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Opening the black box: phylogenetics and morphological evolution of the Malagasy fossorial lizards of the subfamily "Scincinae"

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Abstract

The island of Madagascar harbors a highly endemic vertebrate fauna including a high diversity of lizards of the subfamily "Scincinae," with about 57 species in eight genera. Since limb reduction seems to have been a common phenomenon during the evolution of Malagasy "scincines," diagnosing evolutionary relationships based on morphology has been difficult. Phylogenetic analyses of multiple mitochondrial DNA sequences including the entire ND1, $tRNA^{LEU}$, $tRNA^{ILE}$, $tRNA^{GLN}$ genes, and fragments of the 12S and 16S rRNA and $tRNA^{MET}$ genes were conducted to test the monophyly of the largest genus *Amphiglossus*, and to evaluate the various formal and informal species groupings previously proposed for this species-rich group. A further objective was to determine the phylogenetic placements of the several greatly limb-reduced and limbless Malagasy "scincines" and ascertain whether any of these are derived from within the morphologically plesiomorphic *Amphiglossus*. As limb reduction in skinks is mostly associated with body elongation via an increase in the number of presacral vertebrae, we evaluate the pattern of evolution of the numbers of presacral vertebrae in the context of our phylogeny. We demonstrate that *Amphiglossus* as currently diagnosed is non-monophyletic, and the species fall into two major groups. One of these groups is a clade that contains the included species of the subgenus *Amphiglossus* (*Madascincus*) among other species and is a member of a larger clade containing *Paracontias* and *Pseudoacontias.* In the second group, the nominate subgenus *Amphiglossus* (*Amphiglossus*) forms several subclades within a larger clade that also contains *Androngo crenni* and *Pygomeles braconnieri*, and is sister to *Voeltzkowia*. All analyses provide strong support for the monophyly of *Paracontias* and *Voeltzkowia*. Based on the preferred phylogenetic hypothesis and weighted squared-change parsimony we show that the ancestor of the Malagasy clade was already elongated and had a moderately high number of presacral vertebrae (46–48), which is hypothesized to be the ancestral condition for the whole Malagasy "scincine" clade. We further demonstrate that both multiple increases and reductions of presacral vertebrae evolved in many clades of Malagasy "scincines" and that the use of presacral vertebrae as a major character to diagnose supraspecific units is dubious. Based on our results and published morphological evidence we consider *Scelotes waterloti* Angel, 1930 to be a junior synonym of *Amphiglossus reticulatus* (Kaudern, 1922).

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1. Introduction

Within Squamata, scincid lizards (skinks) are a particularly diverse group with four recognized subfamilies: the Acontinae, Feylininae, Lygosominae, and paraphyletic "Scincinae." The family contains ~1260 species, making it the most speciose lizard family, and among squamates is only outnumbered by the snake family "Colubridae" (>1800 species) (Pough et al., 2004). However, scincid diversity extends beyond pure numbers of species. The family is distributed worldwide and displays a remarkable array of morphological variation including the presumably repeated, convergent evolution of body elongation and extreme limb reduction. The lack of phylogenies for many skink clades has prohibited researchers from examining these phenomena in a phylogenetic context. This is particularly true for the diverse fauna of skinks from Madagascar. Due to their secretive lives and the scarcity of available material in collections, very little is known about the diversity, geographic distribution, and phylogenetic affinities of these lizards. This situation prompted Greer (1970), in his analysis of the subfamily "Scincinae" (="scincines"), to describe the island as a "…'*black box*' *of our analysis of scincine evolution*…." Until relatively recently, only 44 species from seven different "scincine" genera were known from Madagascar (Glaw and Vences, 1994), but the number of described Malagasy "scincines" has risen substantially in the last 10 years. Currently, there are no less than 57 species from eight genera known from Madagascar (e.g., Andreone and Greer, 2002; Nussbaum and Raxworthy, 1995; Sakata and Hikida, 2003a,b; see Appendix).

Because of their fossorial/semi-fossorial nature (many species burrow in leaf litter, sand, soil, or rotten wood), several taxa in the different "scincine" genera have partially or completely lost their limbs (Andreone and Greer, 2002; Nussbaum and Raxworthy, 1995). As limb reduction has presumably evolved multiple times within these skinks, diagnosing evolutionary relationships based on morphology has been difficult. Coupled with the scarcity of museum specimens, this widespread morphological convergence has hindered phylogenetic studies of these lizards. Greer (1970) hypothesized that the Malagasy "scincines" are part of a more inclusive "scincine" group inhabiting sub-Saharan Africa, the Seychelles, and Mauritius. Extensive taxonomic revisions of Malagasy "scincines" were published by Brygoo between 1979 and 1987 (Brygoo, 1979, 1980a,b,c,d, 1981a,b,c, 1983, 1984a,b,c,d,e, 1985, 1987). He provided a re-definition of the largest genus *Amphiglossus* and distinguished two subgenera (*Amphiglossus* and *Madascincus*) based mainly on differences in body size and the number of presacral vertebrae (Brygoo, 1980a,c, 1981a, 1984a,b). Furthermore, he erected the new genus *Androngo* for all species with more than 48 presacral vertebrae (Brygoo, 1981b, 1987). These groupings included several species formerly considered to belong to the African genus *Scelotes*. All Malagasy "*Scelotes*" are now placed in either *Androngo* or *Amphiglossus*, thus restricting *Scelotes* to South and East Africa (Brygoo, 1981a). Of the 37 currently recognized species of *Amphiglossus*, all (except *A. stylus*) lack a reduction of the head scales, generally exhibit slight to moderate limb reduction and body elongation, and all retain an external ear opening (Andreone and Greer, 2002; Brygoo, 1981a, 1985; Glaw and Vences, 1994) Thus, *Amphiglossus* consists of the generally most morphologically plesiomorphic "scincines" of Madagascar and the associated islands to the northwest. The lack of diagnostic derived characters led Andreone and Greer (2002) to speculate that *Amphiglossus* may not be monophyletic.

While the majority of the *Amphiglossus* are either fossorial or leaf litter dwellers, there are three large species (*A. astrolabi*, *A. reticulatus*, and *A. waterloti*) that are primarily either aquatic or semi-aquatic. These were attributed to the nominate subgenus (Brygoo, 1980a,c). The three species *A. melanopleura*, *A. ankodabensis*, and *A. mouroundavae* were placed into the newly erected subgenus *Madascincus* (Brygoo, 1981a, 1984b). However, the majority of species have not been classified into formal subgeneric groupings (Andreone and Greer, 2002; Brygoo, 1988).

Because of the potential difficulty in using morphological characters to determine the phylogenetic affinities of limb-reduced and/or limbless taxa (e.g., Estes et al., 1988; Greer and Cogger, 1985; Lee, 1998) we collected mitochondrial DNA data in order to infer the phylogenetic relationships among the Malagasy "scincines". The main focus of our study is to test the monophyly of *Amphiglossus*, as well as evaluate the various formal and informal species groupings previously proposed for this species-rich group. Our sampling also allows us to determine the phylogenetic placements of the several greatly limb-reduced and limbless Malagasy "scincines" and determine whether any of these are derived from within *Amphiglossus*. And finally, limb reduction in skinks is generally associated with body elongation via increasing numbers of presacral vertebrae. For the Malagasy "scincines," there exists an extensive comparative database for this character. Thus, in the context of our phylogeny, we evaluate the evolution of presacral vertebrae number among the Malagasy "scincines".

Table 1
List of voucher specimens for each species included in the present study, with their respective localities, collection numbers, and accession numbers (12S, 16S, ND1, and associated tRNAs)

