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PHYLOGENETIC SYSTEMATICS AND EVOLUTION WITHIN

THE FAMILY SCINCIDAE

by

Alison Swindle Whiting

A dissertation submitted to the faculty of

Brigham Young University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Integrative Biology

Brigham Young University

April 2004

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GRADUATE COMMITTEE APPROVAL

of a dissertation submitted by

Alison Swindle Whiting

This dissertation has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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Date	Aaron M. Bauer
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As chair of the candidate's graduate committee, I have read the dissertation of Alison Swindle Whiting in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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Keith A. Crandall
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Accepted for the College

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ABSTRACT

PHYLOGENETIC SYSTEMATICS AND EVOLUTION WITHIN THE FAMILY SCINCIDAE

Alison Swindle Whiting

Department of Integrative Biology

Doctor of Philosophy

Scincidae (skinks) comprise one of the largest families of lizards, and the more than 1300 species show great variation in body size and form, and are found worldwide in a diversity of habitats. The group presents many interesting questions ranging from the colonization of oceanic islands, to the evolution of limb loss, yet most of these and other questions remain understudied. The purpose of this dissertation is to use multiple mitochonidrial and nuclear DNA markers in connection with current cladistic methods to address evolutionary questions at many levels within Scincidae.

In chapter one, a molecular phylogenetic study, based on six genes and extensive analyses support Cordylidae+Xantusiidae as its sister group, and confirm the paraphyly of Scincinae. The Acontinae is the sister group to all remaining skinks, while Feylininae is nested within an otherwise monophyletic southern African scincine clade. Limited support for reversal of limb and digit loss is found.

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In chapter two, the monophyly and relationships of Malagasy scincines are investigated using data from seven gene regions. Malagasy scincines are monophyletic, and derived from a single colonization from southern Africa. Our analyses confirm the paraphyly of *Amphiglossus*, and support *Madascincus* as a valid genus. *Madascincus* is sister to a monophyletic *Paracontias*, while *Voeltzkowia* is basal to the remaining *Amphiglossus* and *Pygomeles*.

Chapter three uses seven gene regions to infer relationships within South American *Mabuya*, and to test the hypothesis of two independent colonizations from Africa to the New World. Direct Optimization (DO) is compared with traditional alignment methods, and multilocus maximum likelihood and Bayesian methods are used to date divergence times within the group. Results show that DO consistently finds more optimal tree topologies regardless of the optimality criterion used, and provides the ability to use models throughout the alignment and tree reconstruction process. South American *Mabuya* are not monophyletic, and the two colonization hypothesis is confirmed with parametric bootstrapping. Within the mainland species of *Mabuya*, many taxonomic problems are uncovered including multiple species complexes. Due to the lack of reliable fossil information, dating methods differ greatly in their estimates of divergence dates within *Mabuya*.

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Phylogenetic relationships and limb loss in sub-Saharan African scincine lizards (Squamata: Scincidae)

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Abstract

Skinks are the largest family of lizards and are found worldwide in a diversity of habitats. One of the larger and more poorly studied groups of skinks includes members of the subfamily Scincinae distributed in sub-Saharan Africa. Sub-Saharan African scincines are one of the many groups of lizards that show limb reduction and loss, and the genus *Scelotes* offers an excellent opportunity to look at limb loss in a phylogenetic context. Phylogenetic relationships were reconstructed for a total of 52 taxa representing all subfamilies of skinks as well as other Autarchoglossan families using sequence from six gene regions including; 12S, 16S, and cytochrome *b* (mitochondrial), as well as α-Enolase, 18S, and C-mos (nuclear). The family Scincidae is recovered as monophyletic and is the sister taxon to a (Cordylidae + Xantusiidae) clade. Within skinks the subfamily Acontinae is monophyletic and sister group to all remaining skinks. There is no support for the monophyly of the subfamilies Lygosominae and Scincinae, but sub-Saharan African scincines + *Feylinia* form a well supported monophyletic group. The monophyly of *Scelotes* is confirmed, and support is found for two geographic groups within the genus. Reconstructions of ancestral states for limb and digital characters show limited support for the reversal or gain of both digits and limbs, but conservative interpretation of the results suggest that limb loss is common, occurring multiple times throughout evolutionary history, and is most likely not reversible.
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Keywords: Scincidae; Scincinae; *Scelotes*; mtDNA; Nuclear genes; Phylogeny; Limb loss

1. Introduction

With more than 1300 species, skinks comprise the largest family (Scincidae) of lizards, and include >25% of the world's lizard diversity (Bauer, 1998). Greer (1970b) defined four subfamilies within skinks that are still widely used today. The Acontinae (18 spp.) and Feylininae (4 spp.) are small groups of completely limbless skinks restricted to Africa. The Lygosominae is the largest and most speciose subfamily and is distributed worldwide, but with the majority of its diversity in Australia and Asia. Like the two small subfamilies, the monophyly of the Lygosominae has generally been accepted on the basis of derived morphological features (Greer, 1970b, 1986; Griffith et al., 2000; but see Hutchinson, 1981). The Scincinae is also a large subfamily distributed throughout the Americas and Asia, but with its center of diversity in Africa. Greer (1970b) postulated that scincines were primitive, originated in Africa, and independently gave rise to the other three subfamilies. The recognized paraphyly of the Scincinae has long been an impediment to the resolution of higher order skink relationships. Recently, Greer and Shea (2000) described the shared occurrence of a derived head scale pattern (the "chalcidine" condition) characterizing all non-lygosomine skinks except *Eumeces*, *Scincus*, and *Scincopus* and Griffith et al. (2000) have proposed a fifth subfamily, the Eumecinae, in an attempt to identify monophyletic subgroups within the Scincinae *sensu* Greer (1970b).

One of the most poorly studied groups of scincines consists of the seven genera occurring in sub-Saharan Africa. One of these, *Chalcides*, is chiefly Mediterranean in its distribution, and has been the subject of relatively

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intensive systematic study (Brown and Pestano, 1998; Caputo, 1993; Caputo et al., 1999). Among the remaining taxa, four genera: *Typhlacontias*, *Sepsina*, *Proscelotes*, and *Scelotes*, occur chiefly in southern Africa (south of the Kunene and Zambezi Rivers), while two genera: *Scolecoseps* and *Melanoseps* are restricted to tropical east and central Africa. The affinities of some of these forms, as well as the taxa now allocated to the Acontinae and Feylininae, were considered by de Witte and Laurent (1943). They grouped *Sepsina* with the acontines and *Scelotes*, *Scolecoseps*, *Melanoseps*, and *Typhlacontias* with the feylinines, while regarding *Proscelotes* as ancestral to both lineages. Greer (1970a,b) accepted some of these relationships, but considered *Sepsina* and *Proscelotes* as closely related and regarded acontines, feylinines, and scincines as phylogenetically distinct from one another.

Among the southern African scincines the genus *Scelotes*, with 21 species, is by far the most diverse group. The genus was originally described by Fitzinger (1826), and has been investigated by Hewitt (1921, 1927, 1929), Barbour and Loveridge (1928), de Witte and Laurent (1943), and FitzSimons (1943). The last of these reviews synonymized *Sepsina* with *Scelotes*, but confirmed the placement of Malagasy forms in a separate genus, *Amphiglossus*. Greer (1970a) reduced the total number of *Scelotes* species to 14, revalidating *Sepsina* and including the East African species *uluguruensis* in *Scelotes*. Broadley's recent monograph (1994) brought the total number of species to 21, and postulated certain interspecific relationships based on limb, eyelid, and scale characters. To date there have been no molecular data presented nor formal cladistic analyses conducted for *Scelotes* or for sub-Saharan African scincines as a whole (but see Brown and Pestano, 1998; Caputo et al., 1999; Haacke, 1997 for analyses of *Chalcides* and *Typhlacontias*, respectively). Although an explicit phylogeny of *Scelotes* and its relatives is desirable in its own right, it also provides the basis for the investigation of the evolution of limb reduction, which characterizes many of the African scincines and numerous other clades of lizards (Camp, 1923; Gans, 1975; Lande, 1977; Presch, 1975; Wiens and Slingluff, 2001).

Limb loss or reduction is an interesting phenomenon seen in many clades of squamates including snakes, amphisbaenids, and dibamid, teiid, gymnopthalmid, pygopodid, anguid, cordylid, and scincid lizards. The occurrence of limb loss in multiple squamate lineages leads to questions concerning the evolutionary pattern or stages of limb loss, and the developmental mechanisms and pathways involved (Wiens and Slingluff, 2001). Species within each of the currently recognized subfamilies of skinks, except the Eumecinae, demonstrate complete external limb loss, and it is postulated that limb reduction in some form has occurred more than 30 times within skinks (Bauer, 1998; Greer, 1991). The most speciose lineage to exhibit limb reduction, and that with the finest gradations in loss, is the Australian lygosomine genus *Lerista* (Greer, 1987, 1990, 1991; Hauser, 1996; Kendrick, 1991). Among scincines the greatest variation in limb expression occurs in the southern African genus *Scelotes*, which exhibits a morphocline from fully functional pentadactyl limbs to complete limblessness, with many species showing seemingly transitional stages in reduction of digits and limbs. Due to this variation, *Scelotes* offers an exceptional system in which to study limb loss in a phylogenetic context. In particular, *Scelotes* may be used to test the hypothesis that limb and digital loss is irreversible (Dollo's Law; Gould, 1970).

The purposes of this paper are: (1) test the monophyly of sub-Saharan African scincines, (2) test the monophyly of *Scelotes*, (3) establish a preliminary estimate of phylogeny for sub-Saharan African scincines (specifically *Scelotes*) based on molecular data, and (4) evaluate limb and digital loss in a phylogenetic context within this group.

2. Materials and methods

2.1. Sampling

Taxon sampling focused on sub-Saharan African scincines (5/7 genera), with an emphasis on southern African forms (4/4 genera) and more specifically on the genus *Scelotes* (9/21 spp.). In total, 36 taxa representing all four subfamilies of skinks (*sensu* Greer, 1970b) were sequenced, including Scincinae (7 genera, 18 spp.), Acontinae (2 genera, 3 spp.), Feylininae (1 genus, 1 sp.), and Lygosominae (8 genera, 12 spp.; see Table 1). In order to test the monophyly and placement of Scincidae, representatives from the following Autarchoglossan families were included in the analysis: Xantusiidae (2 spp.), Teiidae (2 spp.), Gymnophthalmidae (2 spp.), Cordylidae (4 spp.), and Lacertidae (3 spp.). *Hemidactylus*, *Gehyra* (Gekkota: Gekkonidae), and *Gambelia* (Iguania: Crotaphytidae) were used to root the tree. Liver, muscle, or tail tissue from each individual was collected into 100% EtOH or salt buffer solution for DNA extraction (see Table 1 for specimen information and GenBank accession numbers).

2.2. Molecular data

Due to the wide range of divergence levels within and among the target taxa, and the breadth of the taxonomic questions being addressed, it was necessary to use multiple mitochondrial and nuclear markers characterized by heterogeneous divergence rates. Moreover, congruence among independent markers provides a better estimate of phylogeny, obviating the concern of gene trees

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Table 1
List of all specimens included in this study, as well as GenBank accession numbers for all sequences used

