineicauda</i> 1	Port Elizabeth, EC, SA	NMB R8598
<i>Acontias m. o. lineicauda</i> 2	Port Elizabeth, EC, SA	NMB R8598
<i>Acontias percivalli tasmani</i> 1	Coega, EC, SA	NMB R8603
<i>Acontias percivalli tasmani</i> 2	Coega, EC, SA	NMB R8604
<i>Acontias gracilicauda gracilicauda</i> 1	Elandsnek farm, FS, SA	NMB R8605
<i>Acontias gracilicauda gracilicauda</i> 2	Elandsnek farm, FS, SA	NMB R8606
<i>Acontias percivalli percivalli</i> 1	Dodoma, TZ	TM 85192
<i>Acontias percivalli percivalli</i> 2	Dodoma, TZ	TM 85193
<i>Acontias percivalli occidentalis</i>	Pumonge Cave, ZM	AMB 6196
<i>Acontias breviceps</i>	Wakkerstroom, MP, SA	NMB R8611
<i>Acontias plumbeus</i>	Ndumo Reserve, KZN, SA, NMB	R8607
<i>Xanthusia vigilis</i>	Arizona, Pierce Ferry, USA	LACM 135220
<i>Lepidophyma flavimaculatum</i>	Escabal, Panama	LACM 128585
<i>Cordylus warreni depressus</i>	un-accessioned material	—
<i>Pseudocordylus m. microlepidotus</i>	un-accessioned material	—
<i>Trachylepis capensis</i>	Grahamstown, EC, SA	NMB R8609
<i>Trachylepis striata punctatissima</i>	Phuthaditjhaba, EC, SA	NMB R8610

Locality abbreviations: WC, Western Cape; EC, Eastern Cape; NC, Northern Cape; FS, Free State; MP, Mpumalanga; KZN, KwaZulu-Natal. All are provinces within South Africa.

Country abbreviations: SA, South Africa; ZM, Zimbabwe; TZ, Tanzania.

a freezer. Voucher specimens of ingroup taxa have been deposited in the Bloemfontein Natural History Museum collection, Free State, South Africa (Table 1).

DNA sequencing and phylogenetic analysis

Total genomic DNA was extracted using a Qiagen DNEasy kit, following the manufacturer's extraction protocol. Following extraction, DNA samples were stored in a fridge at -20°C until required for PCR. 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 (Cunningham *et al.* 1992), while the primers COIa (5'-AGT ATA AGC GTC TGG GTA GTC-3') and COIb (5'-CCT GCA GGA GGA GGA GAT CC-3') were used to amplify the COI gene (Palumbi *et al.* 1991), fragments to investigate the phylogenetic relationship among all the acontine taxa. In addition sequence data derived from two additional mtDNA gene loci (12S rRNA and *cyt b*) were also used in the analyses. For the 12S gene region the primers 12S were A (5'-CTG GGA TTA GAT ACC CCA CTA-3') and 12S B (5'-TGA GGA GGG TGA CGG GCG GT-3') (Kocher *et al.* 1989) while the primers for the *cyt b* region were WWF (5'-AAA YCA YCG TTG TWA TTC AAC TAC-3') and *cyt b* R2 (5'-GGG TGR AAK GGR ATT TTA TC-3') (Whiting *et al.* 2003). In addition, we used the *c-mos* primers, *c-mos* G77.1 (5'-TGG CYT GGT GCW GCA TTG ACT-3') and *c-mos* G79 (5'-CCT TTA AGG AGT TCA GGA GCAC-3'), from Saint *et al.* (1998) to amplify this gene region. While the primer pairs *RAG-IF* (5'-AGY CAR TAY CAY AAR ATG TA-3') and *RAG-IR* (5'-GCR TTN CCD ATR TCT CR TG-3') (Hoegg *et al.* 2004) were used to amplifying the *RAG-1* locus.