Species	Locality	Collection number	Accession number
1. *Amphiglossus astrolabi*	Manantenina Village, Madagascar	UMMZ 208802	AY315474/AY315523/AY315569
2. *Amphiglossus igneocaudatus*	Ibity, Madagascar	UMMZ 217449	AY315475/AY315524/AY315570
3. *Amphiglossus igneocaudatus*	Ibity, Madagascar	ZSM 518/2001	AY315476/AY315525/AY315571
4. *Amphiglossus igneocaudatus*	Itremo, Madagascar	ZSM 521/2001	AY315477/AY315526/AY315572
5. *Amphiglossus intermedius*	Ankarana Reserve, near Ambilobe, Madagascar	UMMZ 201587	AY315478/AY315527/AY315573
6. *Amphiglossus intermedius*	Ampijoroa (Ankarafantsika), Madagascar	ZSM 522/2001	AY315479/AY315528/AY315574
7. *Amphiglossus macrocercus*	Mantady Park, near Moramanga, Madagascar	UMMZ 195924	AY315480/AY315529/AY315575
8. *Amphiglossus macrocercus*	Ankaratra, above Nosiarivo, Madagascar	ZSM 382/2000	AY315484/AY315533/AY315579
9. *Amphiglossus macrocercus*	Andringitra, Andohariana Plateau, Madagascar	ZSM 530/2001	AY315485/AY315534/AY315580
10. *Amphiglossus* cf. *macrocercus*	Ambohimanarivo, Madagascar	ZSM 198/2002	AY315492/AY315541/AY315587
11. *Amphiglossus melanopleura*	Montagne d'Ambre, Antomboka River, Madagascar	UMMZ 208656	AY315481/AY315530/AY315576
12. *Amphiglossus melanopleura*	Andasibe, Madagascar	ZSM 525/2001	AY315482/AY315531/AY315577
13. *Amphiglossus melanurus*	Montagne d'Ambre, Petit Lac, Madagascar	UMMZ 201590	AY315483/AY315532/AY315578
14. *Amphiglossus melanurus*	Maroantsetra, Madagascar	ZSM 245/2002	AY315502/AY315551/AY315597
15. *Amphiglossus mouroundavae*	Antsahamanara, Tsaratanana Massif, Madagascar	MRSN R1866	AY315487/AY315536/AY315582
16. *Amphiglossus mouroundavae*	Montagne d'Ambre, Antomboka River, Madagascar	UMMZ 201592	AY315486/AY315535/AY315581
17. *Amphiglossus nanus*	Andasibe, Lac Vert, Madagascar	ZSM 199/2002	AY315493/AY315542/AY315588
18. *Amphiglossus ornaticeps*	Manantantely Forest, near Tolanaro, Madagascar	UMMZ 196048	AY315488/AY315537/AY315583
19. *Amphiglossus punctatus*	Marojejy Reserve, Manantenina River, Madagascar	UMMZ 208785	AY315489/AY315538/AY315584
20. *Amphiglossus reticulatus*	Berara Forest, Mahajanga Faritany, Madagascar	MRSN R1723	AY315490/AY315539/AY315585
21. *Amphiglossus* sp. (cf. *melanurus*)	Torotorofotsy, Madagascar	UADBA-MV 2001.1313	AY315494/AY315543/AY315589
22. *Amphiglossus* sp.	Ampijoroa (Ankarafantsika), Madagascar	uncatalogued	AY315503/AY315552/AY315598
23. *Amphiglossus splendidus*	Summit of Ambatorongorongo, Madagascar	UMMZ 208789	AY315495/AY315544/AY315590
24. *Amphiglossus stumpffi*	Berara Forest, Mahajanga Faritany, Madagascar	MRSN R1718	AY315497/AY315546/AY315592
25. *Amphiglossus stumpffi*	Montagne d'Ambre, Antomboka River, Madagascar	UMMZ 201595	AY315496/AY315545/AY315591
26. *Amphiglossus tanysoma*	Berara Forest, Mahajanga Faritany, Madagascar	MRSN R1729	AY315498/AY315547/AY315593
27. *Amphiglossus tanysoma*	Antsirasira, Madagascar	MRSN R1865	AY315491/AY315540/AY315586
28. *Amphiglossus tsaratananensis*	Tsaratanana, Matsabory, Madagascar	UMMZ 208798	AY315499/AY315548/AY315594
29. *Amphiglossus waterloti*	Manongarivo Reserve, Ambalafary, Madagascar	UMMZ 201597	AY315500/AY315549/AY315595
30. *Amphiglossus waterloti*	Ampijoroa (Ankarafantsika), Madagascar	ZSM 528/2001	AY315501/AY315550/AY315596
31. *Androngo crenni*	Andasibe, Madagascar	ZSM 288/2002	AY315504/AY315553/AY315599
32. *Paracontias brocchii*	Montagne d'Ambre, Antomboka River, Madagascar	UMMZ 209153	AY315507/AY315556/AY315602
33. *Paracontias hildebrandti*	Montagne d'Ambre, Antomboka River, Madagascar	UMMZ 209166	AY315508/AY315557/AY315603
34. *Paracontias holomelas*	Marojejy Reserve, Manantenina River, Madagascar	UMMZ 201644	AY315509/AY315558/AY315604
35. *Paracontias* sp. nov.	Antsahamanara, Tsaratanana Massif, Madagascar	FAZC, uncatalogued	AY315510/AY315559/AY315605
36. *Proscelotes eggeli*	Lushoto Distr., Mazumbai Forest Reserve, Tanzania	CAS 168959	AY155368/AY155367/AY315608
37. *Proscelotes eggeli*	Korogwe Dist., Korogwe Ambangulu Tea Estate, Tanzania	FMNH 250585	AY315512/AY315561/AY315607
38. *Pseudoacontias menamainty*	Berara Forest, Madagascar	MRSN R1826	AY315511/AY315560/AY315606
39. *Pygomeles braconnieri*	Betioky, Madagascar	UMMZ 229882	AY315513/AY315562/AY315609

40. *Pygomeles braconnieri*	Anakao or Arboretum, near Tulear, Madagascar	ZSM 603/2000	AY315514/AF215235/AY315610
41. *Voeltzkowia fierinensis*	Arboretum, near Tulear, Madagascar	UADBA-MV 2000.569	AY315516/AY315563/AY315612
42. *Voeltzkowia* cf. *fierinensis*	Anakao, Madagascar	ZSM 606-610/2000	AY315519/AY315565/AY315615
43. *Voeltzkowia lineata*	Beraketa, Madagascar	UMMZ 197125	AY315517/AY315564/AY315613
44. *Voeltzkowia lineata*	Anakao, Madagascar	ZSM 611/2000	AY315518/AF215238/AY315614
45. *Cordylus* sp.	Africa	No voucher	AY315471/AY315520/AY315566
46. *Zonosaurus* sp.	Pet trade	TNHC 55947	AY315472/AY315521/AY315567
47. "*Eumeces*" *egregius*	USA	GenBank	AB016606/AB016606/AB016606
48. "*Eumeces*" *fasciatus*	Missouri, Camden Co., Sunrise Beach, USA	SDSU 3836	AY315505/AY315554/AY315600
49. *Eumeces schneiderii*	Pet trade	TNHC 55948	AY315506/AY315555/AY315601
50. *Scincus scincus*	Pet trade	TNHC 55667	AY315515/AY712942/AY315611

Acronyms: CAS for California Academy of Sciences, San Francisco, USA; DMH for David M. Hillis, University of Texas-Austin; FAZC for Franco Andreone Zoological Collection (specimens to be deposited in MRSN); MRSN for Museo Regionale di Scienze Naturali, Torino, Italy; SDSU for San Diego State Univesity; TNHC for Texas Natural History Collection, Austin, Texas, USA; TWR for Tod W. Reeder field number, San Diego, USA; UADBA-MV for Universite d'Antananarivo, Departement de Biologie Animale, Madagascar (Miguel Vences, uncatalogued); UMMZ for University of Michigan, Museum of Zoology, USA; ZFMK for Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany; and ZSM for Zoologische Staatssammlung, München, Germany.

2. Materials and methods

2.1. Choice of terminal taxa

In all, 29 Malagasy "scincine" species were included in this study (with several species represented by multiple individuals; Table 1): *Amphiglossus* (19 species), *Androngo crenni*, *Paracontias* (4), *Pseudoacontias menamainty*, *Pygomeles braconnieri*, and *Voeltzkowia* (3). Additional non-Malagasy "scincines" (*Eumeces* sensu lato [Brandley et al., 2005; Schmitz et al., 2004], *Proscelotes*, *Scincus*) as well as one acontine (*Acontias meleagris*) were sampled as outgroups. Because of the uncertain higher-level relationships among skinks and the fact that the Malagasy "scincines" may not represent a clade, the overall skink phylogeny was simultaneously rooted with one cordylid and one gerrhosaurid (*Cordylus* and *Zonosaurus*, respectively). The Cordylidae and the Gerrhosauridae are generally thought to be closely related to the Scincidae, with these three families forming the Scincoidea (Estes et al., 1988; Lee, 1998; Townsend et al., 2004). General locality and voucher information is provided in Table 1.

2.2. DNA amplification, sequencing, and alignment

DNA was extracted from tissue using QiaAmp kits (Qiagen) or a standard phenol/chloroform/proteinase-K protocol (Hillis et al., 1996). Multiple mitochondrial DNA fragments were amplified including the entire ND1, $tRNA^{LEU}$, $tRNA^{ILE}$, $tRNA^{GLN}$ genes, and partial fragments of the 12S and 16S rRNA and $tRNA^{MET}$ genes. PCR and sequencing primers are given in Table 2. Sufficient PCR product was generated after 33–40 cycles (12S and 16S fragments: 94 °C for 45–60 s, 50–55 °C for 30–45 s, and 72 °C for 30–90 s; ND1 fragment: 94 °C for 60 s, 50–58 °C for 30 s, and 72 °C for 60–90 s). PCR products were purified using Qiaquick purification kits (Qiagen) or PEG/NaCl precipitation. Purified PCR templates were sequenced using dye-labeled dideoxy terminator cycle sequencing on an ABI 377 automated DNA sequencer.