Species	16S (∼600 bp)	Cytb (∼700 bp)	12S (∼1000 bp)	C-mos (∼600 bp)	18S (∼1800 bp)	Enolase (∼250 bp)
Acontinae						
Acontias litoralis	AY217945	AY217791	AY217996	AY217843[a]	AY217893	–
Acontias percivali	AY217946	AY217792	AY217997	AY217844[a]	AY217894	–
Typhlosaurus caecus	AY217947	AY217793	AY217998	AY217845[a]	AY217895	–
Feylininae						
Feylinia grandisquamis	AY217952	AY217798	AY218002[a]	AY217850[a]	AY217900	AY218044
Lygosominae						
Emoia caeruleocauda	AY217962	AY217808	AY218012	AY217859	AY217910	AY218051
Emoia cyanura	AY217968	AY217814	AY218018	AY217865	AY217916	AY218055
Emoia jakati	AY217958	AY217804	AY218008	AY217855	AY217906	AY218047
Eugongylus rufescens	AY217961	AY217807	AY218011	AY217858	AY217909	AY218050
Lamprolepis smaragdina	AY217957	AY217803	AY218007	AY217854	AY217905	AY218046
Lygisaurus novaeguineae	AY217964	AY217810	AY218014	AY217861	AY217912	AY218052
Mabuya hoeschi	AY217963	AY217809	AY218013	AY217860	AY217911	–
Mabuya spilogaster	AY217959	AY217805	AY218009	AY217856	AY217907	AY218048
Mabuya striata	AY217966	AY217812	AY218016	AY217863	AY217914	AY218054
Scincella lateralis	AY217960	AY217806	AY218010	AY217857	AY217908	AY218049
Sphenomorphus simus	AY217967	AY217813	AY218017[a]	AY217864	AY217915	–
Tiliqua gigas	AY217965	AY217811	AY218015	AY217862	AY217913	AY218053
Scincinae						
Eumeces fasciatus	AY217972	AY217818	AY218022[a]	AY217869	AY217920	AY218057
Eumeces inexpectatus	AY217990	AY217837[a]	AY218040[a]	AY217888	AY217939	AY218075
Eumeces laticeps	AY217989	AY217836	AY218039[a]	AY217887	AY217938	AY218074
Melanoseps occidentalis	AY217973	AY217819	–	AY217870[a]	AY217921	AY218058
Proscelotes eggeli	AY155367[b]	AY217829	AY155368	AY217880	AY217931	AY218067
Scelotes anguineus	AY217981	AY217827	AY218030	AY217878	AY217929	AY218066
Scelotes arenicola	AY217988	AY217835	AY218038	AY217886	AY217937	AY218073
Scelotes bipes	AY217979	AY217825	AY218028	AY217876	AY217927	AY218064
Scelotes caffer	AY217985	AY217832	AY218035	AY217883	AY217934	AY218070
Scelotes gronovii	AY217986	AY217833	AY218036	AY217884	AY217935	AY218071
Scelotes kasneri	AY217987	AY217834	AY218037	AY217885	AY217936	AY218072
Scelotes mirus	AF153586[b]	AY217828	AY218031	AY217879[a]	AY217930	–
Scelotes sexlineatus-1	AY217980	AY217826	AY218029	AY217877	AY217928	AY218065
Scelotes sexlineatus-2	AY217983	AY217830	AY218033	AY217881	AY217932	AY218068
Scelotes sexlineatus-3	AY217984	AY217831	AY218034	AY217882	AY217933	AY218069
Scelotes sp.nov.	AY217978	AY217824	AY218027	AY217875[a]	AY217926	AY218063
Scincus scincus	AY217976	AY217822	AY218025	AY217873	AY217924	AY218061
Sepsina angolensis	AY217975	AY217821	AY218024	AY217872	AY217923	AY218060
Typhlacontias brevipes	AY217974	AY217820	AY218023	AY217871	AY217922	AY218059
Typhlacontias punctatissimus	AY217977	AY217823	AY218026	AY217874[a]	AY217925	AY218062
Cordylidae						
Cordylus namaquensis	AY217950	AY217796	AY218000	AY217848[a]	AY217898	–
Gerrhosaurus nigrolineatus	AY217948	AY217794	AY217999	AY217846	AY217896	–
Tracheloptychus petersi	AY217949	AY217795	–	AY217847[a]	AY217897	–
Cordylosaurus subtesselatus	AY217951	AY217797	AY218001	AY217849	AY217899	–
Xantusiidae						
Xantusia vigilis	AY217993	AY217840	AY218042[a]	AF148703[ab]	AY217942	AY218078
Lepidophyma sylvatica	AY217994	AY217841	AY218043	AY217891	AY217943	AY218079
Teiidae						
Cnemidophorus ocellifer	AY217992	AY217839	AY218041[a]	AY217890[a]	AY217941	AY218077
Tupinambis quadrilineatus	AY217991	AY217838	–	AY217889[a]	AY217940	AY218076
Gymnophthalmidae						
Colobosaura modesta	AY217953	AY217799[a]	AY218003[a]	AF420845[ab]	AY217901	–
Leposoma scincoides	AY217954	AY217800	AY218004	AY217851	AY217902	–
Lacertidae						
Mesalina guttulata	AY217969	AY217815	AY218019[a]	AY217866[a]	AY217917	AY218056
Psammodromus algirus	AY217970	AY217816	AY218020[a]	AY217867[a]	AY217918	–

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Table 1 (*continued*)

Takydromus septentrionalis	AY217971	AY217817	AY218021[a]	AY217868[a]	AY217919	–
Gekkonidae						
Hemidactylus frenatus	AY217955	AY217801	AY218005[a]	AY217852	AY217903	–
Gehyra mutilata	AY217956	AY217802	AY218006	AY217853	AY217904	AY218045
Iguania						
Gambelia wislizenii	AY217944	AY217790	AY217995	AY217842[a]	AY217892	–

Specimen ID numbers and localities are listed in Appendix A.
[a] Sequences are not complete for the entire gene region, partial sequences were used for analysis.
[b] Sequences generated in prior studies, taken from GenBank.

versus species trees (Doyle, 1992, 1997; Moore, 1995). Cytochrome *b* (cytb), 12S rDNA, and 16S rDNA are some of the most commonly used mitochondrial genes in vertebrate phylogenetic studies. Cytb appears to be informative at divergence levels up to 80 Mya (Graybeal, 1994) and in this study resolved relationships within *Scelotes*. Due to the secondary structure of ribosomal DNA, 12S and 16S have both conserved and variable regions, making them informative over a large range of divergence times within squamates (i.e., Pellegrino et al., 2001; Reeder and Wiens, 1996). Among the nuclear genes, 18S rDNA has been empirically shown to be useful in resolving higher-level relationships (divergence times of ~300 Mya; Hillis and Dixon, 1991), and in this study is primarily used to infer relationships between skinks and other families of lizards. C-mos is a proto-oncogene that codes for the protein involved in the arrest of oocyte maturation, and has been used to infer relationships at many levels within squamates (Brehm et al., 2001; Carranza et al., 2002; Harris et al., 1999; Pellegrino et al., 2001; Saint et al., 1998). α-Enolase is an enzyme involved in glycolysis and the gene responsible for its production (in the Peking duck) has been shown to consist of 12 exons and 11 introns (Kim et al., 1991). The primers used in this study were designed to specifically amplify a region consisting of intron eight and small portions of exons eight and nine; this region appears to be informative at interspecific levels (Friesen et al., 1997).

DNA was extracted following a standard phenol/chloroform protocol, and purified using Centricon-100 purification columns (Whiting, 2001). DNA templates and controls were amplified using standard PCR techniques in 50 μl reactions (see Table 2 for primer sequences and general PCR profiles), and products were visualized via 2% agarose gel electrophoresis. The target

Table 2
List of primer sequences and sources, and basic PCR conditions used in the amplification of all gene regions

Primer name	Sequence 5′–3′	Reference	PCR conditions
ALL 18S primers		Whiting (2001)	95(12); 94(1), 54(1), 72(1) × 40; 72(5)
18S b5.5	CGCTATTGGAGCTGGAATTACC	This study	
CYTB1	CCATCCAACATCTCAGCATGATGAAA	Palumbi et al. (1991)	95(3); 94(1), 50(1), 72(1) × 40; 72(5)
CB3H	GGCAAATAGGAARTATCATTC	Palumbi et al. (1991)	
CYTB F.1	TGAGGACARATATCHTTYTGRGG	This study	
CYTB2	CCCTCAGAATGATATTTGTCCTCA	Palumbi et al. (1991)	
CYTB R.2	GGGTGRAAKGGRATTTTATC	This study	
12SZ-L	AAAGGTTTGGTCCTAGCCTT	Goebel et al. (1999)	95(3); 94(1), 50(1), 72(1) × 40; 72(5)
12SK-H	TCCRGTAYRCTTACCDTGTTACGA	Goebel et al. (1999)	
12SA-L	AAACTGGGATTAGATACCCCACTAT	Palumbi et al. (1991)	
12S R.4	GACGGCGGTATATAGGCTG	This study	
12S R.6	ATAGTRGGGTATCTAATCCYAGTTT	This study	
cmosG77.1	TGGCYTGGTGCWGCATTGACT	All C-mos primers were modified from Saint et al. (1998)	95(12); 94(1), 56(1), 72(1) × 40; 72(5)
cmosG79	CCTTTAAGGAGTTCAGGAGCAC		
cmosG74.1	GARCWTCCAAAGTCTCCAATC		
cmosG73.1	GGCTRTAAARCARGTGAAGAAA		
Enol L731	TGGACTTCAAATCCCCCGATGATCCCAGC	Friesen et al. (1997)	95(12); 94(1), 56(1), 72(1) × 35; 72(5)
Enol H912	CCAGGCACCCCAGTCTACCTGGTCAAA	Friesen et al. (1997)	
16S F.1	TGTTTACCAAAAACATAGCCTTTAGC	This study	95(3); 94(1), 50(1), 72(1) × 35; 72(5)
16S R.0	TAGATAGAAACCGACCTGGATT	This study	

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products were purified using the Gene Clean III kit (Bio101 Co.) and sequenced using the Perkin Elmer Big Dye cycle sequencing kit. Purified sequencing reactions were analyzed on either an ABI 377, or ABI 3100 automated sequencer. To insure the accuracy of sequences, negative controls were included in every reaction, complementary strands were sequenced, and sequences were manually checked using the original chromatograph data in the program Sequencher 3.1.1 (GeneCodes Co.). All sequences have been deposited on the GenBank database (see Table 1 for accession numbers).

2.3. Analytical methods

2.3.1. Alignment

Alignment is the process of assigning statements of homology, and has been shown to have a large impact on tree reconstruction (Phillips et al., 2000; Wheeler, 1996). Alignment of protein coding genes (c-mos, and cytb) was based on conservation of the amino acid reading frame, using Sequencher 3.1.1. Ribosomal DNA has long proven to be one of the greatest challenges for alignment, and the common practices of aligning data by eye or manually adjusting computer alignments are subjective and can bias the final topology (Wheeler, 1996). Therefore 18S, 16S, 12S, and α-Enolase were all aligned using optimization alignment (OA) in the computer program POY (Gladstein and Wheeler, 1999–2002). OA combines alignment and tree reconstruction into a single step, thereby minimizing assumptions and using the same parameters for both tasks (see Wheeler, 1996, 1999, for a detailed explanation). Each gene is divided into conserved and variable regions (for ribosomal DNA these regions are comparable to secondary structure of stems and loops) that are entered into POY as separate files, meaning all regions can be analyzed individually or together, but alignment is constrained to take place only within each specified region. In this way, morphological or protein coding data can also be entered as a pre-aligned data partition so that no shift in alignment will take place, but those characters will be used in the optimization of all characters on the tree (Frost et al., 2001; Wheeler, 1995, 1996). OA results in a topology, but one can also choose to have an implied alignment produced from the OA tree. In this way, POY is used to produce alignments for further analysis in other programs and under other optimality criteria. All POY analyses were run on an IBM SP 2 supercomputer. Analysis was performed on each gene individually as well as the combined data set using the following search strategy: "-fitchtrees -parallel -noleading -norandomizeoutgroup -impliedalignment -sprmaxtrees 1 -tbrmaxtrees 1 -maxtrees 5 holdmaxtrees 50 -slop 5 -checkslop 10 -buildspr -buildmaxtrees 2 -random 50 -stopat 25 -multirandom -treefuse -fuselimit 10 -fusemingroup 5 -fusemaxtrees 100 -numdriftchanges 30 -driftspr -numdriftspr 10 -drifttbr -numdrifttbr 10 -slop 10 -checkslop 10 -seed -1".

2.3.2. Tree reconstruction

Tree reconstruction via OA was performed in POY (Gladstein and Wheeler, 1999–2002). In order to further explore the data, implied alignments from POY were also analyzed in PAUP* 4.0b10 (Swofford, 1999) under both parsimony (MP) and maximum likelihood (ML) criteria, and using Bayesian analysis in the computer program Mr. Bayes (Huelsenbeck and Ronquist, 2001). All MP searches were performed with equal character weighting, 10,000 random addition sequences with tree bisection reconnection (TBR) branch swapping, and with gaps treated both as missing data and as a fifth state. Under the ML criterion, the appropriate model of nucleotide substitution was selected using Modeltest 3.0 (Posada and Crandall, 1998). The chosen model of evolution was then implemented for ML searches consisting of 100 random addition sequences with TBR branch swapping. All ML searches were performed on an IBM SP2 supercomputer to reduce computational time. The selected nucleotide substitution model was also used in Bayesian analysis, with specific parameter values estimated as part of the analysis, consisting of 1,000,000 generations with four incrementally heated chains, and trees sampled every 20 generations. Stationarity was reached before 3000 generations, and after discarding these first 150 trees (burn in), the 50% majority rule tree was obtained from the remaining 49,850 data points.

2.3.3. Branch support

Posterior probabilities were assessed as part of the Bayesian analysis. For MP analyses Bremer support (Bremer, 1994) and partitioned Bremer support (Baker and DeSalle, 1997) were calculated using Treerot (Sorenson, 1999) and PAUP* 4.0b10 (Swofford, 1999). Nodal support was also assessed using nonparametric bootstrapping as performed in PAUP* 4.0b10, with 10,000 bootstrap replicates of 10 random sequence additions each, and TBR branch swapping for MP trees, and with 100 bootstrap replicates of five random sequence additions each, and TBR branch swapping for ML trees.