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 $10\times \text{Mg}^{2+}$ free buffer, 0.5 μL of a 10 mM dNTP solution and 0.5 μL of the primer sets at 10 mM, 0.1 unit of *Taq* polymerase and 1–3 μL of template DNA. The PCR temperature regime for all four mtDNA 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 . For both the nuclear markers (*c-mos* and *RAG-1*) PCR protocols were similar, except for the use of Hotmaster *Taq* following the protocol suggested by the manufacturer. These two genes were amplified at 52°C for *c-mos* and 53°C for *RAG-1*. PCR products were electrophoresed in a 1% regular agarose gel for 30 min at 70V containing ethidium bromide. Products were visualized under UV light. PCR products were purified using a PCR purification (QiaColumn kit) 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, PerkinElmer, 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 3730 XL automated machine.

Outgroup selection

Two recent studies (Whiting *et al.* 2003; Townsend *et al.* 2004) suggest that the subfamily Acontinae is sister to a clade

Table 2 A list of the Acontinae taxa and outgroups sampled and sequenced in the present study. Some of the mtDNA sequences were downloaded from GenBank from two earlier studies by Daniels *et al.* (2002, 2005). All of the nuclear sequence for both *c-mos* and *RAG-1* were generated during the present study. NS indicates loci not sequenced.

Taxon	GenBank accession numbers for each gene fragment					
	16SrRNA	12SrRNA	COI	cyt <i>b</i>	<i>c-mos</i>	<i>RAG-1</i>
<i>T. caecus</i>	DQ249033	DQ249019	NS	DQ249096	DQ249064	DQ249128
<i>T. lomii</i>	AY028894	DQ249020	DQ249078	DQ249097	DQ249065	DQ249129
<i>T. vermii</i>	AY028895	DQ249021	NS	DQ249098	DQ249066	DQ249130
<i>Acontophiops lineatus</i>	AY028872	DQ249005	AY028851	DQ249084	DQ249039	DQ249104
<i>A. litoralis</i>	AY028871	DQ249013	AY028868	DQ249092	DQ249047	NS
<i>A. l. grayi</i> 1	DQ249029	DQ249007	DQ249074	DQ249086	DQ249042	DQ249107
<i>A. l. grayi</i> 2	DQ249030	DQ249008	DQ249075	DQ249087	DQ249041	DQ249106
<i>A. l. lineatus</i> 1	AY028874	DQ249009	AY028852	DQ249088	DQ249044	DQ249109
<i>A. l. lineatus</i> 2	AY028875	DQ249010	AY028853	DQ249089	DQ249043	DQ249108
<i>A. l. tristis</i> 1	AY028876	DQ249011	AY028866	DQ249090	DQ249045	DQ249110
<i>A. l. tristis</i> 2	AY028877	DQ249012	AY028867	DQ249091	DQ249046	DQ249111
<i>A. m. meleagris</i> 1	AY683700	AY683646	AY683754	AY683825	DQ249056	DQ249120
<i>A. m. meleagris</i> 2	AY683783	AY683659	AY683735	AY683801	DQ249061	DQ249125
<i>A. m. orientalis</i> 1	AY683685	AY683664	AY683741	AY683816	DQ249051	DQ249114
<i>A. m. orientalis</i> 2	AY683684	AY683665	AY683742	AY683817	DQ249050	DQ249115
<i>A. m. o. lineicauda</i> 1 PE	AY683713	AY683680	AY683766	AY683793	DQ249059	DQ249123
<i>A. m. o. lineicauda</i> 2 PE	AY683714	AY683679	AY683767	AY683792	DQ249060	DQ249124
<i>A. m. o. lineicauda</i> 1 PA	AY683705	AY683650	AY683759	AY683809	DQ249058	DQ249122
<i>A. m. o. lineicauda</i> 2 PA	AY683706	AY683652	AY683760	AY683810	DQ249057	DQ249121
<i>A. p. tasmani</i> 1	AY683691	AY683669	AY683746	AY683821	DQ249055	DQ249116
<i>A. p. tasmani</i> 2	AY683692	AY683670	AY683745	AY683822	DQ249054	DQ249117
<i>A. g. gracilicauda</i> 1	AY683682	AY683643	AY683736	AY683790	DQ249049	DQ249113
<i>A. g. gracilicauda</i> 2	AY028870	DQ249014	AY028862	DQ249093	DQ249048	DQ249112
<i>A. p. percivali</i> 1	AY683695	DQ249015	AY683749	DQ249271	DQ249054	DQ249119
<i>A. p. percivali</i> 2	AY683696	DQ249016	AY683750	DQ249272	DQ249055	DQ249118
<i>A. p. occidentalis</i>	DQ249032	DQ249018	DQ249077	DQ249095	DQ249063	DQ249127
<i>A. breviceps</i>	DQ249031	DQ249017	DQ249076	DQ249094	DQ249062	DQ249126
<i>A. plumbeus</i>	DQ249028	DQ249006	DQ249073	DQ249085	DQ249040	DQ249105
<i>X. vigilis</i>	DQ249035	DQ249024	DQ249081	DQ249101	DQ249069	DQ249133
<i>L. flavimaculatum</i>	DQ249036	NS	NS	DQ249102	DQ249070	DQ249134
<i>C. w. depressus</i>	DQ249038	DQ249026	DQ249083	DQ249103	DQ249072	NS
<i>P. m. microlepidotus</i>	DQ249037	DQ249025	DQ249082	NS	DQ249071	NS
<i>T. capensis</i>	AY028888	DQ249022	DQ249079	DQ249099	DQ249067	DQ249131
<i>T. s. punctatissima</i>	DQ249034	DQ249023	DQ249080	DQ249100	DQ249068	DQ249132