The ND1 protein-coding sequences were aligned by eye. The 12S, 16S, and tRNA data were aligned with reference to published secondary structure maps (12S: Titus and Frost, 1996; 16S: Gutell and Fox, 1998; and tRNAs: Kumazawa and Nishida, 1993). To assess positional homology in the 12S and 16S loops, each data set was aligned under varying pairwise and multiple gap costs (6, 9, and 12) using ClustalX (Thompson et al., 1997). Nucleotide positions that changed under one or more different gap costs were considered ambiguously aligned and were excluded from the phylogenetic analyses (Gatesy et al., 1993; Milinkovitch and Lyons-Weiler, 1998). In some regions of the 12S and 16S data, the ability to align the data for the skinks was improved if the

Table 2
Primer used in the present study

Primer name	Sequence (5′ → 3′)	Position[a]	Source
tPhe	AAA GCA CRG CAC TGA AGA TGC	44	Wiens and Reeder (1997)
12a	AAA CTG GGA TTA GAT ACC CCA CTA T	526	Kocher et al. (1989)
12g	TAT CGA TTA TAG GAC AGG CTC CTC TA	630	Leaché and Reeder (2002)
12e	GTR CGC TTA CCM TGT TAC GAC T	984	Wiens and Reeder (1997)
16aR2	CCC GMC TGT TTA CCA AAA ACA	1928	Reeder (2003)
16d	CTC CGG TCT GAA CTC AGA TCA CGT AG	2456	Reeder (1995)
16dR	CTA CGT GAT CTG AGT TCA GAC CGG AG	2481	Leaché and Reeder (2002)
ND1-INTF	CTA GCW GAA ACM AAY CGA GCC CC	3309	This study
ND1-INTF2	AAY CGV GCV CCW TTY GAC CTW ACA GA	3323	This study
ND1-INTR2	CRA AKG GGC CDG CTG CRT AYT CTA C	3356	This study
ND1-INTR	TAT TCT GCT AGG AAG AAW AGG GCG	3379	This study
TMet	TCG GGG TAT GGG CCC RAR AGC TT	3836	Leaché and Reeder (2002)

[a] Position of the terminal 3′ base of the "*Eumeces*" *egregius* mt genome (GenBank Accession No. NC_000888; Kumazawa and Nishida, 1999).

sequences for *Cordylus* and *Zonosaurus* were removed (their corresponding data replaced with "?" in these regions). Because our explicit goal is to test the relationships of *Amphiglossus* and its relatives, and not the monophyly of Scincidae, we feel the exclusion of these two taxa is justified. All DNA sequences have been deposited in GenBank (Table 1).

2.3. Phylogenetic analyses

Phylogenetic analyses were conducted using maximum parsimony (MP), maximum likelihood (ML), and partitioned Bayesian methods. MP and ML analyses were implemented in PAUP* 4.0b10 (Swofford, 2002). The MP heuristic search consisted of 1000 random addition sequence replicates, TBR branch swapping, and gaps coded as missing data. The ML phylogeny was estimated following a successive approach similar to that described by Swofford et al. (1996) and Wilgenbusch and de Queiroz (2000), with Modeltest 3.0 (Posada and Crandall, 1998) being used to test alternative models of sequence evolution. An initial ML tree was constructed using the JC model (Jukes and Cantor, 1969; as-is stepwise addition, TBR branch swapping). The best model (and model parameters) estimated by Modeltest from this initial tree were used in a subsequent ML heuristic tree search (20 random addition sequence replicates, TBR branch swapping). If the resulting ML tree differed from the initial starting tree, then all models were retested on the new tree, followed by a new ML tree search. This process was iterated until the $-\ln L$ stabilized.

All partitioned Bayesian analyses were implemented with MrBayes 3b4 (Huelsenbeck and Ronquist, 2001). Because different genes and gene regions may be under very different biochemical constraints, they may also evolve under very different models of evolution. It has been demonstrated previously that applying different models to different subsets of the data (i.e., partitioned or mixed-model analyses) may yield better estimates of phylogeny (as measured by $-\ln L$) and, in some cases, improved estimates of posterior probabilities (Brandley et al., 2005; Nylander et al., 2004). Thus, we took advantage of the ability of MrBayes 3b4 to perform partitioned analyses.

Because numerous partitioning strategies are possible, we employed the method of Brandley et al. (2005) and used the Bayes factor to select among a priori selected partitioning strategies. Our goal was to choose a partitioning strategy that modeled the data well, but did not include extraneous partitions. We selected six partitioning strategies ranging from six total partitions to no partitions (i.e., a traditional, single-model analysis) (Table 3). All partition strategies are denoted with a capital P and a numerical subscript identifying the number of data partitions (e.g., P_1, P_6, etc.). Additional subscript letters identify multiple partitioning strategies that have the same number of data partitions but partition the data differently (e.g., P_{4A}, P_{4B}, etc.). We then used the Bayes factor to compare the results of the most-partitioned analysis to the alternative strategies with fewer partitions. If a strategy using fewer partitions was not strongly different from the most partitioned, then this strategy was chosen as the best partitioning scheme (i.e., the one that best modeled the data, but with the fewest partitions). Bayes factors were estimated by calculating the difference of the ln-transformed harmonic means of the posterior likelihoods between the two analyses being tested (Newton and Raftery, 1994). Harmonic means were estimated using the *sump* command in MrBayes. We used a 2ln Bayes factor >10 as the criterion

Table 3
Identification of partitioning strategies used in the partitioned Bayesian analyses

ID	Partitioning strategy
P_6	ND1 by codon; separate 12S, 16S, and tRNAs
P_{4A}	ND1 by codon; combined 12S, 16S, and tRNAs
P_{4B}	ND1, 12S, 16S, and tRNAs
P_2	ND1, combined 12S, 16S, and tRNAs
P_1	All data combined

for strong support (Brandley et al., 2005; Huelsenbeck and Imennov, 2002; Kass and Raftery, 1995).

Models for each partition were determined using the likelihood-ratio test implemented by MrModeltest (Nylander, 2002). All partitioned Bayesian analyses consisted of 2×10^7 generations (started on random trees) and four incrementally heated Markov chains (using default heating values), sampling the Markov chains at intervals of 1000 generations. The first 4×10^6 generations were discarded as "burn-in" and we confirmed stationarity by tracking the posterior probabilities of individual clades through time using the *cump* and *slide* command in Converge v0.1 (Warren et al., 2003). Stationarity was assumed when the cumulative posterior probabilities of all clades stabilized. To ensure the Bayesian analyses were not trapped on local optima, three separate analyses were performed (per partitioning strategy), mean $-\ln L$ scores were compared for each of the three runs, and posterior probability estimates for each clade were compared between the three analyses using scatterplots created by the *compare2trees* command in Converge. If apparent convergence on the same optimum was determined for all three analyses, the postburn-in trees for the three analyses were combined.

The percentage of samples (pooled for a given data set) recovering any particular clade represents that clade's posterior probability (Huelsenbeck and Ronquist, 2001; Huelsenbeck et al., 2001). Unlike non-parametric bootstrap proportions which are known to be conservative estimates of clade confidence (Hillis and Bull, 1993), recent simulation studies (e.g., Alfaro et al., 2003; Erixon et al., 2003; Wilcox et al., 2002) have demonstrated that Bayesian posterior probabilities are less biased estimators of confidence and thus generally represent much closer estimates of true clade probabilities (referred to as "*Pp*" throughout). Also, whereas the Bayesian approach may be more sensitive to signal in the sequence data (i.e., provide higher confidence for short internodes; Alfaro et al., 2003), there is also an increased chance of the Bayesian method assigning higher confidence to incorrectly inferred short internodes because of the stochastic nature of the underlying model of evolution (Alfaro et al., 2003; Erixon et al., 2003). Given this, clades with $Pp \geqslant 0.95$ were generally considered strongly (significantly) supported, but with the caveat that relatively high posterior probabilities for short internodes (particularly those that might receive low bootstrap values) may be overestimates of confidence.

Nodal support for the MP analyses was inferred using the non-parametric bootstrap (5000 pseudoreplicates, 100 random addition sequences/pseudoreplicate, and TBR branch swapping). Clades with bootstrap values (referred to as "*BS*" throughout) of $\geqslant 70\%$ were considered strongly supported (Hillis and Bull, 1993).