2.3.4. Sensitivity analysis

Sensitivity analysis provides an alternative assessment of nodal support in that it allows one to explore the sensitivity of the data and specific relationships and conclusions to perturbations of analytical parameters. Relationships that appear in all or most of the sensitivity analyses are those that are robust to varied assumptions of alignment and tree reconstruction parameters. Each gene region was analyzed individually in POY using multiple parameter sets (see Table 3), and all data were then combined and analyzed under these same param-

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Table 3
Optimization alignment results

Parameter set	1:1:1	2:1:1	2:2:1	3:1:1	3:2:1	3:3:1	4:1:1	4:2:1	4:3:1	4:4:1
18S length	171	203	254	231	286	338	260	317	368	419
16S length	2104	2449	3201	2698	3562	4268	2906	3843	4655	5320
12S length	4579	5356	6914	5886	7669	9123	6425	8280	9948	11,441
cmos length	992	992	1289	992	1289	1505	992	1289	1505	1879
Cytb length	4257	4257	6117	4257	6117	6782	4257	6117	6782	9872
Enol length	585	778	956	926	1133	1294	1055	1291	1476	1635
Combined length	13,029	14,610	19,311	16,580	21,805	25,529	17,851	22,273	28,276	33,054
ILD metric	0.02617	0.0394	0.03	0.0959	0.08	0.936	0.1096	0.051	0.125	0.075

The ILD metric is computed from individual and combined tree lengths and attempts to find the topology that best fits all individual data partitions, therefore the parameter set (in this case 1:1:1) with the smallest ILD metric is preferred. Parameter sets refer to the cost assigned a given change (Gap:Tv:Ts), and tree length results are listed for individual and combined analyses for each parameter set.

eter sets. In an attempt to minimize incongruence between data sets, an ILD metric was computed for each parameter set by subtracting the sum of the individual tree lengths from the combined tree length, and then dividing by the combined tree length (Phillips et al., 2000; Wheeler et al., 2001). In this way, the ILD metric is not used as a statistical test of incongruence or to determine the cause of incongruence, but rather as a method of finding the parameter set resulting in the topology that best fits all individual data partitions. Therefore, the parameter set with the smallest ILD metric was chosen as the best estimate of relationships, while trees from all parameter sets were used to evaluate the stability of specific relationships across the parameter landscape.

2.3.5. Reconstructing ancestral states

Parsimony is the most widely used method for reconstructing ancestral character states and testing hypotheses of character evolution. Parsimony attempts to minimize the number of changes in ancestral character states, while making relatively few assumptions about the evolutionary processes involved (Cunningham et al., 1998; Maddison and Maddison, 1992; Schluter et al., 1997; Swofford and Maddison, 1992). Because parsimony reconstruction minimizes change and does not incorporate branch length information, it may fail when rates of character evolution are high, or divergence times between taxa are great (Cunningham, 1999; Cunningham et al., 1998; Frumhoff and Reeve, 1994; Pagel, 1994; Schluter et al., 1997; Shultz et al., 1996). Maximum likelihood methods combine branch lengths with terminal character states to determine rates of change for characters and reconstruct a probability for each ancestor having a specific character state. In this study, ancestral character states were reconstructed using both parsimony and likelihood methods, and differences in the resulting reconstructions were addressed.

Parsimony reconstructions were performed in MacClade 4.0 (Maddison and Maddison, 2000), for both fore and hind limb characters. In an attempt to look at both the complete loss of limbs, as well as the assumption of a gradual loss of digits through evolutionary time, one binary character was coded for the presence or absence of limbs, while a second multistate character was coded for the number of digits per limb. This resulted in two fore limb characters and two hind limb characters, and ancestral states were reconstructed with characters treated as unordered, ordered, and irreversible. Different optimizations were evaluated by the difference in the number of steps required for each.

Maximum likelihood reconstructions were performed in the program Discrete 4.0 (Pagel, 1999), which is designed for two discretely coded binary characters. This program allows one to test for correlated evolution, as well as reconstruct ancestral character states using both one and two rate models (forward and reverse rates of character change can be set independently). Discrete was run using the topology and branch lengths generated in the ML analysis, and fore and hind limbs were coded as present = 0 or absent = 1. Likelihoods for each node of interest were calculated using "local" estimates by setting the state equal to 0 and 1 successively (Pagel, 1999). Due to the widely held view that complex characters such as limbs are more easily lost than gained (Gould, 1970; Omland, 1997; Waters et al., 2002), analyses were run under various forward (limb loss) and reverse (limb gain) rate parameters: forward and reverse parameters unrestricted, forward rate = reverse rate of change, and the forward rate equaling 10 and 100 times the reverse rate. All analyses were run multiple times to ensure accuracy.

3. Results

3.1. Molecular data

The molecular data collected include approximately 5000 bases across six gene regions for 52 taxa (see Table 1). Uncorrected pairwise sequence divergence for each gene across all taxa, within skinks, within sub-Saharan African scincines, and within *Scelotes* are shown in

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Table 4
Uncorrected pairwise sequence divergence across various taxonomic levels for each molecular marker used in this study

Gene region	All taxa (%)	Skinks (%)	Sub-Saharan scincines (%)	*Scelotes* (%)
18S	4.5	2.6	0.97	0.06
C-mos	27.8	13.1	8.0	2.3
16S	23.6	17.3	13.6	8.7
Enol	34.9	23.6	13.1	2.6
12S	33	24.7	22.9	15.7
Cytb	56	27	22.8	21.2

Table 4. These divergence profiles reflect great variation in the rates of evolution among the markers, and suggest their phylogenetic utility at different taxonomic levels.

3.2. Optimization alignment

Tree lengths for all optimization alignment (OA) searches are shown in Table 3. We combined all data to provide the best estimate of phylogeny (Chippindale and Wiens, 1994; Eernisse and Kluge, 1993; Kluge, 1989; Kluge and Wolf, 1993; Kluge, 1998; Nixon and Carpenter, 1996), and topologies from individual gene analyses were not evaluated separately, but only used in calculating the ILD metric. The parameter set of 1:1:1 (gap cost:transversion cost:transition cost) minimized incongruence among data sets (as shown by the ILD metric in Table 3). One tree (length 13,029) resulted from the OA search, and is shown in Fig. 1. The implied alignment from this topology was analyzed under MP in PAUP*, with gaps coded as a fifth state and as missing data, and both resulted in a topology identical with the OA tree (proportional branch lengths change slightly with the handling of gaps).

The OA and MP topologies (Fig. 1) recover a monophyletic Scincidae (clade S) with strong support (bootstrap proportion [BP] = 100%, Bremer index [BI] = 58), and a (Xantusiidae + Cordylidae) clade as its sister group (BP = 89; BI = 41; Fig. 1). Within skinks, the subfamily Acontinae is strongly supported as monophyletic (clade A; BP = 100; BI = 79) and is the sister group to the rest of the family (BP = 100; BI = 32). The remaining skinks are divided into two main clades, one consisting of lygosomines + *Eumeces* and *Scincus* (clade B; BP = 98; BI = 24), and the other including sub-Saharan African scincines + *Feylinia* (clade C; BP = 99; BI = 17). Within clade B there are two distinct clades, one composed of (*Scincella* + *Sphenomorphus*) as sister group to North American *Eumeces*, and the other with *Scincus* basal to multiple taxa including *Tiliqua*, *Mabuya*, *Lamprolepis*, *Eugongylus*, *Lygisauria*, and *Emoia*. Clade C is also split into two smaller clades, one consisting of (*Feylinia* + *Melanoseps*) as sister group to *Typhlacontias*, and the other composed of a monophyletic *Scelotes* with *Proscelotes* as its sister taxon, and *Sepsina* basal to this entire group.

3.3. Maximum likelihood and Bayesian analysis

Modeltest analysis indicates that GTR + G + I is the appropriate model of nucleotide substitution for the combined data set, with G = 0.6648, I = 0.5134, base frequencies of A = 0.3109, C = 0.2765, G = 0.1822, T = 0.2304, and transition/transversion rates of A–C = 2.7463, A–G = 4.7317, A–T = 2.0502, C–G = 0.6971, and C–T = 10.6625. ML analysis with the above-stated model recovered a single tree ($-\ln l$ score 55382.9834) with a topology identical to the MP analysis except for the placement of *Scelotes caffer* and *Scelotes gronovii*, whereas Bayesian analysis (under the model stated above) recovered a topology identical to the MP topology. Estimates of nodal support for trees recovered in the ML and Bayesian analyses were roughly equivalent to those for the MP analyses across all but two clades, in which ML estimates were lower and Bayesian estimates were higher, respectively (see Table 5).

3.4. Sensitivity analysis

Many monophyletic groups are recovered in all analyses including: Scincidae (clade S), Acontinae (clade A), (Scincinae + Lygosominae + Feylininae) (clade B + C), *Scelotes*, (*Proscelotes* + *Scelotes*), (*Feylinia* + *Melanoseps*), ((*Feylinia* + *Melanoseps*) + *Typhlacontias*), and (sub-Saharan African scincines + *Feylinia*) (clade C), whereas other relationships were dependent on parameters of tree reconstruction, most notably the placement of *Sepsina* (see Table 6). *Sepsina* is always a basal component of clade C, but it shifts between the (*Proscelotes* + *Scelotes*) and the (*Feylinia* + *Melanoseps* + *Typhlacontias*) clades as a function of alignment parameters. The monophyly of clade B, while supported by many of the sensitivity analyses, is questionable as sampling in this study was not designed to address this question, and the placement of *Scincus* and *Eumeces* are problematic.

3.5. Character reconstruction

When limbs are coded as two binary characters (presence or absence of fore and hind limbs, respectively), the cost of parsimony reconstruction is five steps under all optimization modes (data not shown). Coding fore and hind limb characters for the number of external digits missing (state 0 = five digits, state 1 = 1 digit missing, etc.), produces multistate characters that can be treated as ordered or unordered. Unordered reconstruction of forelimb digit characters has a cost of 7 and includes support for two instances of limb gain (*Scelotes mirus* with five digits and *S. caffer* with two digits), with multiple equivocal nodes (see

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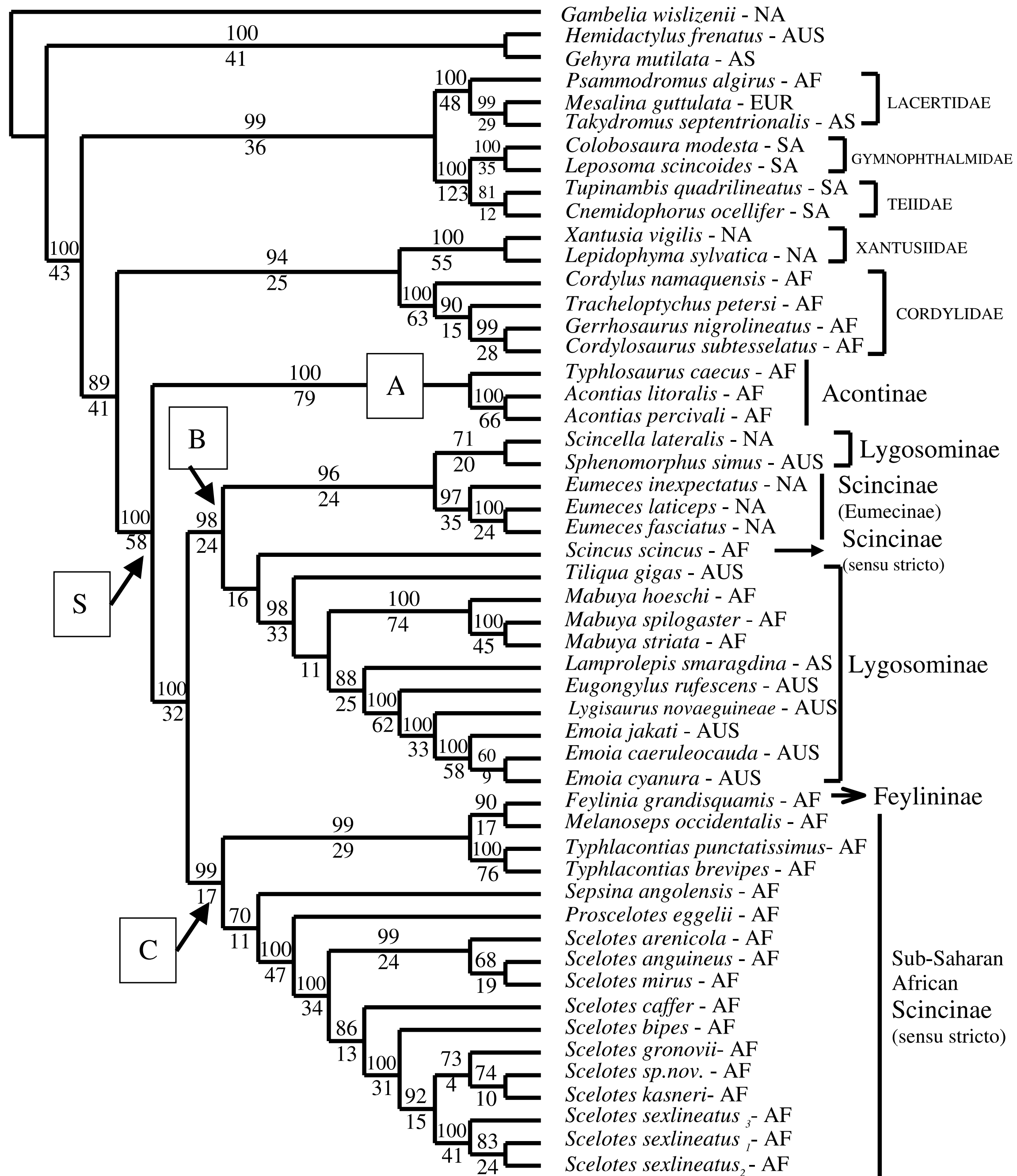

Fig. 1. Optimization alignment (parameter set 1:1:1) and Parsimony (gaps coded as 5th state) topology, cost 13,029. Numbers above branches are bootstrap support (values below 50% are not shown); numbers below branches are Bremer support values. Clade S, Scincidae; clade A, Acontinae; clade B, Lygosominae + *Eumeces* + *Scincus*; and clade C, sub-Saharan African scincines + *Feylinia*. Species names are followed by the continent of origin: AF, Africa; AS, Asia; AUS, Australia; NA, North America; SA, South America; and EUR, Europe (specific locality information is listed in Appendix A).