Unless otherwise indicated, all taxa abbreviated with an *A.* are classified as belonging to the genus *Acontias*.

that contains all the remaining skink families and the two clades together as sister to the Cordylidae and the Xanthusiidae. Consequently, we used two nonacontine skink species (*Trachylepis capensis* and *Trachylepis striata punctatissima* — formerly *Mabuya*), and two cordylids (*Cordylus warreni depressus* and *Pseudocordylus microlepidotus microlepidotus*) and two night lizards (*Xanthusia vigilis* and *Lepidophyma flavimaculatum*) as outgroups.

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) from which a consensus sequence was created. The 16S rRNA and 12S 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. We excluded the highly variable loop regions from the analyses. The protein-coding mitochondrial COI and *cyt b* sequences as well as the nuclear protein-coding gene sequences for *c-mos* and *RAG-1* were aligned manually. No insertion or deletions were observed. The new data from the 16S rRNA, 12S rRNA *cyt b* and COI gene regions were deposited in GenBank (Table 2). In addition, sequences from the earlier study by Daniels *et al.* (2005) were included for 16S rRNA, 12S rRNA, COI and *cyt b* (Table 2). All the *c-mos* and *RAG-1* sequences were newly generated and deposited in GenBank (Table 2).

Maximum parsimony (MP), maximum likelihood (ML) and Bayesian approaches were used to estimate phylogenetic relationships among the subject taxa. MP and ML phylogenetic analyses were executed in PAUP*4 version beta 10

(Swofford 2002). 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 best fit substitution model was calculated using MODELTEST version 3.06 (Posada & Crandall 1998). The best-fit maximum likelihood score was chosen using Akaike's 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 & Anderson 2002; Nylander *et al.* 2004). For the ML analyses heuristic searches with TBR branch swapping and 100 random additions of taxa were also performed.

Uncorrected ('p') sequence divergence values were calculated between samples. Phylogenetic confidence in the nodes recovered from parsimony was estimated by parametric bootstrapping (Felsenstein 1985), analysing 1000 pseudo-replicates of data sets, while due to computational constraints only 100 pseudo-replicates were performed for ML. Bayesian inferences were used to investigate optimal tree space using the program MrBayes 3.0b4 (Ronquist & Huelsenbeck 2003). For each analysis, four Markov chains were run, with each chain starting from a random tree and 5 million generations generated. Sampling from the chain occurred every 5000th tree for each of the three partitions (nDNA, two loci; mtDNA, four loci, followed by the total evidence). In these combined analyses, loci were partitioned according to substitution models selected using MODELTEST, using unlinked parameters. A 50% majority rule consensus tree was generated from the trees retained (after the burn-in trees were discarded using likelihood plots), with posterior probabilities (*pP*) for each node estimated by the percentage of time the node was recovered. For the Bayesian analyses, data sets were run a minimum of four times to test that they converge on the same topology. Since we explored the compatibility of the four mitochondrial and two nuclear loci separately using MP, ML and a Bayesian framework and found topological congruence among these methods within these two data sets (results not shown), we present the topologies and analyses resulting from these two data sets plus the total evidence analyses. To test alternative phylogenetic hypothesis we performed the Shimodaira & Hasegawa (1999) test (comparing the unconstrained tree to the null hypothesis) ($-\ln L_0 - \ln L_1 = \Delta - \ln L$) as implemented in PAUP*4 version beta 10.