Table 4
Number of presacral vertebrae (PSV) for the Malagasy "scincines" and *Proscelotes eggeli* (sub-Saharan "scincine")

Taxon	PSV No.[a]	Reference
Amphiglossus astrolabi	38; 37–38	Brygoo (1980a)
Amphiglossus igneocaudatus	37; 35–39	Brygoo (1984d)
Amphiglossus intermedius	38; 37–40	Brygoo (1984d)
Amphiglossus macrocercus	40; 39–43	Brygoo (1984a)
Amphiglossus melanopleura	30; 29–31	Brygoo (1981a, 1984a)
Amphiglossus melanurus	43; 35–45	Brygoo (1984a)
Amphiglossus mouroundavae	30; 29–30	Brygoo (1984b)
Amphiglossus nanus	33; 31–34	Andreone and Greer (2002)
Amphiglossus ornaticeps	42; 42–45	Brygoo (1984e)
Amphiglossus punctatus	32[b]; 30–33	Raxworthy and Nussbaum (1993)
Amphiglossus reticulatus	37	Brygoo (1980a)
Amphiglossus splendidus	36; 33–36	Brygoo (1981a, 1985)
Amphiglossus stumpffi	41; 39–42	Brygoo (1984d)
Amphiglossus tanysoma	52; 52–53	Andreone and Greer (2002)
Amphiglossus tsaratananensis	36	Brygoo (1981b)
Amphiglossus waterloti	37; 37–38	Brygoo (1980a),Raxworthy and Nussbaum (1993)
Androngo crenni	56; 54–57	Brygoo (1981a)
Paracontias brocchii	63; 63–64	Brygoo (1980b)
Paracontias hildebrandti	51; 50–55	Andreone and Greer (2002), Brygoo (1980b)
Paracontias holomelas	57; 57–58	Brygoo (1980b)
Proscelotes eggeli	43; 41–44	Allen Greer (unpublished data)
Pseudoacontias menamainty	67	Andreone and Greer (2002)
Pygomeles braconnieri	62; 57–65	Brygoo (1984c)
Voeltzkowia fierinensis	47; 46–50	Brygoo (1981c)
Voeltzkowia lineata	50; 48–54	Brygoo (1981c)

[a] Modal number of presacral vertebrae (except where noted), followed by range (when present).
[b] Mean number of presacral vertebrae.

2.4. Ancestral reconstruction of number of presacral vertebrae

Evolutionary changes in the number of presacral vertebrae were evaluated by mapping these attributes onto the preferred partitioned Bayesian phylogeny (with branch lengths estimated from the mean posterior density; see Section 3). Data on the number of presacral vertebrae are available in the literature for all the described Malagasy "scincine" species included in this present study (see Table 4). Ancestral character state reconstructions were performed in Mesquite v1.0 (Maddison and Maddison, 2003).

When multiple individuals of a given species are examined, a range of presacral vertebrae numbers (Table 4) is often present, but usually a "common" number is evident. Thus, in our analysis we used the modal number of presacral vertebrae. The evolution of presacral vertebrae number was reconstructed using the method of weighted squared-change parsimony (Maddison, 1991) which weights the minimized sum of the squared-changes by dividing this measure by a given branch length (implemented in Mesquite). Also, for those species represented by multiple individuals, the "single" species branch length was based on the summed branch lengths of all individuals. This weighted squared-change parsimony method of evaluating the evolution of continuous data was preferred over a more traditional parsimony approach of mapping discrete attributes onto a phylogeny. A discrete parsimony approach could be implemented by assigning a unique character state for each possible number of vertebrae between 30 and 67 and ordering the array. However, Mesquite (as well as MacClade v4; Maddison and Maddison, 2000) can handle only 26 discrete character states. Though information is lost, it was possible to recode the presacral vertebrae numbers into ordered character state bins of two vertebrae; thus, reducing the effective number of unique character states to 19. The overall general patterns and results of such a discrete parsimony approach were essentially the same (results not shown) as those from the weighted squared-change parsimony approach; however, the ancestral reconstructions at many of the internal nodes were equivocal. Presacral vertebrae numbers will be abbreviated "PSV" throughout the text. All reported ancestral reconstructions are rounded to the nearest whole number.

3. Results

3.1. Phylogenetic analyses

The complete alignment consisted of 2732 bp. In total, alignments for 370 positions were ambiguous; thus, the analyzed sequences constituted a matrix of 2362 characters. Of these, 1130 sites were variable and 935 were parsimony-informative.

The maximum parsimony analysis inferred one most parsimonious tree (tree length = 6438; Fig. 1). The single optimal tree from the single-model ML analysis ($TVM + I + \Gamma$; $-\ln L = 29899.195$; parameters provided in Table 5; tree not shown) and the majority-rule consensus tree from the partitioned Bayesian analysis (Fig. 2) were essentially the same. The only difference involved the placement of *Amphiglossus tsaratananensis*, with the ML analysis placing this species as the sister lineage to the clade containing *A. tanysoma*, *A. ornaticeps*, *A. melanurus*, and *Androngo crenni*. All phylogenetic analyses (MP, ML, and Bayesian) clearly show that *Amphiglossus* is non-monophyletic. On the other hand, all analyses provide strong support for the monophyly of *Paracontias* and *Voeltzkowia*.

The results of the partitioned Bayesian analyses and estimated Bayes factor comparisons are provided in Table 6. Partitioning the ND1 data by codon position had a dramatic effect on the mean $-\ln L$ (compare P_6 and P_{4A} to the other strategies), a result consistent with Brandley et al. (2005). Employing six partitions resulted in an improvement of mean $-\ln L$ that was very strongly better than any of the alternative strategies according to the Bayes factor. Thus, the six-partition analysis is our preferred partitioning strategy and all discussion of Bayesian phylogeny and clade posterior probabilities will be limited to the results of this analysis.

From this point, we will primarily present the specific relationships inferred by the partitioned Bayesian analysis employing six partitions (Fig. 2) since it better models the evolution of the molecular data (though we also mention the MP support values for the different clades). The partitioned Bayesian analysis strongly supports two separate monophyletic groups that together contain all species of "*Amphiglossus*" (Fig. 2), with *Androngo* and *Pygomeles* being nested within one of these major clades. One major clade contains the included species of the subgenus *Madascincus* ("*A.*" *melanopleura* and "*A.*" *mouroundavae*), as well as "*A.*" *intermedius*, "*A.*" *stumpffi*, "*A.*" *igneocaudatus*, and "*A.*" *nanus*. We will refer to this clade as the *Madascincus* group hereafter. The second major clade contains the three large semi-aquatic species of the nominate subgenus *Amphiglossus* ("*A.*" *astrolabi*, "*A.*" *reticulatus*, and "*A.*" *waterloti*), the remaining as yet unclassified "*Amphiglossus*" species, *Androngo crenni*, and *Pygomeles braconnieri*. We will refer to this clade as the nominate *Amphiglossus* group hereafter.

"*Amphiglossus*" *splendidus* is placed as sister taxon to *Pygomeles*, though without strong support ($BS < 50$; $Pp = 0.90$). Additionally "*A.*" *splendidus* and *Pygomeles* form a clade exclusive of the rest of the nominate *Amphiglossus* group, though the level of support for this specific placement within the nominate *Amphiglossus* group is weak. The only included member of *Androngo* is nested

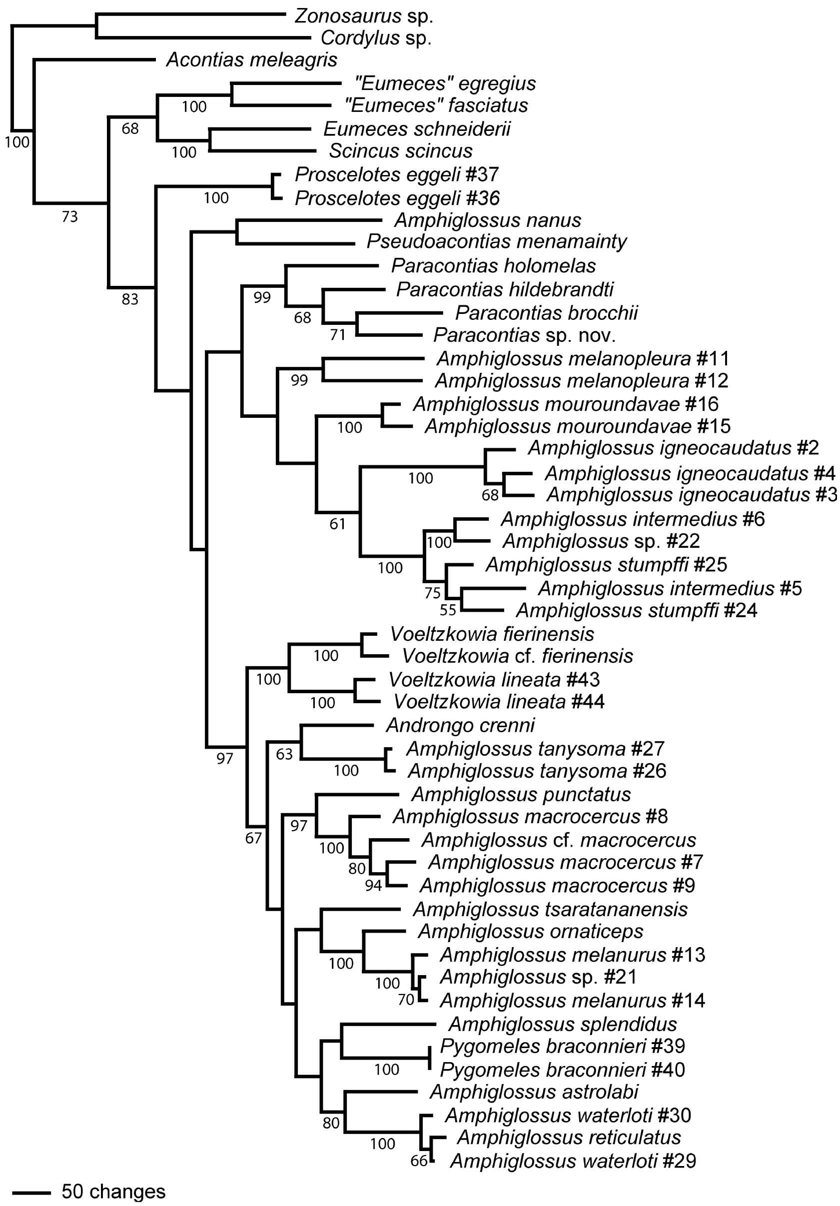

Fig. 1. Maximum parsimony phylogram based on 2362 bp of mitochondrial DNA gene sequences. Values below the nodes are bootstrap values in percent (5000 pseudoreplicates, 100 random addition replicates/pseudoreplicate; values below 50% not shown).