Fig. 2a). Ordering the forelimb digit character requires 23 steps and still supports reversals, while forcing irreversibility has a cost of 27 (see Figs. 2b and c). The reconstruction of the hind limb digit character shows similar results, with an unordered cost of nine supporting one reversal with many equivocal nodes, an ordered cost of 24, and an irreversible cost of 28 (data not shown).

Likelihood reconstruction results in probabilities for ancestral states, which can provide more confidence in results but also leads to more ambiguity in reconstructions than a parsimony analysis. When rates for limb gain and loss of are allowed to change freely on the tree, support is found for two limb gains (95–100% probability) just as in parsimony reconstructions, but this support becomes ambiguous (<85%) when the rate of

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Table 5
Nodal support values for selected relationships

Relationship	Optimization alignment—partitioned Bremer support: 18S/16S/12S/Enol/C-mos/cytb = total Bremer support	MP-bootstrap%	ML-bootstrap%	Bayesian-posterior probability
Monophyly of Scincidae	5/19/22/0/9/3 = 58	100	100	0.99
Monophyly of Acontinae (clade A)	3/28/32/0/14/2 = 79	100	100	1.0
Monophyly of *Scelotes*	0/10/16/7/2/−1 = 34	100	100	1.0
Proscelotes + *Scelotes*	0/17/10/3/8/9 = 47	100	100	1.0
Sepsina + (*Proscelotes* + *Scelotes*)	0/10/3/1/−1/−2 = 11	70	55	1.0
Feylinia + *Melanoseps*	4/0/0/11/1/1 = 17	90	82	1.0
(*Feylinia* + *Melanoseps*) + *Typhlacontias*	7/2/11/2/4/3 = 29	99	100	1.0
Acontinae sister to remaining Scincidae	0/14/10/0/6/2 = 32	100	100	0.99
Sub-Saharan African scincines + *Feylinia* (clade C)	0/5/5/3/2/2 = 17	99	100	1.0

Table 6
Results of sensitivity analysis indicating clade stability under a range of optimization alignment parameters (gap cost:transversion cost:transition cost), maximum parsimony (MP), maximum likelihood (ML), and Bayesian analysis (Bayes)

Relationship	1:1:1	2:1:1	2:2:1	3:1:1	3:2:1	3:3:1	4:1:1	4:2:1	4:3:1	4:4:1	MP	ML	Bayes
Monophyly of Scincidae	X	X	X	X	X	X	–	X	X	X	X	X	X
Monophyly of Scincinae	–	–	–	–	–	–	–	–	–	–	–	–	–
Monophyly of Lygosominae	–	–	–	–	–	–	–	–	–	–	–	–	–
Monophyly of Acontinae (clade A)	X	X	X	X	X	X	X	X	X	X	X	X	X
Acontinae as sister group to remaining skinks	X	X	X	X	X	X	–	X	X	X	X	X	X
Sub-Saharan African. scincines + *Feylinia* (clade C)	X	X	X	X	X	X	X	X	X	X	X	X	X
Lygosominae + *Eumeces* + *Scincus* (clade B)	X	X	X	X	X	X	–	–	X	X	X	X	X
Monophyly of *Scelotes*	X	X	X	X	X	X	X	X	X	X	X	X	X
Scelotes + *Proscelotes*	X	X	X	X	X	X	X	X	X	X	X	X	X
Sepsina + (*Proscelotes* + *Scelotes*)	X	–	–	–	–	–	–	X	X	–	X	X	X
Sepsina + ((*Feylinia* + *Melanoseps*) + *Typhlacontias*)	–	X	X	X	X	X	X	–	–	X	–	–	–
Feylina + *Melanoseps*	X	X	X	X	X	X	X	X	X	X	X	X	X
(*Feylinia* + *Melanoseps*) + *Typhlacontias*	X	X	X	X	X	X	X	X	X	X	X	X	X
Sister group to Scincidae	Xa + C	Xa + C	Xa + C	C + L	Xa + C	scinc	Xa + C	C	Xa + C	Xa + C	Xa + C	Xa + C	Xa + C

Xa, Xantusiidae; C, Cordylidae; L, Lacertidae; and scinc, remaining Scincomorpha. Presence of a relationship is denoted with X.

limb gain is constrained to be equal to limb loss. When the rate of limb loss is set at 10 times (or more) that of limb gain, the reconstruction of ancestral states is unambiguous, and matches the irreversible parsimony reconstruction (Fig. 2c) for both fore and hind limbs (data not shown).

4. Discussion

4.1. Taxonomic implications

4.1.1. Sister group to skinks

While the monophyly of Scincidae is confirmed in all analyses, the sister group to skinks does vary in sensitivity analyses (see Table 6). Past studies within Scincomorpha have found strong support for a sister group relationship between skinks and cordylids (Scincoidea) (Estes et al., 1988; Odierna et al., 2002; Schwenk, 1988; Vicario et al., 2003), but the placement of Xantusiidae has been problematic (Estes, 1983; Estes et al., 1988; Evans and Chure, 1998; Lang, 1991; Lee, 1998; Macey et al., 1997; Presch, 1988; Rieppel, 1980), although some studies have found support for the sister group relationship of skinks and xantusiids (e.g., Harris et al., 1999, 2001; Presch, 1988). The final results of this study support (Cordylidae + Xantusiidae) as the primary outgroup to skinks, and generally support the Estes et al. (1988) topology for Scincomorpha (with the placement of Xantusiidae as the only exception).

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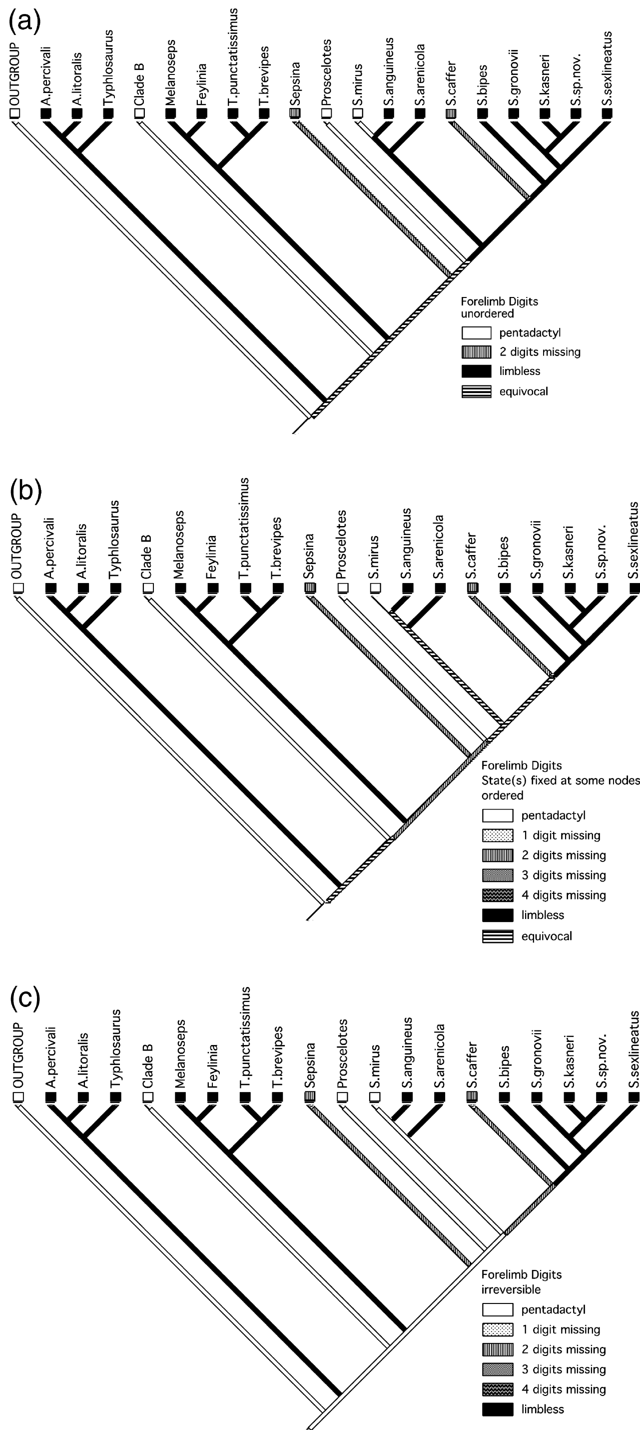

Fig. 2. Parsimony reconstructions for forelimb digit character: 0—pentadactyl, 1—1 digit missing, 2—2 digits missing, etc. *Note.* in order to simplify figures the entire tree has not been shown. (a) Unordered character reconstruction with a total cost of 7, showing support for limb gain (*S. mirus* and *S. caffer*). (b) Ordered character reconstruction with a cost of 24. (c) Irreversible character reconstruction with a total cost of 28.

4.1.2. Monophyly of subfamilies

This study only provides support for the monophyly of the skink subfamily Acontinae. A single representative of Feylininae is included, so monophyly of this subfamily cannot be tested, but the relationship of *Melanoseps* + *Feylinia* is strongly supported. This is in partial agreement with Greer's hypothesis that the Feylininae were "derived from the *Scelotes–Melanoseps–Scolecoseps* line of scincines (Greer, 1985, p. 143)." Further sampling will determine if Feylininae should be subsumed within Scincinae, or if *Melanoseps* and *Typhlacontias* should be included in Feylininae. Based on our limited sampling, neither Scincinae nor Lygosominae is monophyletic. Members of the genus *Eumeces* (only North American taxa sampled) are supported as the sister group of representative *Sphenomorphus* Group lygosomines, while *Scincus* is weakly supported as the sister taxon of the remaining lygosomines, representing both the *Eugongylus* and *Mabuya* Groups (*sensu* Greer, 1979, 1989).

The paraphyly of Scincinae is not unexpected. Greer (1970b) initially suggested that each of his other subfamilies (Acontinae, Feylininae, and Lygosominae) was derived from within scincines. Scincine paraphyly has more recently been proposed by Griffith et al. (2000), who erected a new subfamily, Eumecinae, to accommodate a putatively monophyletic group of chiefly North American, Central American, and East Asian *Eumeces* that they regarded as basal to lygosomines plus remaining scincines. Although our results suggest that Eumecinae is not the sister group of the remaining Scincidae, its relatively basal position among the scincine + lygosomine clade (exclusive of the sub-Saharan African scincines) does receive support within the framework of our limited taxon sampling.

The non-monophyly of Lygosominae, however, is a surprising result. Greer (1970b, 1986) has provided several morphological synapomorphies of this group and these have been accepted, although not rigorously tested, by virtually all subsequent workers (e.g., Griffith et al., 2000; Honda et al., 2000). Hutchinson (1981), based on immunologically derived data, argued however that the *Sphenomorphus* group was only distantly related to other lygosomines, a conclusion with which we concur. Our results strongly suggest that the chalcidine head scale pattern of Greer and Shea (2000) is primitive within skinks or that it has evolved independently in acontines and in the African scincines. These results must be regarded as tentative, however, as the sampling in this study was designed to test only the monophyly of sub-Saharan African scincines and not that of the entire subfamily, or of lygosomines.

Although Greer's (1970b) hypothesis of the origin of all other skinks from within scincines is not supported by our results, his hypothesis of an original southern African diversification for the family followed by expansion through Asia and Australia is supported with the basal position of acontines within Scincidae, and the sister group relationship of sub-Saharan African

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scincines (including *Feylinia*) to the remaining scincines and lygosomines sampled.