Results

Combined mitochondrial gene topology (16S rRNA, 12S rRNA, COI and cyt b)

All three tree-building methods recovered a highly congruent topology. For the MP analyses, a single topology was recovered, with a tree length of 2516 steps (CI = 0.56, RI = 0.76) derived from 714 parsimony-informative characters

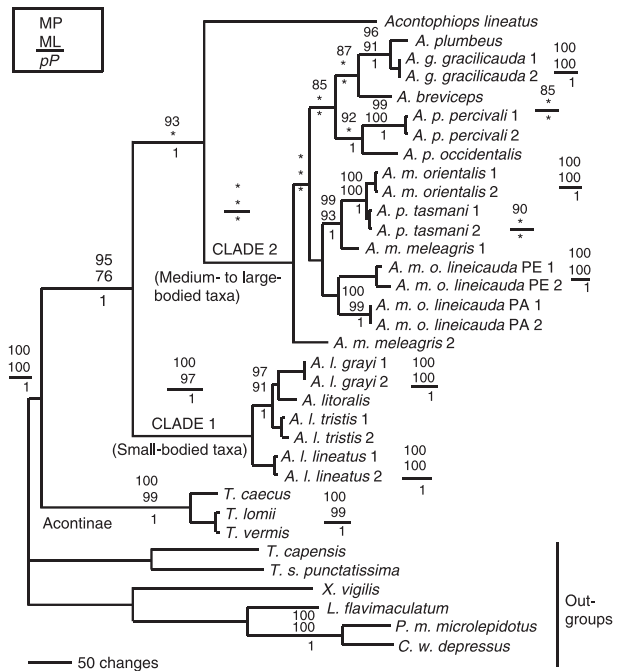


Fig. 1 Maximum parsimony (MP) phylogram (CI = 0.56, RI = 0.76) derived from the four combined mitochondrial (mtDNA) markers (16S rRNA, 12S rRNA, COI and *cyt b*). Values above the nodes for each clade represent the bootstrap values obtained for MP and maximum likelihood (ML), respectively, while the posterior probabilities (*pP*) obtained from the Bayesian inferences are shown below the nodes. Nodes marked with an asterisk indicate bootstrap values and posterior probabilities below 75% and 1, respectively.

from a total of 1960 characters (16S rRNA = 408b, 12S rRNA = 375 bp, COI = 610 and *cyt b* = 567; Fig. 1). The bootstrap topology recovered the monophyly of the Acontinae, demonstrating the monophyly and basal placement of *Typhlosaurus* (100% bootstrap support). In addition the small-bodied *Acontias* taxa (clade 1) was monophyletic. Here the sister relationship between *A. lineatus tristis*, *A. litoralis* and *A. lineatus grayi* (clade 1) was well supported (100% bootstrap support). A second group of *Acontias* taxa (comprising the medium- and large-bodied species — clade 2), forms a sister clade to the monotypic *Acontophiops lineatus*. Within clade 2 the sister relationships between the medium-bodied skinks *A. percivali percivali* and *A. p. occidentalis* is confirmed (with 92% bootstrap support), while *A. breviceps* was sister to a clade containing *A. g. gracilicauda* and *A. plumbeus* with 87% and 96% bootstrap support, respectively. The clade comprising these taxa has 88% bootstrap support. In addition, the sister relationships between *A. meleagris meleagris* (Robben Island), *A. percivali tasmani* and *A. m. orientalis* has 93% bootstrap support. The ML substitution model with the following parameters: base composition is A = 0.3277, C = 0.2775, G = 0.1500 and T = 0.2448, while the rate matrix was R(a)