Table 5
Model parameters used in the maximum likelihood analysis

$-\ln L$	Base frequencies				Substitution rates						Rate parameters	
	A	C	G	T	$A \leftrightarrow C$	$A \leftrightarrow G$	$A \leftrightarrow T$	$C \leftrightarrow G$	$C \leftrightarrow T$	$G \leftrightarrow T$	Γ	I
29899.195	0.3847	0.3053	0.1109	0.1991	1.9989	15.7570	1.5378	0.7659	15.7570	1.0000	0.6277	0.4626

within the nominate *Amphiglossus* group and is consistently placed as the sister taxon to "*A.*" *tanysoma* ($BS = 63$; $Pp = 1.0$). *Voeltzkowia* is strongly supported as monophyletic ($BS = 100$; $Pp = 1.0$) and is placed as the sister clade to the nominate *Amphiglossus* group ($BS = 67$; $Pp = 0.99$). *Paracontias* is also a well-supported monophyletic group ($BS = 99$; $Pp = 1.0$) and is placed as the sister clade to the *Madascincus* group.

The partitioned Bayesian analysis places *Pseudoacontias menamainty* as the sister taxon of the *Paracontias* + *Madascincus* group clade (Fig. 2). However, this specific placement of *Pseudoacontias* is not strongly supported in the partitioned Bayesian analysis and the MP analysis weakly supports a relatively more basal position within the Malagasy "scincine" clade, as sister taxon to "*A.*" *nanus* (Fig. 1).

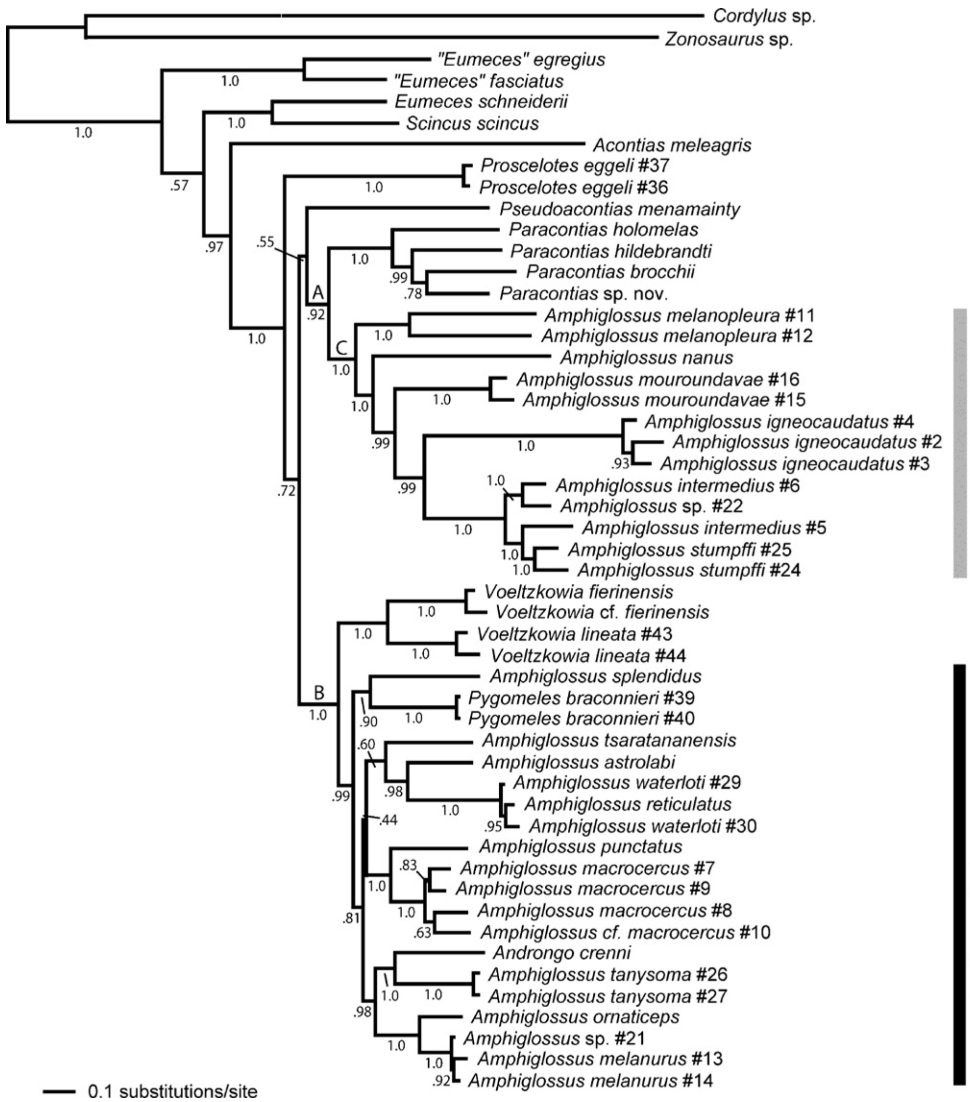

Fig. 2. Fifty percentage majority-rule consensus of trees sampled from the posterior distribution (at stationarity) of the most-partitioned analysis (strategy P_6), and our best estimate of Madagascar "scincine" lizard phylogeny. Branch lengths are calculated from means of the posterior probability density. Values below the nodes represent posterior probabilities estimated from all trees sampled at stationarity. Clades A, B, and C refer to clades in Fig. 3.

Table 6
2ln Bayes factor results for comparisons among each partitioning strategy

	Partitioning strategies				
	P_6	P_{4A}	P_{4B}	P_2	P_1
P_6	—				
P_{4A}	**29.7**	—			
P_{4B}	**503.6**	473.9	—		
P_2	**569.5**	539.8	65.9	—	
P_1	**840.4**	810.7	336.8	270.9	—

2ln Bayes factors ⩾10 are considered very strongly different (Kass and Raftery, 1995).

3.2. Evolution of presacral vertebrae

Based on the preferred phylogenetic hypothesis (Fig. 2) and weighted squared-change parsimony, 47 presacral vertebrae (PSV) is hypothesized to be the ancestral condition for the Malagasy "scincine" clade (Fig. 3). While the placement of the relatively basal *Pseudoacontias* is weakly supported (Figs. 1 and 2), there is relatively strong support for its exclusion from the two major basal clades (i.e., Clade A = *Paracontias* + *Madascincus* group [$Pp = 0.92$] and Clade B = *Voeltzkowia* + nominate *Amphiglossus* group [$Pp = 1.0$]) (Fig. 2). The potential alternate placements of the greatly elongated *Pseudoacontias* (~67 PSV) as the sister taxon of Clade B (Fig. 3) or the sister taxon of all remaining Malagasy "scincines" does not greatly change the hypothesized ancestral number of presacral vertebrae (46–48 PSV). Thus, evidence appears strong that the ancestral Malagasy "scincine" possessed a relatively high number of presacral vertebrae compared to the