4.1.3. Acontinae

Acontinae is a monophyletic group (Daniels et al., 2002; Greer, 1970b) comprised of three genera and 18 spp., all of which are completely limbless and burrowing. Previous hypotheses suggested that Acontinae was a derivative of the *Sepsina–Proscelotes* group of scincines (Greer, 1985). The strongly supported basal position of acontines within Scincidae is therefore a surprising result. It has also been suggested that acontines may be more closely related to dibamids than to other skinks (Rieppel, 1980, 1984), as they share many derived characters with *Dibamus* and some with *Anelytropsis* (Estes et al., 1988; Greer, 1985; Rieppel, 1984). Dibamid relationships have also been suggested for *Feylinia* (Boulenger, 1884; Camp, 1923; Cope, 1885). No dibamids were included in this study so these hypotheses cannot be tested, but the results found here suggest that they cannot be related to both acontines and *Feylinia*, and this only adds further intrigue to the debate over their placement.

4.1.4. Lygosominae

Although the sampling in this study was not designed to address questions of lygosomine relationships, Greer's *Sphenomorphus* group is supported by the sister group relationship of *Scincella* and *Sphenomorphus*, and the *Eugongylus* group is supported by the clade consisting of *Eugongylus*, *Lygisaurus*, and *Emoia*. The sampled members of the *Mabuya* group (*Lamprolepis*, *Mabuya*, and *Tiliqua*); (Greer, 1979, 1989) do not appear to be monophyletic, but rather constitute several lineages basal to the *Eugongylus* group. The paraphyly of the *Mabuya* group was also reported by Honda et al. (1999) based on the analysis of 12S and 16S rRNA data. Our overall results regarding lygosomine relationships are in general agreement with those of Honda et al. (2000), who also found that the *Sphenomorphus* group is basal to other lygosomines (as did Greer, 1979, 1989). Honda et al. (2000) also found support, albeit weak, for the monophyly of a clade consisting of the *Eugongylus* group of Greer (1979) plus a restricted *Mabuya* group. The *Egernia* group (*sensu* Greer, 1979), regarded by Greer (1989) as part of a larger *Mabuya* group, was found to be basal to this clade by both Honda et al. (2000) and this study.

4.1.5. Sub-Saharan African scincines

The placement of *Sepsina* varies in sensitivity analyses, but is well supported in the final tree. Greer (1970a) divided southern African scincines into two groups, with *Sepsina* and *Proscelotes* forming a primitive group based on presence of a large postorbital bone, open supratemporal fenestra, and small interparietal scale that does not contact the supraocular scales. *Sepsina* also retains the primitive character of pterygoid teeth. These morphological characters lend support to the placement of *Sepsina* as basal to (*Proscelotes* + *Scelotes*). The *Typhlacontias*, *Melanoseps*, *Feylinia* clade is a highly derived group modified for burrowing with almost complete limb loss, relatively short tail lengths, and loss of external ear openings. Greer (1970b) noted the morphological similarity between *Typhlacontias* and *Feylinia*, but could not distinguish convergence from homology; our data support the interpretation that the shared similarities between the two genera are synapomorphic. The long branch lengths within this group in the maximum likelihood tree (tree not shown) indicate large evolutionary distances between these taxa, but identical relationships are recovered in parsimony and likelihood analysis (with high nodal support), and in every sensitivity analysis, suggesting that their position in the phylogeny is well supported by these data. Our findings thus contradict the suggestion that *Sepsina* (or *Sepsina* and *Proscelotes*) are allied to acontines and that *Scelotes* and *Melanoseps* were members of a lineage that gave rise to feylinines (de Witte and Laurent, 1943; Greer, 1985).

4.1.6. Scelotes

The monophyly of *Scelotes* is among the most well supported results of this study (Fig. 1; Table 6). There is slight variation in the placement of two species (*S. caffer* and *S. gronovii*) among analyses, but beyond that relationships within the genus are stable. There is a geographic split in the genus, with the eastern and the western species forming separate clades. The species with western distributions are well sampled in this study, and appear to be closely related (except *S. caffer*) as shown by the short branch lengths in the maximum likelihood tree (tree not shown). All species of this clade have an opaque or transparent window in the lower eyelid, small ear openings and, with the exception of the basal *S. caffer*, have lost the forelimb entirely and retain only two digits on the hind limb (one in *S. gronovii*). The species with eastern distributions are not well sampled, therefore little can be said of this group. Although he did not perform a cladistic analysis, Broadley (1994) proposed that *S. mirus* was the most primitive of the eastern species and *S. arenicola* the most derived, based on a presumed progressive loss of digits and limbs. This study does not support a progressive loss of digits and places *S. arenicola* basal to the eastern group with *S. mirus* more derived, although this may be due to lack of sampling in this group. Within the genus, *S. caffer* is most enigmatic in its placement, coming out basal to either the eastern or western clade in various sensitivity analyses. *S. caffer* is distributed in scattered populations in the eastern and western cape of South Africa, in

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contrast to the majority of *Scelotes* species which have small but continuous distributions. The entire fragmented range of *caffer* needs to be explored as it may be the link between the eastern and western groups, or may represent a complex of species (Branch and Bauer, 1995).

4.2. Limb loss

Due to their complex nature, it has been argued that limbs can be lost but not regained (Gans, 1975; Greer, 1991; Presch, 1975). One can imagine, however, a scenario in which a developmental pathway is truncated or turned off, thereby resulting in a limbless organism, but one that still possesses all of the information to grow a limb (Galis et al., 2001). If it is true that limb development is plastic, then phylogenetic relationships based exclusively on limb and digital characters need to be revaluated with larger character sets. In this study, parsimony reconstruction of digit characters supports the reversal from limbless to limbed, but the difference between the cost of this reconstruction and the irreversible reconstruction is only four steps (Fig. 2). Likelihood reconstructions also show some level of support for reversal when parameters are free, but when the rate of limb loss becomes higher than the rate of limb gain, no support for reversal remains. On the basis of known cases of hyperphalangy among squamates, Greer (1992) estimated that the loss of a single phalanx is about 5.3 times more common than a gain. Therefore, the phylogenetic results of this study do not provide conclusive evidence that limb development is a plastic trait showing equally probable forward and reverse changes throughout evolutionary time. Rather, a conservative interpretation supports the age-old idea that limbs have been lost many times for many reasons, but not regained. On the other hand, our results show no evidence for the progressive loss of digits within *Scelotes*, and weakly support plasticity of digit number (the eastern clade of *Scelotes*). At this time, reversibility of digital and phalangeal loss has only been proposed in *Lerista* (Hauser, 1996; Kendrick, 1991), and these results remain controversial.

5. Conclusions

This study is the first to use molecular data to investigate relationships among sub-Saharan African scincines, and is the largest sampling of genes ever generated for skinks. Within sub-Saharan African scincines *Scelotes*, *Proscelotes*, and *Sepsina* form one clade, while *Typhlacontias*, *Melanoseps*, and *Feylinia* compose a second, primarily limbless clade. These results and the monophyly of sub-Saharan African scincines provide the necessary outgroup information and will be the foundation for all further study within the genera that compose this group. Relationships within *Scelotes* were also investigated in an attempt to better understand the evolution of limb loss. Although sampling was not ideal, some support was found for the reversal of limb and digit loss. These results stress the need for more comprehensive study of the morphological and developmental pathways involved in limb production.

This large molecular data set not only clarifies relationships within sub-Saharan African scincines but also provides insight into higher level relationships within skinks. The monophyly of Scincidae is confirmed, and the primary outgroup to the family supported by these data is a (Xantusiidae + Cordylidae) clade. Within skinks the subfamily Acontinae is monophyletic while the Lygosominae and Scincinae are not. While these results are not entirely unexpected, this study has shown the great need for a comprehensive look at phylogenetic relationships within skinks and the taxonomic revisions needed at the subfamilial level.

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Appendix A

List of all specimen identification numbers and localities. Museum abbreviations follow Levinton et al. (1985) except as follows: AMB, Aaron M. Bauer (specimens to be deposited in AMS); AJL-FN, Angelo J. Lambiris Field number; Bezy, Robert Bezy field number; LG, Miguel T. Rodrigues field number, NJK, Nathan J. Kley field number; Pettrade, specimen obtained through the pet trade; No Voucher, no voucher specimen taken (the lizard was identified, non-destructively sampled, and released)

Species	Specimen ID #	Locality
Acontinae		
Acontias litoralis	CAS 206800	South Africa: Northern Cape Province; vic. McDougall Bay water tank
Acontias percivali	YPM 12687	Unknown
Typhlosaurus caecus	AMB 6817	South Africa: Northern Cape Province; 9.9 km S. of Lambertsbaai
Feylininae		
Feylinia grandisquamis	NJK 0069	Unknown
Lygosominae		
Emoia cyanura	BYU 47334	Fiji: Viti Levu; Sigatoka
Emoia caeruleocauda	BYU 47567	Papua New Guinea: Gulf Province; Ivimka Research Station, Lakekamu Basin
Emoia jakati	BYU 47357	Papua New Guinea: Milne Bay Province; Alotau International Hotel grounds
Eugongylus rufescens	BYU 46974	Papua New Guinea: Eastern Highlands Province; Herowana Village
Lamprolepis smaragdina	BYU 47331	Unknown
Lygisaurus novaeguineae	BYU 47351	Papua New Guinea: Gulf Province; Ivimka Research Station, Lakekamu Basin
Mabuya hoeschi	CAS 206963	Namibia: Kunene region; Khorixas Dist.; Sesfontein Rd., 52 km N. of Palmweg
Mabuya spilogaster	CAS 206938	Namibia: Erongo Region; Karibib Dist.; Usakos-Hentiesbaai Rd., 10 km E. of Spitzkop turnoff
Mabuya striata	CAS 206970	Namibia: Kunene Region; Opuwo Dist.; Opuwo Rd., 87.6 km N. of Palmweg-Sesfontein Rd
Scincella lateralis	BYU 47335	Florida: Liberty Co.; Camel Lake Recreational Area
Sphenomorphus simus	BYU 47016	Papua New Guinea: Gulf Province; Ivimka Research Station, Lakekamu Basin
Tiliqua gigas	BYU 46821	Papua New Guinea: Gulf Province; Kakoro Village, Lakekamu Basin
Scincinae		
Eumeces laticeps	BYU 47336	Florida; Duval Co., Little Talbot Island
Eumeces inexpectatus	BYU 46699	Florida; Duval Co., Little Talbot Island
Eumeces fasciatus	BYU 46698	Florida; Holmes Co., Ponce de Leon Springs
Melanoseps occidentalis	CAS 207873	Equatorial Guinea: Bioko Id.; Cast Road, ca. 5 km S. of Luba
Proscelotes eggeli	CAS 168959	Tanzania: Tanga Region; Lushoto Dist.; West Usambara Mnts., Mazumbai Forest Reserve
Scelotes anguineus	AJL-FN 452	South Africa: Eastern Cape Prov.; Port Elizabeth
Scelotes arenicola	CAS 209635	South Africa: KwaZulu Natal Prov.; Kosi Bay Nature Reserve, NW Corner of Lake Nhlange
Scelotes bipes	CAS 224005	South Africa: Western Cape Prov.; ~4.6 km N. of Grootbaai, Bloubergstrand on Melkbos Rd.
Scelotes caffer	CAS 206859	South Africa: Northern Cape Prov.; Brandberg, Farms Kourootje and Kap Vley, De Beers Mining area

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Appendix A (*continued*)

Species	Specimen ID #	Locality
Scelotes gronovii	CAS 206990	South Africa: Western Cape Prov.; 18.5 km N. of jct rd R365 on R27 towards Lambertsbaai
Scelotes kasneri	CAS 206991	South Africa: Western Cape Prov.; 18.5 km N. of jct rd R365 on R27 towards Lambertsbaai
Scelotes mirus	No Voucher	Swaziland: Malolotja Reserve
Scelotes sexlineatus-1	CAS 206813	South Africa: Northern Cape Prov.; Port Nolloth
Scelotes sexlineatus-2	CAS 206819	South Africa: Northern Cape Prov.; McDougall Bay
Scelotes sexlineatus-3	CAS 206854	South Africa: Northern Cape Prov.; Brandberg,, Farms Kourootje and Kap Vley, De Beers Mining area
Scelotes sp.nov	CAS 223934	South Africa: Western Cape Prov.; ~4.6 km N. of Grootbaai, Bloubergstrand on Melkbos Rd
Scincus scincus	YPM 12686	Unknown
Sepsina angolensis	SMW 6694	Namibia: Kunene Reg.; Kamanjab District
Typhlacontias brevipes	CAS 206947	Namibia: Erongo Reg.; Walvis Bay Dist.; S. bank of Kuiseb Rv. Near Rooibank Rd
Typhlacontias punctatissimus	CAS 223980	Namibia: Kunene Reg; ~1.1 km N. of Munutum Rv, at Skeleton Coast Park east boundry
Cordylidae		
Cordylus namaquensis	CAS 223964	Namibia: Karas Reg.; Karasburg Dist.; Farm Narudas, ~0.3 m N. of house
Gerrhosaurus nigrolineatus	No Voucher	Pettrade
Tracheloptychus petersi	YPM 12691	Unknown
Cordylosaurus subtesselatus	AMB 6861	Namibia: Karas Reg.; Karasburg Dist.; Farm Narudas, Rd. at river crossing
Xantusiidae		
Xantusia vigilis	Bezy6248	Arizona: Yavapai Co.; 0.8 miles (by Hwy 93) SE Nothing
Lepidophyma sylvatica	ENEPI 4011	Mexico: San Luis Potosi; 27 km (by Hwy 80) NE Ciudad del Maiz
Teiidae		
Tupinambis quadrilineatus	LG1132	Brazil: Goias; Niquelandia
Cnemidophorus ocellifer	MZ 78779	Brazil: Mato Grosso; Barra do Garcas
Gymnophthalmidae		
Colobosaura modesta	MZ 8956	Brazil: Goias; Niquelandia
Leposoma scincoides	LG1409	Brazil: Bahía; Una
Lacertidae		
Mesalina guttulata	No Voucher	Egypt: Harraat al Harrah
Psammodromus algirus	No Voucher	Portugal: Tua
Takydromus septentrionalis	No Voucher	China: Zhousan Islands
Gekkonidae		
Hemidactylus frenatus	No Voucher	Papua New Guinea: Central Province; Port Moresby Airways Hotel
Gehyra mutilata	AMB6582	Malaysia: West Malaysia; Pulau Pinang, Summit of Penang Hill
Iguania		
Gambelia wislizenii	BYU 47329	Utah: Emery Co.; San Rafael Swell, Ding Dang Canyon

Note. Specimens obtained through the pet trade and those with unknown locality data were only used when they could be reliably identified, and lack of specific locality information would not change results or conclusions.