[A-C] = R(c) [A-T] = R(d) [C-T] = R(f) [G-T] = 1, R(b) [A-G] = 3.7836, R(e) [C-T] = 4.5585, while the I = 0.4515, and Γ (gamma shape parameter) = 1.20 (-ln L = 14206.44; AIC = 28426.89). The bootstrap support for the same clades evident with the MP analyses was also evident with the ML analyses, confirming the monophyly of the Acontinae (76% bootstrap support), as well as the monophyly of *Typhlosaurus* (99% bootstrap support) and *Acontias* taxa in clade 1 (97% bootstrap support). Similarly, the posterior probability Bayesian topology recovered the same clades with good statistical support ($pP = 1$).

Combined nuclear sequence topology (c-mos and RAG-1)

All three tree-building methods (MP, ML and Bayesian inferences) recovered the same topology. For the MP analyses, six trees were recovered with CI = 0.86, RI = 0.90, tree length of 313 while the total number of parsimony-informative characters was 233 from a potential 1144 characters (400 bp and 744 bp for *c-mos* and *RAG-1*, respectively) (Fig. 2). The resulting topology recovered the monophyly of the Aconinae

(100% bootstrap support), as well as the monophyly of *Typhlosaurus* (100% support) and that of *Acontias*, albeit with low bootstrap support. *Acontophiops* was sister taxon to *Acontias*. Within *Acontias*, the small-bodied taxa (clade 1) again formed a well-supported monophyletic group (with 96% support). For the ML analyses the substitution model was TrN + I (-ln L = 3590.01; AIC = 7190.03) was selected using the AIC criteria with the following parameters. The base frequency was, A = 0.2702, C = 0.2260, G = 0.2194 and T = 0.2844, the rate matrix was R(a) [A-C] = R(c) [A-T] = R(d) [C-G] = R(f) [G-T] = 1, R(b) [A-G] = 4.84 and R(e) [C-T] = 4.03 with the proportion of invariable sites (I) = 0.51. The bootstrapped ML topology recovered the monophyly of the Acontinae (100% bootstrap support), monophyly of *Typhlosaurus* (with 92% bootstrap support), as well as the monophyly of the small-bodied *Acontias* taxa (clade 1) (94% bootstrap support). The Bayesian topology recovered the same clades with good statistical support, with both the monophyly of the subfamily, *Typhlosaurus* and *Acontias* taxa in clade 1 having a $pP = 1$.

Combined evidence topology

The MP analyses recovered a single tree with a tree length of 2574, CI = 0.55, RI = 0.72, based on the 947 parsimony-informative characters from the combined evidence data sets (Fig. 3). As with the separate analyses of the nuclear and the mitochondrial data, the monophyly of the Acontinae is well supported. Similarly, the monophyly of *Typhlosaurus* and the small-bodied *Acontias* taxa (clade 1) have 100% bootstrap support. The monotypic genus *Acontophiops lineatus* is sister taxon to a clade comprising all medium- to large-bodied taxa (clade 2). Within the medium- to large-bodied *Acontias* there is a clear differentiation between the medium- and large-bodied skinks, with the large-bodied taxa (clade 2) forming a well-supported clade (with 88% bootstrap support). The substitution model selected for the ML analyses was TrN + I + Γ , with base frequencies of A = 0.3108, C = 0.2651, G = 0.1678 and T = 0.2563, the rate matrix was R(a) [A-C] = R(c) [A-T] = R(d) [C-G] = R(f) [G-T] = 1, R(b) [A-G] = 3.9103, R(e) [C-T] = 4.4607, while the I = 0.3628, and $\Gamma = 0.5880$ (-ln L = 18275.61; AIC = 36565.23). The ML bootstrap topology supported the same major clades evident with the MP analyses. Similarly, the posterior probability Bayesian topology recovered the same clades with good statistical support ($pP = 1$). Constraining the two main clades observed within *Acontias* to be monophyletic recovers a topology that is statistically significantly worse than our best estimate (18 190.08–18 444.52 = 254.44; $P < 0.05$). We can thus reject the monophyly of *Acontias*, corroborating the genetic distinction of the two clades.