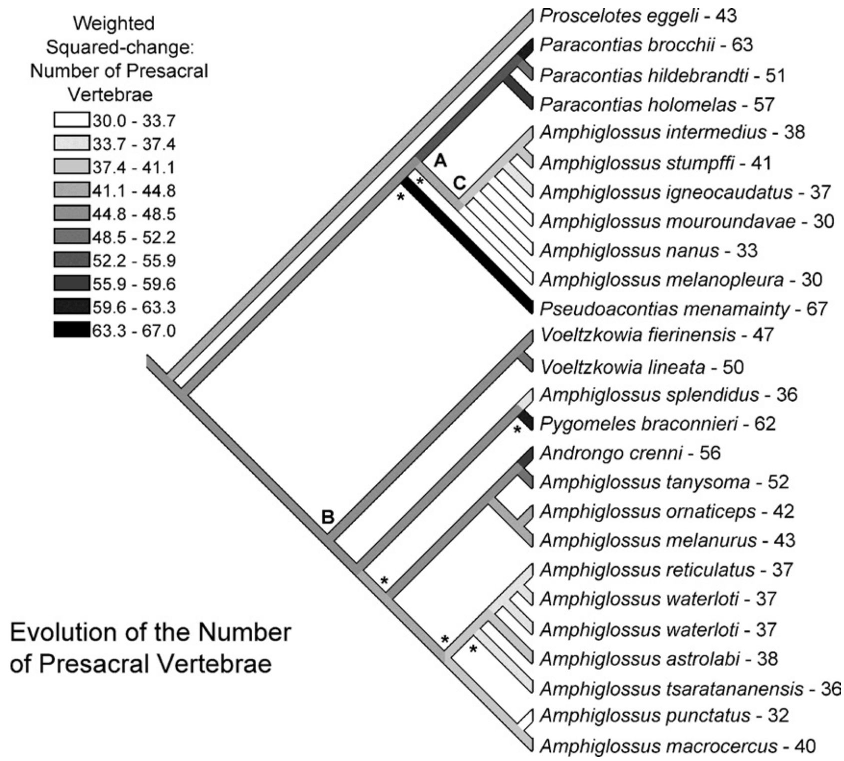

Fig. 3. Phylogenetic reconstruction of presacral vertebrae number for the Malagasy "scincines." Modes of presacral vertebrae numbers are mapped onto the consensus tree of the six-partition Bayesian analysis (Fig. 2), with those taxa lacking data pruned from the phylogeny. Numbers following species names are the modal number of presacral vertebrae (see Table 4 for observed ranges). Asterisks along branches denote weakly supported clades. Labels A, B, and C refer to clades in Fig 2.

lowest numbers exhibited by some "*Amphiglossus*" species nested well within different parts of the phylogeny (e.g., ~30 PSV in "*A.*" *melanopleura* and "*A.*" *mouroundavae*; ~32 PSV in "*A.*" *punctatus*).

According to the scenario suggested by our results, the ancestor of the Malagasy "scincines" was elongate with a moderate number of presacral vertebrae (46–48 PSV) and evolution to the extremes exhibited within this clade (high and low PSV) has occurred independently multiple times. The highest numbers of presacral vertebrae have independently evolved in the following lineages or clades: *Pseudoacontias menamainty* (67 PSV), *Pygomeles braconnieri* (62 PSV), *Paracontias* (51–63 PSV), and *Androngo crenni* (56 PSV). The evolution of an elevated number of presacral vertebrae has also occurred in *Voeltzkowia* (e.g., to 50 PSV in *V. lineata*). And finally, the highest number of PSV exhibited by any of the "*Amphiglossus*" species is 52 and is exhibited by "*A.*" *tanysoma*, which is strongly placed as the sister taxon of the elongate *Androngo crenni* ($BS = 63$; $Pp = 1.0$).

There is strong phylogenetic evidence for the evolution to a relatively low number of presacral vertebrae (30–40) occurring independently within the major Clades A and B (Fig. 3). This conclusion is based on the assumption that the ancestor of the overall Malagasy clade possessed a moderately high number of presacral vertebrae (46–48 PSV; further elaboration on this assumption in Section 4). Within Clade A, all the "*Amphiglossus*" represent a strongly supported clade, with some of the basal-most members exhibiting the lowest numbers of presacral vertebrae among Malagasy "scincines" (i.e., "*A.*" *melanopleura* and "*A.*" *mouroundavae*; 30 PSV). The weighted squared-change parsimony reconstruction for the ancestor of this "*Amphiglossus*" clade is 41 presacral vertebrae, with a subsequent reduction to 30 in "*A.*" *melanopleura*. There is also a further hypothesized reduction in the common ancestor of the clade containing the remaining "*Amphiglossus*" of Clade A (exclusive of "*A.*" *melanopleura*), with additional independent reductions to much lower numbers of presacral vertebrae in "*A.*" *nanus* and "*A.*" *mouroundavae* (30 and 33 PSV, respectively).

Within Clade B, there also appears to have been multiple independent reductions in presacral vertebrae number (as well as multiple increases). However, because many of the inferred relationships within this major clade are weakly supported, identifying the exact number and sequence of reduction events is difficult. Even so, one of the lowest numbers of presacral vertebrae exhibited by a Malagasy "scincine" is found within Clade B (i.e., "*Amphiglossus*" *punctatus* with 32 PSV; other "*Amphiglossus*" species with similar PSV number are members of Clade A). Given the strong support of the nested position of "*A.*" *punctatus* within Clade B, where all the remaining species possess ⩾36 presacral vertebrae (and the basal-most species generally possess many more), it appears there is strong evidence once again that significant and recurrent reduction of presacral vertebrae has occurred among the Malagasy "scincines."

4. Discussion

Relationships among species of "*Amphiglossus*" have been controversial (Glaw and Vences, 1994; Raxworthy and Nussbaum, 1993) mainly because of their morphological similarity. The data presented in this study provide the first molecular phylogeny of the "scincines" of Madagascar and provide an independent means of testing the different taxonomic schemes.

4.1. "Amphiglossus" phylogeny

One of the most intriguing results from this study is that the genus "*Amphiglossus*" as currently recognized is not-monophyletic, with two very distinct radiations. The members of the two previously classified subgenera (*Amphiglossus*/*Madascincus*) are each part of different larger groups containing many additional "*Amphiglossus*" species of very different morphologies. The subgenus *Amphiglossus* (sensu Brygoo, 1980a,c; "*A.*" *astrolabi*, "*A.*" *waterloti*, and "*A.*" *reticulatus*) is strongly supported as a clade. However, the taxa of the subgenus *Madascincus* (sensu Brygoo, 1984b) do not form a clade exclusive of other species of "*Amphiglossus*"; but these species are members of a larger major clade including some other "*Amphiglossus*", a group we refer to as the *Madascincus* group (Fig. 2).

Since the mtDNA provides strong evidence that "*Amphiglossus*" is not monophyletic, formal generic taxonomic changes are needed in order to have a classification that reflects the evolutionary history of the group. Since all the included members of the two previously recognized subgenera (*Amphiglossus* and *Madascincus*) are, respectively, restricted to each of the two strongly supported major clades (i.e., nominate *Amphiglossus* group and *Madascincus* group), it is tempting to elevate these subgenera to generic status and apply the names (i.e., *Amphiglossus* sensu stricto and *Madascincus*) to these major clades (which will ultimately likely be the case). However, while some informal phenetic groups of species have been proposed (e.g., Glaw and Vences, 1994), widespread convergence in morphological attributes (e.g., color patterns, presacral vertebrae number; see below) reduces our confidence in being able to assess the phylogenetic affinities (i.e., taxonomic allocations) of those species for which we currently lack samples (∼50% of recognized species of "*Amphiglossus*"). Thus, formal taxonomic recommendations will be postponed until additional "*Amphiglossus*" species, as well as a couple of other Malagasy "scincine" genera missing in our data set (e.g., *Cryptoscincus* or the only very recently described *Sirenoscincus*), can be included in future studies. Below we further discuss the phylogenetic relationships within and/or among some of the formal and informal species groups previously proposed within "*Amphiglossus*".

The first of these proposed groups corresponds to the large aquatic/semi-aquatic species ("*A.*" *astrolabi*, "*A.*" *reticulatus*, and "*A.*" *waterloti*) placed into the nominate subgenus by Brygoo (1980a,c). Our data strongly support the monophyly of these species and their nested position within the nominate *Amphiglossus* group. Because of their strong morphological similarities, the validity of the specific status of "*A.*" *waterloti* (with respect to "*A.*" *reticulatus*) has been discussed by several authors (Andreone and Greer, 2002; Brygoo, 1980c; Glaw and Vences, 1994; Raxworthy and Nussbaum, 1993). The main differences between these two species are the number of longitudinal scale rows at mid-body and the number of ventral scales (Brygoo, 1980c). Recent data from several new specimens significantly narrows the gaps for these characters (Andreone and Greer, 2002), but both forms continued to be recognized as distinct taxa. Our data support a very close relationship between the two forms, with "*A.*" *waterloti* possibly paraphyletic with respect to "*A.*" *reticulatus.* There is also a relatively low level of genetic differentiation between the three sampled individuals, with the levels of divergence being essentially equal to or less than that observed between multiple conspecific individuals sampled from other "*Amphiglossus*" species (e.g., "*A.*" *mouroundavae*, "*A.*" *melanurus*, and "*A.*" *igneocaudatus*; Fig. 2). All this recent morphological and molecular evidence leads us to consider the two forms as conspecific; thus, "*A.*" *waterloti* (Angel, 1930) becomes a subjective junior synonym of "*A.*" *reticulatus* (Kaudern, 1922).