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MALAGASY SKINKS (SQUAMATA: SCINCIDAE) AND THE PHYLOGENETIC UTILITY OF GAPDH

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ABSTRACT: Malagasy skinks are a poorly known group, and relationships within the group have not been critically evaluated. In this paper we present the first phylogeny of Malagasy scincine lizards, based on modern cladistic analysis of data from seven molecular markers. We also evaluate the phylogenetic utility of the nuclear intron Glyceraldehyde-3-phosphate dehydrogenase (Gapdh) in lizards. Our analysis confirms the paraphyly of "*Amphiglossus*", and supports *Madascincus* as a valid genus. The generic status of another, unnamed clade of "*Amphiglossus*" is supported by some analyses. *Paracontias* is monophyletic, but the subgenera *Malacontias* and *Paracontias* are not. *Voeltzkowia* is basal to two groups of "*Amphiglossus*" and *Pygomeles*. Malagasy scincines are monophyletic and originate from a

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single colonization event from Africa. Gapdh is phylogenetically useful in skinks at the specific and generic levels.

Key words: *Amphiglossus*, α-Enolase, Gapdh, Madagascar, Phylogeny, Scincidae, Scincinae

Knowledge of the herpetofauna of Madagascar is still in the discovery phase with many species known from a single specimen or location, and new information about distributions, behavior, and basic biology being published every year (Andreone, et al., 2000; Andreone and Raxworthy, 1998; Andreone, et al., 2001; Glaw and Vences, 1996; Krüger, 1999; Raxworthy and Nussbaum, 1994). With many nocturnal or burrowing species, the ten genera of skinks (Scincidae) are probably the most poorly studied group of reptiles in Madagascar (Glaw and Vences, 1994), as shown by the description of four new species of *Amphiglossus* in 1993 (Raxworthy and Nussbaum, 1993), one new *Pseudoacontias* in 1995 (Nussbaum and Raxworthy, 1995), the recent description of nine new species and one new genus including five *Amphiglossus*, three *Paracontias*, and one *Pseudoacontias* (Andreone and Greer, 2002), and *Sirenoscincus yamagishii* (Sakata and Hikida, 2003a). The two genera belonging to the subfamily Lygosominae (*Mabuya* and *Cryptoblepharus*) are widespread and fully limbed, whereas the seven genera from the subfamily "Scincinae" are all endemic to the Malagasy region, and all show limb reduction to some degree (Glaw and Vences, 1994; note that Greer's (1970) subfamily Scincinae has been shown to be paraphyletic, but within southern Africa and Madagascar it remains a useful term).

Amphiglossus Duméril and Bibron 1839 was the first genus of Malagasy scincines described with *Amphiglossus astrolabi* as the type species. Boulenger (1887) moved *A. astrolabi*

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to the reduced limb genus *Scelotes*, distributed throughout southern Africa, where most limbed Malagasy scincines were placed from that time on (e.g., Angel, 1942; de Witte and Laurent, 1943). Greer (1970) placed the Madagascan members of *Scelotes* as incertae cedis, but in a series of 19 papers from 1979-1987 Brygoo resurrected *Amphiglossus*, moving the Malagasy species of *Scelotes* into that genus or *Androngo* Brygoo, 1982, depending on the number of presacral vertebrae. Andreone and Greer (2002) moved three of the species of *Androngo* back to *Amphiglossus*, leaving the former monotypic, and revising *Amphiglossus* to include species showing digit reduction. Of all the scincid genera present in Madagascar, *Amphiglossus* is currently the largest with 37 species (35 endemic to Madagascar, one endemic to the Comoro Islands, and one endemic to the Glorioso Islands, northwest of Madagascar).

The two completely limbless Malagasy genera include *Cryptoscincus* Mocquard, 1894, which is monotypic and known only from the two type specimens, and *Paracontias* Mocquard, 1894, with eight species divided into three subgenera (Brygoo, 1980b). *Voeltzkowia* Boettger, 1893 is composed of three completely limbless species (currently placed in the nominate subgenus), and two species with very rudimentary hindlimbs that were originally described in the genus (now subgenus) *Grandidierina* (Brygoo, 1981b). The newly described monotypic *Sirenoscincus* Sakata and Hikida, 2003 shows similarity in some scale characters to *Voeltzkowia* but is unique in having forelimbs with no hindlimbs (Sakata and Hikida, 2003a). *Pseudoacontias* Bocage, 1889 was originally described as completely limbless from a single type specimen of *P. madagascariensis*, which was destroyed by fire in 1978 (Brygoo, 1980b). The status of the genus remained uncertain until 1995 when a second species (*P. angelorum*) was described with no forelimbs and “flaplike hindlimbs with no toes” (Nussbaum and Raxworthy, 1995, p. 94). In 2002 a third species was described (*P. menamainty*) with “a button like scale”

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representing the forelimbs, and no hindlimbs (Andreone and Greer, 2002, p. 161), and most recently *P. unicolor*, a completely limbless species was described from Nosy Be (Sakata and Hikida, 2003b). While the validity of the genus is no longer in question, *Pseudoacontias* remains enigmatic as all four described species are known from single specimens. *Pygomeles* A. Grandidier, 1867, also contains one limbless species (known from the two types) and one species with rudimentary hindlimbs (Brygoo, 1984c).

Despite much research effort (Angel, 1942; Brygoo, 1979, 1980a, b, c, d, 1981a, b, c, d, 1983a, b, 1984a, b, c, d, e, 1985, 1986, 1987; de Witte and Laurent, 1943), relationships between Malagasy and African scincines and within the Malagasy group are unknown and have never been critically evaluated. In 1943 de Witte and Laurent presented a tree depicting relationships for all African and Madagascan skink genera showing limb reduction, but there is no explanation of how the tree was derived. de Witte and Laurent show Malagasy scincines as monophyletic with *Proscelotes* and *Sepsina* described as the most primitive of the African genera, and the most closely related to the Malagasy forms. Within Malagasy genera, *Amphiglossus* was considered primitive and described as giving rise to the Malagasy "*Acontias*" (*Pseudoacontias*, *Pseudacontias*, and *Paracontias*), as well as the group of *Voeltzkowia*, *Pygomeles*, *Grandidierina* and *Cryptoscincus*. Hewitt (1929) also concluded that the Madagascan "*Acontias*" were derived from *Amphiglossus* and only distantly related to the African forms. In his review of the scincine lizards of sub-Saharan Africa and the surrounding islands, Greer (1970) did not attempt to discuss the Malagasy complex in detail, but did state that they were closest to the mainland *Proscelotes* and *Sepsina* based on a small interparietal that does not contact the supraoculars and a well developed post orbital bone. The most complete treatment of Malagasy scincines was Brygoo's series of papers (1979-1987), and while he did make some

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statements about similarity of specific genera, he did not explicitly address the relationships among genera. Brygoo's most widely used character was the number of presacral vertebrae (used to defined the genus *Androngo* and the subgenera and groups of *Amphiglossus*, although some of these groups overlap in their number of presacral vertebrae) (Brygoo, 1981d, 1984b, d, e, 1987) and while it is generally accepted that a low number of presacral vertebrae is the primitive condition, the usefulness and validity of this character has been questioned (Andreone and Greer, 2002). Andreone and Greer (2002) also point out that *Amphiglossus* as currently defined, is composed of "the most generally primitive members" of Malagasy scincines, and is therefore "almost certainly a paraphyletic group" (pg.163).

It is well known that multiple molecular markers provide greater support and more robust estimates of organismal phylogeny than reliance on a single marker (e.g.; Baker and DeSalle, 1997; Thornton and DeSalle, 2000; Van Den Bussche, et al., 2003; Whiting, et al., 2003). Individual gene trees may differ for many reasons, but when multiple genes are analyzed in combination random homoplasy from each marker will be dispersed over different parts of the tree, and the true phylogenetic signal of all markers should emerge (e.g.; Kluge, 1989, 1998; Thornton and DeSalle, 2000). For many years the majority of molecular markers used in vertebrate phylogenetic studies have been mitochondrial, due to the ease of primer design and amplification, as well as rapid rates of evolution. Because of the lack of significant recombination and matrilineal inheritance, the mitochondrial genome has been referred to as a single linked locus (Avise, 1994; Kluge, 1989), therefore nuclear markers are a necessary addition to provide a well corroborated phylogeny. Nuclear introns offer an excellent option for phylogenetic studies at lower taxonomic levels because they are non-coding, and the lack of functional constraints may lead to high rates of evolution (Creer, et al., 2003; Palumbi and

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Baker, 1994). With the use of exon primed intron crossing (EPIC) markers, primers are designed to anneal to highly conserved flanking exons and amplify a small portion of each as well as the intervening intron (Friesen, et al., 1999; Friesen, et al., 1997). In principle, primers can be readily designed from conserved exons and used on a variety of taxonomic groups, and the origin of PCR products can be verified by comparison of exon sequences. Glyceraldehyde-3-phosphate dehydrogenase (Gapdh) is one of the key enzymes involved in glycolysis, and analysis of single stranded conformational polymorphisms (SSCPs) in intron XI revealed 5 alleles in 59 marbled murrelets, two different alleles in four Kittlitz's murrelets, and one additional allele in three long-billed murrelets (Friesen, et al., 1997). These results, along with successful amplification in a reptile and a mammal, suggest that Gapdh may be useful in phylogenetic studies of many vertebrate groups. In this study we explore the phylogenetic utility of intron XI of Gapdh in skinks, and use this region in combination with several other nuclear and mitochondrial gene regions to present a molecular phylogenetic hypothesis for many of the Malagasy taxa.

Materials and Methods

Sampling:

Taxon sampling included the Malagasy genera *Amphiglossus* (14 spp.), *Paracontias* (3 spp.), *Pygomeles* (1 sp.), and *Voeltzkowia* (1 sp.); representatives from the genera *Cryptoscincus*, *Androngo, Sirenoscincus* (all monotypic), and *Pseudoacontias* (4 spp., known only from holotypes) were not available for inclusion in this study (see Table 1 for specimen information). The southern African genera *Scelotes* (9 spp.), *Typhlacontias* (2 spp.), *Melanoseps* (1 sp.), *Proscelotes* (1 sp.), and *Sepsina* (1 sp.), were included to test previously hypothesized relationships between southern African and Malagasy genera. In order to place the Malagasy

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taxa within skinks as a whole, species from *Trachylepis* (formerly African *Mabuya*), *Sphenomorphus*, and *Tiliqua* (subfamily Lygosominae); *Feylinia* (subfamily Feylininae); and *Eumeces*, and *Scincus* (subfamily "Scincinae") were included. Based on prior study (Whiting et al., 2003), the subfamily Acontinae has been shown to be basal within skinks and was therefore used to root all analyses.