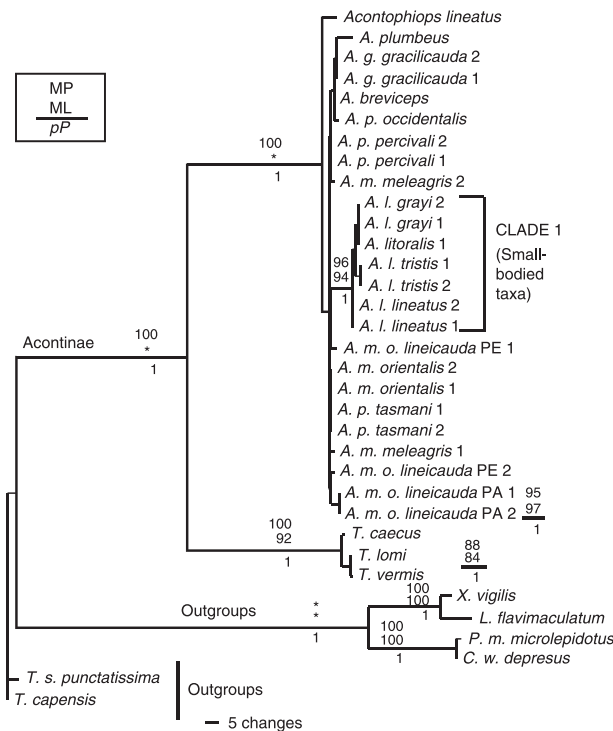


Fig. 2 Maximum parsimony (MP) phylogram (CI = 0.86, RI = 0.90) derived from the combined nuclear (nDNA) markers (*c-mos* and *RAG-1*). Values above the nodes for each clade represent the bootstrap values obtained for MP and maximum likelihood (ML), respectively, while the posterior probabilities (pP) obtained from the Bayesian inferences are shown below the nodes. Nodes marked with an asterisk indicate bootstrap values and posterior probabilities below 75% and 1, respectively.

Discussion

All three phylogenetic methods employed (MP, ML and Bayesian analyses) converged on a similar topology for both

values representing the greatest distance between specimens with *RAG-1* between the four genera was 2.4% between clade 1 and clade 2 (*Acontias*), 2.4% between *Acontias* and *Typhlosaurus* and 2%, 1.4%, 2.1% between *Acontophiops*, clade 1, *Acontias* and *Typhlosaurus*, respectively. The uncorrected distance value with *cyt b* between clade 1 and *Acontias* was 11%, while between clade 1 and *Typhlosaurus* it was 14%, and between *Acontias* and *Typhlosaurus* the divergence value was 13%. These results suggest that taxa within clade 1 have divergence values comparable to those observed between genera. These *cyt b* divergence values are comparable to previously estimated mean genetic distances (12–13.6%) for congeneric reptile taxa previously estimated (Johns & Avise 1998; Harris 2002). In addition, taxa in clade 1 are distributed only within two southern Africa biomes, while taxa in clade 2 have a broader geographical distribution. Collectively, the large suite of independent characters that distinguish these two *Acontias* clades suggests long-term historical separation of these two evolutionary lineages. Since *Acontias sensu stricto* is comprised of two monophyletic groups, we formally partition the genus into two genera. For the medium- to large-bodied taxa (in clade 2 — comprising *A. g. gracilicauda*, *A. breviceps*, *A. p. occidentalis*, *A. p. percivali*, *A. m. meleagris*, *A. m. orientalis*, *A. p. tasmani* the morph *lineicauda* and *A. plumbeus*) the generic name *Acontias* is retained, since the taxa in this clade are taxonomically the first described, thus they have precedence over the new genus. We assign the small-bodied taxa (in clade 1 — comprising *A. litoralis*, *A. lineatus lineatus*, *A. lineatus tristis* and *A. lineatus grayi*) to a new genus *Microacontias* gen. nov., the description of which follows below.

Microacontias gen. nov.