Besides the previously recognized subgenera, a few other phenetic groups of "*Amphiglossus*" have been recognized. One of these is characterized by a conspicuous dark lateral stripe (Glaw and Vences, 1994) and contains four recognized species ("*A.*" *igneocaudatus*, "*A.*" *intermedius*, "*A.*" *polleni*, and "*A.*" *stumpffi*) generally distributed in western and northwestern Madagascar (with one questionable locality of "*A.*" *polleni* on the east coast). Our analysis strongly supports the monophyly of this group (="*A.*" *igneocaudatus* species group of Brygoo, 1984d), as well as its placement within the *Madascincus* group. Previously, there has been doubt as to the taxonomic status of the Ibity population of "*A.*" *igneocaudatus* (Brygoo, 1984d), with Raxworthy and Nussbaum (1993) noting morphological similarity to "*A.*" *intermedius*. In our study, the mtDNA strongly groups the Ibity individuals of "*A.*" *igneocaudatus* with the Itremo "*A.*" *igneocaudatus*, and the individuals of this species are genetically quite divergent from "*A.*" *intermedius* (Fig. 2). And finally, our analysis does not support a close relationship between the two "*A.*" *intermedius* individuals from different localities (Fig. 2), with the Ampijoroa individual being more closely related to a sympatric unidentified species. Obviously, the species limits within the "*A.*" *igneocaudatus* group need to be further evaluated.

Brygoo (1981a) informally recognized another phenetic group of "*Amphiglossus*" for four brownish medium-sized species ("*A.*" *melanurus*, "*A.*" *macrocercus*, "*A.*" *gastrostictus*, and "*A.*" *poecilopus*) from eastern and central Madagascar. Brygoo (1984a) also hypothesized that the northern "*A.*" *tsaratananensis* may also be a member of this group. We provide strong evidence that the species of this phenetic group included in our study ("*A.*" *melanurus*, "*A.*" *macrocercus*, and "*A.*" *tsaratananensis*) do not form a clade (Figs. 1 and 2).

A final informal group of "*Amphiglossus*" was noted by Glaw and Vences (1994) for several species (i.e., "*A.*" *andranovahensis*, "*A.*" *ardouini*, "*A.*" *frontoparietalis*, "*A.*" *ornaticeps*, and "*A.*" *splendidus*) possessing transverse markings on the head and/or body. Brygoo (1984e, 1985) also postulated affinities between members of this group (i.e., "*A.*" *andranovahensis* and "*A.*" *ornaticeps*; "*A.*" *ardouini*, "*A.*" *frontoparietalis*, and "*A.*" *splendidus*; respectively). However, our data do not support a close relationship between the two species included in our study. "*Amphiglossus*" *ornaticeps* is strongly placed as the sister lineage to a small clade containing "*A.*" *melanurus* and an unidentified "*Amphiglossus*" species, and "*A.*" *splendidus* is weakly placed with *Pygomeles* (Figs. 1 and 2).

4.2. Phylogenetic affinities of the limb-reduced genera

The monophyly of the greatly limb-reduced *Voeltzkowia* is strongly supported by the mtDNA data (Figs. 1 and 2) and it is placed as the sister taxon of the nominate *Amphiglossus* group. Traditionally, this small clade (containing five described species) confined to the arid regions of southwestern and western Madagascar (Glaw and Vences, 1994) has been divided into two distinctive subgenera, *Voeltzkowia* (completely limbless; represented by *V. lineata*) and *Grandidierina* (forelimbs absent, hindlimbs greatly reduced; represented by *V. fierinensis* and an undescribed species). The rare limbless *Cryptoscincus*, probably from southwestern Madagascar, is one of only two Malagasy "scincine" genera not represented in this study, but has been hypothesized to be closely related to *Voeltzkowia* (Brygoo, 1981c).

The limbless genus *Paracontias* is also strongly supported as a clade and appears to be most closely related to the "*Amphiglossus*" of the *Madascincus* group. Traditionally, three subgenera (i.e., *Angelias*, *Malacontias*, and *Paracontias*) have been recognized to accommodate four species. Recently, Andreone and Greer (2002) described three new *Paracontias* species, but did not place these into any of the previously recognized subgenera. Though they did not conduct an explicit phylogenetic analysis, they questioned whether the few characters used to define these subgeneric groups actually diagnosed monophyletic taxa. Andreone and Greer (2002) suggested the possibility that *Malacontias* or a *Malacontias* + *Paracontias* group could be a "true lineage" (=clade?). However, though our sampling within the genus *Paracontias* is limited (only two of the subgenera represented: *Malacontias* [*P. hildebrandti*, *P. holomelas*] and *Paracontias* [*P. brocchii*]), our data do not support a monophyletic *Malacontias*.

Very little is known about the biology of the enigmatic largely limbless *Pseudoacontias* (Andreone and Greer, 2002; Nussbaum and Raxworthy, 1995), with each of the four species being known only from their holotypes. Glaw and Vences (1994) suggested a possible close relationship between the genera *Pseudoacontias* and *Paracontias*. Our data do not provide strong support for such a hypothesis. The partitioned Bayesian analysis weakly places *Pseudoacontias* as the sister taxon to the *Paracontias* + *Madascincus* group clade (Fig. 2), but this taxon could almost as likely be placed as the sister taxon to the *Voeltzkowia* + nominate *Amphiglossus* group clade or even as the sister taxon to all the Malagasy "scincines." Even though our data cannot precisely determine the specific phylogenetic placement of *Pseudoacontias*, there is support for a relatively basal separation from the other Malagasy "scincine" clade because of the relatively strong support for its exclusion from the large *Paracontias* + *Madascincus* and *Voeltzkowia* + nominate *Amphiglossus* clades.

The elongated, limb-reduced genera *Pygomeles* and *Androngo* are nested within the strongly supported nominate *Amphiglossus* group. The specific placement of *Pygomeles braconnieri* (forelimbs absent; short hindlimbs with single toes) within this group is weakly supported, but all analyses suggest a possible close relationship to the pentadactylous "*Amphiglossus*" *splendidus*. *Androngo* was originally erected to accommodate four "*Amphiglossus*" species (i.e., "*A.*" *trivittatus*, "*A.*" *alluaudi*, "*A.*" *crenni*, and "*A.*" *elongatus*) with varying degrees of limb reduction (but none completely limbless) and greater than 48 presacral vertebrae (Brygoo, 1987). The single *Androngo* species included in our study, *Androngo crenni*, was placed as the sister species of "*Amphiglossus*" *tanysoma*. This corroborates the taxonomic decision of Andreone and Greer (2002) who transferred three of the four *Androngo* species (i.e., *An. alluaudi*, *An. crenni*, and *An. elongatus*) back to "*Amphiglossus*." We were unable to evaluate the phylogenetic placement of Androngo trivittatus. However, based on two morphological features (i.e., postnasal scale absent and relatively high number of presacral vertebrae [53–56 in *An. trivittatus*]) and geographic distribution, Andreone and Greer (2002) postulated a possible close relationship between *An. trivittatus* and *Pygomeles braconnieri*.

4.3. The evolution of presacral vertebrae number in Malagasy "scincines"

Body elongation and limb-reduction are generally correlated phenomena that have occurred repeatedly during scincid lizard evolution. In general, body elongation (=increase in relative snout-vent-length) in skinks is the result of increases in the number of presacral vertebrae. Malagasy "scincines" exhibit great diversity in the degree of body elongation, with the number of presacral vertebrae ranging from a low of 29–30 in the "*Amphiglossus*" species traditionally placed in the subgenus "*Madascincus*" (e.g., Brygoo, 1984b) to as high as 82 in *Pseudoacontias angelorum* (Nussbaum and Raxworthy, 1995). The number of presacral vertebrae is known for all the described species in our study, with the modal number ranging from 30 to 67 (see Table 4 and Fig. 3). Given these extensive comparative data, it is possible for us to investigate the evolution of presacral vertebrae number within the Malagasy "scincine" clade from a phylogenetic perspective.