Molecular data:

DNA was extracted from liver or muscle tissue preserved in 95-100% ethanol using the Qiagen DNeasy kit (Valencia, CA). DNA templates and controls were amplified using standard PCR techniques in 50 μl reactions, and products were visualized via 2% agarose gel electrophoresis. Primers and protocols for the amplification of 18S rDNA, 16SrDNA, 12S rDNA, α-Enolase (Enol), C-mos, and cytochrome b (cytb) are listed in Whiting *et al.* (2003). Gapdh was amplified using the primers L890 and H950, which are designed to amplify intron XI and portions of exons 11 and 12 (Friesen, et al., 1997), using amplitaq gold® (Perkin Elmer), 2.5% DMSO and the following cycling profile: 95˚(12:00); 94˚(1:00), 65˚(1:00), 72˚(1:00) x 35 cycles; 72˚(5:00). Target products were purified using the Montage™ PCR_{96} Filter Plate and Kit (Millipore Co.) and sequenced using the Perkin Elmer Big Dye® version 3 cycle sequencing kit. Sequencing reactions were purified using Sephadex® in MultiScreen ™ Durapore PVDF plates (Millipore Co.). Purified sequencing reactions were analyzed on either an ABI 3100, or ABI 3730 automated sequencer. To insure the accuracy of sequences, negative controls were included in every reaction, complementary strands were sequenced, and sequences were manually checked using the original chromatograph data in the program Sequencher® 4.0 (GeneCodes Co.).

Alignment and tree reconstruction:

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Alignment and tree reconstruction was done using direct optimization (DO) in the program POY v.3.0 (Gladstein and Wheeler, 2003). Protein coding genes (C-mos, and cytb) were aligned based on conservation of the amino acid reading frame using Sequencher® 4.0 (GeneCodes Co.), and entered into POY as "prealigned data". POY analyses were run with gaps, transversions, and transitions all equally weighted, all gene regions included, on an IBM 1320 Linux cluster supercomputer using the following search strategy: "-molecularmatrix 111.txt -repintermediate -catchslaveoutput -fitchtrees -parallel -noleading -norandomizeoutgroup -impliedalignment -sprmaxtrees 1 -tbrmaxtrees 1 -maxtrees 5 -holdmaxtrees 50 -slop 5 -checkslop 10 -buildspr -buildmaxtrees 2 -replicate 200 -multirandom -treefuse -fuselimit 10 -fusemingroup 5 -fusemaxtrees 100 -numdriftchanges 30 -driftspr -numdriftspr 10 -drifttbr -numdrifttbr 10 -slop 10 -checkslop 10 -seed -1". The best trees resulting from the prior search, were then used as the starting tree for ten separate iterations of swapping to ensure the optimal tree was found. For comparison, additional alignments of ribosomal DNA and nuclear introns (18S, 16S, 12S, Enol, and Gapdh) were performed in ClustalX (Thompson, et al., 1997) using the default settings.

DO combines alignment and tree reconstruction into a single step using the same parameters, therefore alignments done in POY also resulted in a tree topology (see Wheeler, 1996; 1999, for a detailed explanation).

Tree reconstructions for alignments from ClustalX were performed using maximum parsimony (MP) in PAUP* 4.0b10 (Swofford, 1999), and Bayesian analysis in Mr. Bayes 3.0 (Huelsenbeck and Ronquist, 2001). MP searches were performed with equal character weighting, 10,000 random addition sequences with tree bisection reconnection (TBR) branch swapping, and with gaps treated both as missing data and as a fifth state. Prior to Bayesian

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analysis, the appropriate model of nucleotide substitution was selected for each gene region using Modeltest 3.0 (Posada and Crandall, 1998). The chosen models of evolution were then implemented in a partitioned Bayesian analysis consisting of 2,500,000 generations with four incrementally heated chains, and trees sampled every 1000 generations. Stationarity was determined as the point at which likelihood scores plateaued, and trees recorded prior to that point were discarded as the "burn in". Bayesian analyses were run three separate times to ensure consistency.

Branch support:

Posterior probabilities were assessed as part of the Bayesian analysis, and Bremer support was calculated as part of the DO analysis in POY. For MP topologies Bremer support (Bremer, 1994) and partitioned Bremer support (Baker and DeSalle, 1997) were calculated using Treerot (Sorenson, 1999) and PAUP* 4.0b10. Nodal support was also assessed for MP trees using nonparametric bootstrapping as performed in PAUP*, with 10,000 pseudoreplicates, two random sequence additions per pseudoreplicate, and TBR branch swapping.

Analysis of Gapdh:

We used PAUP* to calculate base composition, pairwise sequence divergence, and numbers of transitions and transversions in the Gapdh sequences. SWAN 1.0 (Proutski and Holmes, 1998) was used to evaluate the sequence variation along the length of the Gapdh region used in this study. In order to assess substitutional saturation in Gapdh, the number of transitions was divided by the length of the sequence, and plotted against the corrected distance for each pairwise sequence compairison, the same was done for transversions. Modeltest was used to determine the appropriate model of nucleotide substitution used to correct pairwise distances. Tree reconstruction for the individual analysis of Gapdh was performed in PAUP* using MP

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with the same search strategies listed for the combined analysis. Bootstrap and Bremer support were also calculated as described for the combined analysis.

RESULTS

Molecular data:

The molecular data used in this study include approximately 5500 bases across seven gene regions for 45 taxa. For all non-Madagascan lizards, sequences for 18S, 16S, 12S, C-mos, cytb, and Enol are the same as those used in (Whiting, et al., 2003) and were taken from GenBank. GenBank accession numbers for the sequences newly generated for this paper are as follows: Gapdh: AY391229-391251, Enol: AY391212-391228, 18S: AY391195-391211, C-mos: AY391178-391194, 12S: AY391123-391141, 16S: AY391142-391159, and cytb: AY391160-391177. Uncorrected pairwise sequence divergence (based on implied alignment from POY) across all taxa, within scincines, and within Malagasy scincines is shown for each gene in table 2. These divergence profiles reflect the phylogenetic utility of individual markers at different taxonomic levels. We combined all data to provide the best estimate of phylogeny (Chippindale and Wiens, 1994; Eernisse and Kluge, 1993; Kluge, 1989, 1998; Kluge and Wolf, 1993; Nixon and Carpenter, 1996), and topologies from individual gene analyses were only evaluated for identification of possible contamination and well supported conflict between data sets.

Direct Optimization:

One tree (length 8747) resulted from the DO search, and is shown in Fig. 1. Malagasy scincines form a well supported monophyletic group with the genus *Amphiglossus* recovered as three distinct clades, two of which are strongly supported, while *Paracontias* is monophyletic.

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There is strong support for *Paracontias* + a clade of *Amphiglossus* consisting of *A. stumpffi*, *A. intermedius*, *A. igneocaedatus*, *A. mouroundavae*, and *A. melanopleura* (clade A). The remaining Malagasy taxa form a single clade with *Voeltzkowia* basal to a *Pygomeles* + *A. astrolabi* and *A. waterloti* clade, and a second clade of all remaining *Amphiglossus* species (clade B).

The southern African genera *Scelotes* and *Proscelotes* are basal to Malagasy scincines (clade C), while all other southern African genera and *Feylinia* make up the sister group to this clade. Lygosomine species + *Scincus* and *Eumeces* form a single group (clade E) which is sister to a strongly supported southern African and Malagasy scincine clade (clade D). Both Bremer and bootstrap values are high for most nodes, although within clade B the placement of *Pygomeles* and *Amphiglossus mandokava* are only weakly supported.

Clustal Alignment:

Alignment in ClustalX results in a data set of 5543 characters, 1455 of which are parsimony informative (with gaps as 5th state). Parsimony analysis with gaps treated as a 5th state results in 1 tree of length 10587. This topology supports only clades A (99% bootstrap support) B (93% bootstrap) and D (78% bootstrap) from the DO analysis, but all other relationships between major clades have less than 60% bootstrap support. When gaps are coded as missing data, three trees of length 9064 are recovered. The strict consensus topology also supports clades A (72%), B (99%), and D (79%), but is much more congruent with the DO topology. All topologies resulting from the ClustalX alignment show less resolution and lower branch support in comparison with the DO topology.

Bayesian analysis:

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Modeltest analysis indicates that the appropriate models of nucleotide substitution for the ClustalX aligned data are as follows: GTR+G+I for 12S, 16S, and cytb, TrN+I for 18S, HKY+G for cmos and gapdh, and K80+G for Enol. Stationarity was reached before 500,000 generations, and after discarding these first 500 trees (burn in), the 50% majority rule tree was obtained from the remaining 2000 data points.

The Bayesian topology is similar to the MP analysis (gaps coded as missing data - see Fig. 2) with clades A, B, and D present and well supported, and a monophyletic Malagasy scincines. The most obvious difference between the Bayesian and DO topologies is the recovery of all southern African taxa as a monophyletic clade sister to the Malagasy species. Posterior probabilities are quite high across the tree, except within clade B.

Glyceraldehyde-3-phosphate dehydrogenase:

The POY aligned Gapdh dataset consisted of 400 bases, with uncorrected pairwise sequence divergence across all taxa quite high at 35.6% (see Table 2), but dropping to 17.7% and 15.1% within scincines and Malagasy species respectively. When aligned in ClustalX, Gapdh sequence divergence increases to 44.5% across all taxa, 28.3% within scincines, and 22.5% among Malagasy species. Mean nucleotide ratios were unbiased (A=0.25103, C=0.16597, G=0.26254, and T=0.31046) according to Chi-squared tests performed in PAUP* ($\chi^2 = 32.515$, df = 72; P = 1.0). Results from the SWAN 1.0 variability analysis indicate a relatively uniform level of variation throughout the length of intron XI (data not shown).

Independent MP analysis of the Gapdh data recovers a tree largely congruent with the final DO topology, although the position of *Paracontias* and *Voeltzkowia* do change within their respective clades (see Fig. 3). An examination of partitioned Bremer support values on the combined tree reveals that 70% of the support given by Gapdh is at the intrageneric or specific

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level, while 30% support relationships between genera. The saturation plot for Gapdh is shown in Fig. 4.

DISCUSSION

Alignment:

As is typically the case when a thorough investigation of a data set is carried out, results varied based on the alignment method used. Equally weighted DO searches (gap, transversion, and transition cost of 1) consistently resulted in shorter trees (8747 vs. 10587) than the same data aligned in ClustalX and analyzed in PAUP* (equal character weighting with gaps coded as 5th state). Because the raw data and the tree reconstruction parameters are the same in both cases, the differences in final tree scores must be due to the alignment. In a parsimony framework the tree which requires the fewest ad hoc assumptions, or the tree with the shortest length, is by definition the most optimal. Therefore, we favor the topology resulting from the DO algorithm in POY (Fig. 1), and adopt it as our working hypothesis.

Taxonomic Implications:

In all analyses, Malagasy scincines form a strongly supported monophyletic group originating from a single colonization event from southern Africa, confirming the removal of Malagasy species from *Scelotes*, and the hypotheses of Hewitt (1929) and deWitte and Laurent (1943) of a Malagasy clade distinct from the African scincines. The placement of *Proscelotes* + *Scelotes* as sister group to the monophyletic Malagasy clade lends credence to statements by de Witte and Laurent (1943) and Greer (1970b) that *Proscelotes* and *Sepsina* are the closest African relatives to the Malagasy scincines.

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The relationships within Malagasy scincines are much more complex than previously thought; with a paraphyletic "*Amphiglossus*" forming a minimum of three separate groups. *Amphiglossus igneocaudatus*, *A. intermedius*, *A. mouroundavae*, *A. melanopleura* and *A. stumpffi* form the sister group to *Paracontias* (clade B, Fig. 1), and a clade sufficiently distinct to warrant generic status. This group includes representatives of the subgenus *Madascincus* Brygoo, 1982 as well as members of the *igneocaudatus* group of *Amphiglossus* as defined by Brygoo (1984b). The former group (including *A. melanopleura* – type species, *A. mouroundavae* and *A. ankodabensis*) was diagnosed by Brygoo (1984b) as being pentadactyl, having the interparietal small or absent, a SVL of < 80 mm, and fewer than 35 presacral vertebrae. Two additional species, *A. punctatus* (recovered here in clade B, SH test p=0.000), and *A. minutus*, have subsequently been described and assigned to *Madascincus* (Raxworthy and Nussbaum, 1993; Glaw and Vences, 1994). The former, however, differs substantially from all other members of the group (Glaw and Vences, 1994). Brygoo (1984d) described the *igneocaudatus* group of *Amphiglossus* (consisting of *A. igneocaudatus, A. intermedius, A. polleni,* and *A. stumpffi*) based on the presence of a dark lateral band, 35-45 presacral vertebrae, and four well developed pentadactyl limbs. Aside from the plesiomorphic trait of unreduced digital complement, the two clusters share few obvious diagnostic features. Their grouping suggests that this lineage is morpholoigically diverse, with more robust-limbed, shorter-bodied basal members and more elongate, shorter-limbed derived members (the *igneocaudatus* group). With the inclusion of the *igneocaudatus* group, however, Brygoo's (1982, 1984b) diagnosis of *Madascincus* requires revision. We here recognize *Madascincus* as a valid genus, which may be defined as those pentadactyl Malagasy skinks sharing a closer ancestry with *Paracontias*, than with other skinks.