Type species. *Acontias lineatus lineatus* (Peters, 1879)

Content. *Microacontias lineatus lineatus* (Peters, 1879), *M. lineatus grayi* (Boulenger, 1887), *M. lineatus tristis* (Werner, 1911) and *M. litoralis* (Broadley & Greer 1969).

Characterization and diagnosis. Small slender bodied, with a snout vent length (SVL) ranging from 119 mm to 148 mm, tail flattened below, transparent lower eyelid, the enlarged flat rostrum, four to five upper labials, midbody scale rows 12–14. Restricted to the Succulent and Nama Karoo biome along the South African west coast.

Etymology. The name *Microacontias* gen. nov. (micro = small; *aconτίας* = serpentine form) reflects the small-bodied nature of these fossorial skink taxa, relative to the other aconine taxa, while ‘*aconτίας*’ is retained to reflect its superficial serpentine resemblance.

Within *Microacontias* gen. nov. the systematic affinities should be explored further, particularly since *M. litoralis* is

nested within the *M. lineatus lineatus* group, suggesting that this taxon is either a subspecies in this group, or that each of the subspecies within the *M. l. lineatus* group be considered independent operational taxonomic units and elevated to full species status. A large-scale systematic endeavour is planned to explore systematic relationships among taxa within this genus.

Genus: Acontias

Type species. *Acontias meleagris* (Linnaeus, 1758)

Content. *Acontias meleagris* (Linnaeus, 1758), *A. plumbeus* (Bianconi, 1849), *A. percivali percivali* (Loveridge, 1923), *A. breviceps* (Essex, 1925), *A. gracilicauda* (Essex, 1925), *A. percivali occidentalis* (FitzSimons, 1935), *A. p. tasmani* (Hewitt, 1937), *A. m. orientalis* (Hewitt, 1937), *A. g. namaquensis* (Hewitt, 1938) and *A. poecilus* (Bourquin & Lambiris, 1996).

Characterization and diagnosis. Morphologically, these taxa have SVLs that range from 225 mm to 490 mm, while the midbody scale rows range from 14 to 20 within this group (Broadley & Greer 1969). Biogeographically this group is distributed further along the west and south coasts of southern African eastwards into the interior of the subcontinent that includes Botswana, Namibia and Zimbabwe extending into south-eastern Kenya. Within this group, systematic affinities, particularly in the highly polymorphic *Acontias meleagris* complex (comprising *A. m. meleagris*, *A. m. orientalis*, the morph *lineicauda* and *A. p. tasmani*), warrant additional study (Daniels *et al.* 2005). A study in currently in progress that will attempt to delineate species boundaries within this complex.

Convergence in apparent diagnostic features appears widespread among fossorial taxa. For example, two independent studies performed by Whiting *et al.* (2004) and Schmitz *et al.* (2005) suggest that the Malagasy fossorial skink genus *Amphiglossus* as currently defined is not monophyletic and is comprised of two genetically highly distinct groups. These results suggest that morphological characters currently used in the taxonomy of fossorial skinks are homoplastic and warrant closer scrutiny. The apparent lack of well-defined synapomorphies at least for some genera is clearly an obstacle in determining the diversity of fossorial groups. The description of this new genus within *Acontias* suggests that a number of previously defined generic groupings in other skinks may indeed be artificial units, and that a number of the southern African skink genera may contain considerable taxonomic diversity obscured by symplesiomorphic morphological features. From an evolutionary perspective, the morphological differences between *Acontias* and *Microacontias* gen. nov. pose some interesting questions. We hypothesize that it is likely that the differences in body size have led to the development of reproductive differences between these two ecomorphological groups that are likely

enforced by resource partitioning among sympatric taxa. We recommend that where taxonomically ill-defined paraphyletic groups have been recorded with mtDNA sequences, they should be confirmed with the use of nDNA sequences; and where congruent, the appropriate taxonomic changes made to reflect current trends in phylogenetic hypotheses. Such studies are likely to uncover a wealth of new genera and taxa that have previously been obscured by convergent characters, particularly among fossorial groups. Considering that most fossorial taxa have limited vagility, and a large number are point endemics, the effective conservation of this faunal group and their vulnerability to extinction underscore the need for a sound taxonomy that accurately reflects diversity and evolutionary history.

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