Greer (1989) has suggested that the scincid ancestral number of presacral vertebrae is 26, a number exhibited by many phylogenetically diverse scincid species. As previously mentioned, the lowest presacral vertebrae number exhibited by any Malagasy "scincine" is 29, suggesting the common ancestor of the Malagasy clade likely exhibited slightly more presacral vertebrae than the postulated ancestral scincid. Given this, a major question remains; namely, does the lowest number of presacral vertebrae exhibited by some extant Malagasy "scincines" represent the ancestral condition for the whole group? Andreone and Greer (2002) have hypothesized that 29–30 presacral vertebrae is ancestral for "*Amphiglossus*" and that "*Amphiglossus*" contains some of the most "primitive members" of the Malagasy "scincine" clade. Though they also acknowledge that "*Amphiglossus*" is likely non-monophyletic, their ideas suggest (at least implicitly) that 29–30 presacral vertebrae is the ancestral condition for the Malagasy "scincines." However, based only on the Malagasy taxa sampled in our study, the weighted squared-change parsimony reconstruction of the ancestral condition is 47–48 presacral vertebrae (depending on the placement of *Pseudoacontias menamainty*; see Section 3.1). The inclusion of the elongated *Proscelotes eggeli* (a sub-Saharan "scincine") only slightly lowers the possible range of the ancestral condition to 46–48 presacral vertebrae. MtDNA data from a more extensive study of "scincine" phylogeny (Brandley et al., 2005) strongly supports the phylogenetic placement of the Malagasy "scincines" within a major clade containing other "scincines" from sub-Saharan and North Africa, the Seychelles, and southern Europe and south-southwest Asia. The very elongate and limbless *Feylinia* (of the subfamily Feylininae) is also a member of this more inclusive major clade. However, the exact relationship between the Malagasy "scincines" and these other skinks is uncertain. Given the importance of closely related outgroup taxa for reconstructing the ancestral condition at the ingroup node (=Malagasy clade; Maddison et al., 1984), it is important to note that all of the non-Malagasy skinks in this more inclusive major clade are elongate, with many species being greatly limb-reduced (e.g., *Melanoseps*, *Sphenops*, and *Typhlacontias*). Of these non-Malagasy skinks, some members of "*Chalcides*" probably exhibit the lowest number of presacral vertebrae (i.e., ranging between 34 and 65; Caputo et al., 1995; Greer et al., 1998). Even if these "*Chalcides*" with the lowest number of presacral vertebrae are used as the outgroup to the Malagasy "scincines" (an unlikely hypotheses given "*Chalcides*" is a member of a relatively strongly supported exclusive clade containing the elongated *Sphenops* of north-northeast Africa and other elongated sub-Saharan "scincines"; Brandley et al. (2005)), there is essentially no change in the reconstructed ancestral condition (45–46 vs 46–48 PSV). Thus, regardless of which of these non-Malagasy skinks are actually most closely related to the Malagasy clade, it is very unlikely that any other potential outgroup relationship would drastically change the ancestral parsimonious reconstruction presented in this study. Thus, we are confident that the ancestor of the Malagasy clade was already elongated and had a moderately high number of presacral vertebrae.

The evolutionary implication for our hypothesized ancestral condition of the Malagasy clade is that the lowest exhibited numbers of presacral vertebrae are actually derived through loss or reduction in PSV number. While the loss of presacral vertebrae appears to have occurred independently in multiple "*Amphiglossus*" lineages, in no cases does the starting ancestral condition in the beginning of a sequence of loss events exceed the hypothesized ancestral condition for the Malagasy clade (i.e., 46–48 PSV). In other words, our parsimonious ancestral reconstructions do not support the reduction (=reversal) of presacral vertebrae numbers in any lineages that possess a derived increased presacral vertebrae condition (i.e., >48 PSV). Within the Malagasy clade, it appears that once a lineage has started increasing the number of presacral vertebrae from the hypothesized ancestral Malagasy condition (which has occurred independently multiple times), there are no subsequent reversals in these groups. The only apparent exception to this "rule" may be in *Paracontias*. Based on our preferred phylogeny (Fig. 2), the parsimony character reconstruction suggests there has been a slight reduction of presacral vertebrae number in *P. hildebrandti* (53–54 → 51 PSV). However, the slight apparent difference falls within the range of presacral vertebrae variation exhibited within *P. hildebrandti* (50–55 PSV; Andreone and Greer, 2002; Brygoo, 1980b). It is interesting that there are two additional *Paracontias* species (*P. rothschildi*, 46 PSV and *P. milloti*, 47 PSV; Andreone and Greer, 2002; tissues lacking) that exhibit presacral vertebrae numbers that are essentially the same as the hypothesized ancestral Malagasy condition. Future studies that may include these missing species could be very useful for further investigating the pattern of presacral vertebrae evolution leading to and within *Paracontias*.

The patterns of evolution of the number of presacral vertebrae within the Malagasy "scincine" clade also have implications for taxonomy. Some past taxonomic decisions within this clade have been largely based on the observed number of presacral vertebrae. Within "*Amphiglossus*," Brygoo (1984b) described the subgenus "*Madascincus*" for those small-bodied species with the lowest number of presacral vertebrae (29–30 PSV). The "*Madascincus*" species included in this study (i.e., "*A*." *melanopleura* and "*A*." *mouroundavae*) are not each other's closest relative, but are nested within a clade (Clade C; Fig. 3) containing other "*Amphiglossus*" species with >30 presacral vertebrae. Another taxon previously recognized based on presacral vertebrae number is *Androngo* (PSV > 48; Brygoo, 1987). Unfortunately, we were able to include only one species of *Androngo* (i.e., *An. crenni*; sensu Brygoo, 1981b, 1987) in our study of Malagasy "scincine" phylogeny. However, our data strongly support the placement of *An. crenni* (54–57 PSV) as closely related to a species of "*Amphiglossus*" exhibiting a large number of presacral vertebrae (i.e., "*A*." *tanysoma*; 52–53 PSV). In general, given our strong results that the number of presacral vertebrae have independently decreased and increased multiple times within the Malagasy "scincine" clade, we agree with Andreone and Greer (2002) that the number of presacral vertebrae should not be the sole (or major) character used to diagnose groups within the Malagasy "scincine" clade.

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Appendix A

List of all species of "Scincinae" known to occur on Madagascar, with previously supposed subgeneric assignments.

Amphiglossus alluaudi (Brygoo, 1981) (formerly *Androngo*)
Amphiglossus (*Madascincus*) *ankodabensis* (Angel, 1930)
Amphiglossus andranovahensis (Angel, 1933)
Amphiglossus anosyensis Raxworthy and Nussbaum, 1993
Amphiglossus ardouini (Mocquard, 1897)
Amphiglossus astrolabi Duméril and Bibron, 1839
Amphiglossus crenni (Mocquard, 1906) (formerly *Androngo*)
Amphiglossus decaryi (Angel, 1930)
Amphiglossus elongatus (Angel, 1933) (formerly *Androngo*)
Amphiglossus frontoparietalis (Boulenger, 1889)
Amphiglossus gastrostictus (O'Shaugnessy, 1879)
Amphiglossus igneocaudatus (Grandidier, 1867)
Amphiglossus intermedius (Boettger, 1913)
Amphiglossus macrocercus (Günther, 1882)
Amphiglossus macrolepis (Boulenger, 1888)
Amphiglossus mandady Andreone and Greer, 2002
Amphiglossus mandokava Raxworthy and Nussbaum, 1993
Amphiglossus (*Madascincus*) *melanopleura* (Günther, 1877)
Amphiglossus (*Madascincus*) *mouroundavae* (Grandidier, 1872)
Amphiglossus melanurus (Günther, 1877)
Amphiglossus minutus Raxworthy and Nussbaum, 1993
Amphiglussus nanus Andreone and Greer, 2002
Amphiglossus ornaticeps (Boulenger, 1896)
Amphiglossus poecilopus (Barbour and Loveridge, 1928)
Amphiglossus polleni (Grandidier, 1869)
Amphiglossus praeornatus Angel, 1938
Amphiglossus punctatus Raxworthy and Nussbaum, 1993
Amphiglossus reticulatus (Kaudern, 1922)
Amphiglossus splendidus (Grandidier, 1872)
Amphiglossus spilostichus Andreone and Greer, 2002
Amphiglossus stumpffi (Boettger, 1882)
Amphiglossus stylus Andreone and Greer, 2002
Amphiglossus tanysoma Andreone and Greer, 2002
Amphiglossus tsaratananensis (Brygoo, 1981)
Amphiglossus waterloti (Angel, 1930), syn. nov.

Androngot. trivittatus (Boulenger, 1896)
  Androngo trivittatus trilineatus (Angel, 1942)

Cryptoscincus minimus Mocquard, 1906

Pygomeles braconnieri Grandidier, 1867
Pygomeles petteri Pasteur and Paulian, 1962

Paracontias (*P.*) *brocchii* Mocquard, 1894
Paracontias (*Angelias*) *milloti* Angel, 1949
Paracontias (*Angelias*) *rothschildi* Mocquard, 1905
Paracontias (*Malacontias*) *hildebrandti* (Peters, 1880)
Paracontias (*Malacontias*) *holomelas* (Günther, 1877)
Paracontias hafa Andreone and Greer, 2002
Paracontias manify Andreone and Greer, 2002
Paracontias tsararano Andreone and Greer, 2002

Pseudoacontias angelorum Nussbaum and Raxworthy, 1995
Pseudoacontias madagascariensis Bocage, 1889
Pseudoacontias menamainty Andreone and Greer,
Pseudoacontias unicolor Sakata and Hikida, 2003

Sirenoscincus yamagishii Sakata and Hikida, 2003

Voeltzkowia (*Grandidierina*) *fierinensis* (Grandidier, 1869)
Voeltzkowia (*Grandidierina*) *petiti* (Angel, 1924)
Voeltzkowia (*V.*) *lineata* (Mocquard, 1901)
Voeltzkowia (*V.*) *mira* Boettger, 1893
Voeltzkowia (*V.*) *rubrocaudata* (Grandidier, 1869)

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