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The largest assemblage of "*Amphiglossus*" in this study includes *A. mandokava, A. melanurus, A. ornaticeps, A. tsaratananensis, A. punctatus, A. macrocercus*, and one currently undescribed species. Within this clade, *A. melanurus* and *A. ornaticeps* are strongly supported as monophyletic as well as *A. punctatus*, *A. macrocercus* and *A.* sp. These clades do not correspond to any previously hypothesized groups, and with the exception of *A. punctatus*, none of these species belong to either of Brygoo's designated subgenera.

Amphiglossus astrolabi and *A. waterloti* are both large (>200mm SVL) species that are found in aquatic or semiaquatic environments, have been observed foraging under water (Raxworthy and Nussbaum, 1993), and are recovered as well supported sister taxa in all analyses. *Amphiglossus reticulatus* is only known from the type specimen, but appears to be closely allied with *A. waterloti* and was collected in a swampy area (Brygoo, 1980c), therefore it is most likely a member of this clade. This group corresponds to Brygoo's subgenus *Amphiglossus*, defined by the presence of 37-38 presacral vertebrae, 28-34 scale rows around midbody, and > 200 mm SVL (Brygoo, 1981d). One of the most distinctive synapomorphies of this group is the position of the nostril centrally above the first upper labial, which may be an adaptation to an aquatic lifestyle (Brygoo, 1981d; Raxworthy and Nussbaum, 1993). *Pygomeles* is the sister group of this semi-aquatic assemblage, but as there are no obvious morphological synapomorphies and nodal support is low, this result remains tentative. Given the weak support for the placement of both *Pygomeles* and *A. mandokava* within clade B we are unable to falsify the monophyly of clade B "*Amphiglossus*" and conservatively retain the existing generic allocations for all members of this group.

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Paracontias is recovered as a strongly supported clade, but the subgenera *Malacontias* (composed of *P. holomelas* and *P. hildebrandti*) and *Paracontias* (*P. brocchi*) are not supported as *P. brocchi* and *P. holomelas* are sister taxa relative to *P. hildebrandti*.

In this study *Voeltzkowia* is recovered as basal within clade B of Malagasy scincines, and appears to be an old and distinct lineage (as shown by the long branch length in Fig. 1), but the inclusion of additional species from the genus would be needed to test this hypothesis. This placement of *Voeltzkowia* challenges the widely held view that "*Amphiglossus*" is a primitive assemblage giving rise to all other Malagasy scincines (Andreone and Greer, 2002; Angel, 1942; de Witte and Laurent, 1943; Hewitt, 1929), and is yet another example that limb reduction and loss are common among skinks and may not be suitable characters for phylogenetic reconstruction (Hauser, 1996; Whiting, et al., 2003).

The genus *Cryptoscincus* is only known from the two type specimens, but morphologically seems to be very closely related to *Voeltzkowia* (Brygoo, 1981b; Glaw and Vences, 1994) and would presumably also group in a basal position within clade B. *Androngo* was recently reduced to a single species (Andreone and Greer, 2002), with affinities to *Pygomeles* or "*Amphiglossus*". We can not comment on the composition or placement of *Androngo* as none of the relevant species were included in this study.

The primitive number of presacral vertebrae for Malagasy scincines is assumed to be 26 (Andreone and Greer, 2002; Glaw and Vences, 1994), with more advanced species evolving greater numbers of vertebrae. When the number of presacral vertebrae for each species is considered in light of phylogeny, no clear pattern of vertebral increase emerges. Therefore, these data support the idea that a high number of presacral vertebrae have evolved multiple times and

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therefore this character should be used with caution for phylogenetic inference (Andreone and Greer, 2002).

Outside of the Malagasy skinks, clade D and E are very similar to the results found in previous studies (Whiting, et al., 2003), and reconfirm both the paraphyly of the “Scincinae” and the monophyly of an Afro-Malagasy “scincine” clade (including *Feylinia*).

Phylogenetic Utility of Gapdh:

The high substitution rates of many intron sequences make them good candidates for independent tests of mtDNA based “shallow history” phylogenetic hypotheses. Likewise, the lack of functional constraints on intron evolution can result in large and frequent indels at deeper levels of divergence and can make sequence alignment ambiguous. Therefore it is important that the divergence level of the chosen intron is appropriate for the specific study.

Saturation does appear to be a problem for Gapdh when corrected pairwise sequence divergence reaches between 0.06 and 0.08 (see Fig. 4). In this data set, pairwise sequence divergences of ~0.06 correspond to the intergeneric taxonomic level, which is the same point at which Bremer support decreases. Based on sequence divergence levels, partitioned Bremer support, and individual analysis, intron XI of the nuclear Gapdh gene appears to be phylogenetically informative within Malagasy skinks at the intrageneric level. There also appears to be some support for relationships among genera, but sequence divergence rapidly increases with expansion to higher taxonomic levels. Therefore, these data suggest intron XI of Gapdh will be phylogenetically informative within skinks, and by extension perhaps many other squamate groups, at the specific and generic levels.

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Table 1: List of all specimen identification numbers and localities. Museum abbreviations follow (Levinton, et al., 1985) with the following exceptions: AMB = Aaron M. Bauer field number (specimens to be deposited in AMS), AJL-FN = Angelo J. Lambiris field number, NJK = Nathan J. Kley field number, No Voucher = no voucher specimen taken, (the lizard was identified, nondestructively sampled and released), RAN = Ronald Nussbaum field number (specimens to be deposited in UMMZ).

Species	**Specimen ID #**	**Locality**
Acontias litoralis	CAS 206800	South Africa: Northern Cape Province; vic..McDougall Bay water tank.
Acontias percivali	YPM 12687	Unknown
Amphiglossus astrolabi	UMMZ 208804	Madagascar: Antsiranana; Antalaha, 2 Km E. of Antanandavehely
Amphiglossus igneocaudatus	UMMZ 217449	Madagascar: Antananarivo; Antsirabe, Ibity
Amphiglossus intermedius	RAN 42624	Madagascar: Antsiranana; Antalaha, Ankavanana river
Amphiglossus macrocercus	UMMZ 208645	Madagascar: Fianarantsoa; Ivohibe, Andringitra, Iatara river
Amphiglossus mandokava	UMMZ 208654	Madagascar: Antsiranana; Sambava, Marojejy Reserve, Manantenina river
Amphiglossus melanurus	UMMZ 208708	Madagascar: Fianarantsoa; Ivohibe, Andringitra, Iatara river
Amphiglossus melanopleura	FA 1863	Madagascar:Manarikoba-Antsahamanara

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Amphiglossus melanopleura	FA 1859	Madagascar: Andasin'I Governera
Amphiglossus mouroundavae	UMMZ 208738	Madagascar: Antsiranana; Sambava, Marojejy Reserve, Manantenina river
Amphiglossus ornaticeps	UMMZ 208743	Madagascar: Antsiranana; Sambava, Marojejy Reserve, Manantenina river
Amphiglossus punctatus	UMMZ 208787	Madagascar: Fianarantsoa; Ivohibe, Andringitra, Sahavatoy river
Amphiglossus sp.	UMMZ 208848	Madagascar: Fianarantsoa; Ivohibe, Andringitra, Kimora river
Amphiglossus stumpffi	UMMZ 208797	Madagascar: Antsiranana; Nosy Be, Lokobe Reserve, Ampasindava
Amphiglossus tsaratananensis	UMMZ 208798	Madagascar: Mahajanga; Bealanana, Tsaratanana, Matsabory
Amphiglossus waterloti	UMMZ 201597	Madagascar: Antsiranana; Ambanja, Manongarivo Reserve, Ambalafary
Eumeces laticeps	BYU 47336	Florida; Duval Co., Little Talbot Island.
Eumeces inexpectatus	BYU 46699	Florida; Duval Co., Little Talbot Island.
Eumeces fasciatus	BYU 46698	Florida; Holmes Co., Ponce de Leon Springs.
Feylinia grandisquamis	NJK 0069	Unknown
Melanoseps occidentalis	CAS 207873	Equatorial Guinea: Bioko Id.; coast road ca. 5 km S. of Luba.

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Paracontias brocchi	UMMZ 209153	Madagascar: Antsiranana; Antsiranana, Montagne D'ambre, Antomboka river
Paracontias hildebrandti	UMMZ 209166	Madagascar: Antsiranana; Antsiranana, Montagne D'ambre, Antomboka river
Paracontias holomelas	UMMZ 201644	Madagascar: Antsiranana; Sambava, Marojejy reserve, along the Manantenina river
Proscelotes eggeli	CAS 168959	Tanzania: Tanga Region; Lushoto Dist.; West Usambara Mnts., Mazumbai Forest Reserve
Pygomeles braconnieri	UMMZ 197125	Madagascar: Toliara; Amboasary, Beraketa
Scelotes anguineus	AJL-FN 452	South Africa: Eastern Cape Prov.; Port Elizabeth.
Scelotes arenicola	CAS 209635	South Africa: KwaZulu Natal Prov.; Kosi Bay Nature Reserve, NW corner of Lake Nhlange.
Scelotes bipes	CAS 224005	South Africa: Western Cape Prov.; ~4.6 km N of Grootbaai, Bloubergstrand on Melkbos rd.
Scelotes caffer	CAS 206859	South Africa: Northern Cape Prov.; Brandberg,, Farms Kourootje and Kap Vley, De Beers Mining area.
Scelotes gronovii	CAS 206990	South Africa: Western Cape Prov.; 18.5 km N of jct rd R365 on R27 towards Lambertsbaai.

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Scelotes kasneri	CAS 206991	South Africa: Western Cape Prov.; 18.5 km N of jct rd R365 on R27 towards Lambertsbaai.
Scelotes mirus	No Voucher	Swaziland: Malolotja Reserve.
Scelotes sexlineatus	CAS206819	South Africa: Northern Cape Prov.; McDougall Bay.
Scelotes montispectus	CAS223934	South Africa: Western Cape Prov.; ~4.6 km N of Grootbaai, Bloubergstrand on Melkbos rd.
Scincus scincus	YPM 12686	Unknown
Sepsina angolensis	SMW 6694	Namibia: Kunene Reg.; Kamanjab District
Sphenomorphus simus	BYU 47016	Papua New Guinea: Gulf Province; Ivimka Research Station, Lakekamu Basin.
Tiliqua gigas	BYU 46821	Papua New Guinea: Gulf Province; Kakoro Village, Lakekamu Basin.
Trachylepis spilogaster	CAS 206938	Namibia: Erongo Region; Karibib Dist.; Usakos-Hentiesbaai rd., 10km E. of Spitzkop turnoff
Typhlacontias brevipes	CAS 206947	Namibia: Erongo Reg.; Walvis Bay Dist.; S. bank of Kuiseb rv. Near Rooibank rd
Typhlacontias punctatissimus	CAS 223980	Namibia: Kunene Reg; ~1.1 km N of Munutum Rv, at Skeleton Coast Park east boundry

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Typhlosaurus caecus	AMB 6817	South Africa: Northern Cape Province; 9.9 Km S. of Lambertsbaai.
Voeltzkowia lineata	RAN 34923	Madagascar: Toliara; Betioky

Note: Specimens with unknown locality data were reliably identified and outside of the specific group of interest, therefore lack of specific locality information will not likely change results or conclusions.

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Table 2: Uncorrected pairwise sequence divergence based on POY implied alignment across all taxa, African and Malagasy scincines (clade D; Fig. 1), and Malagasy taxa, for each molecular marker used in this study.

Gene region	Sequence length (bp)	All taxa	African and Malagasy scincines	Malagasy scincines
Gapdh	400	35.6%	17.7%	15.1%
Enol	276	17.9%	13.1%	8.2%
18S	1803	1.5%	1.0%	0.11%
16S	659	15.9%	15.5%	13.7%
12S	1150	25.5%	24.4%	18.3%
Cytb	732	26.8%	24.2%	23.7%
C-mos	594	10.8%	8.2%	4.9%

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Figure Legends:

Figure 1: Direct optimization (parameter set 1:1:1) topology, cost 8747. Numbers above branches are Bremer support values, numbers below branches are parametric bootstrap support values (calculated in PAUP* on MP topology from implied alignment; values below 50% are not shown). Upper case letters identify major clades discussed in the text.

Figure 2: Bayesian analysis of combined dataset aligned in ClustalX. 50% majority rule of 2000 trees, with posterior probabilities listed above branches. Nodes marked with black arrows are those that are not present in the MP analysis (gaps missing) of ClustalX aligned data, and parametric bootstrap support values for the MP tree are shown below the branches (values below 50% are not shown).

Figure 3: Maximum Parsimony tree for Gapdh from POY implied alignment, with gaps coded as a 5th state. This is the strict consensus of four trees length 461, numbers above branches are bootstrap support (values below 50% are not shown).

Figure 4: Saturation Plot. The number of transitions (Ts) and transversions (Tv) divided by the sequence length for all pairwise compairisons of Gapdh, and plotted against the HKY+G (the model of nucleotide substitution identified by ModelTest for these data) corrected pairwise distances.

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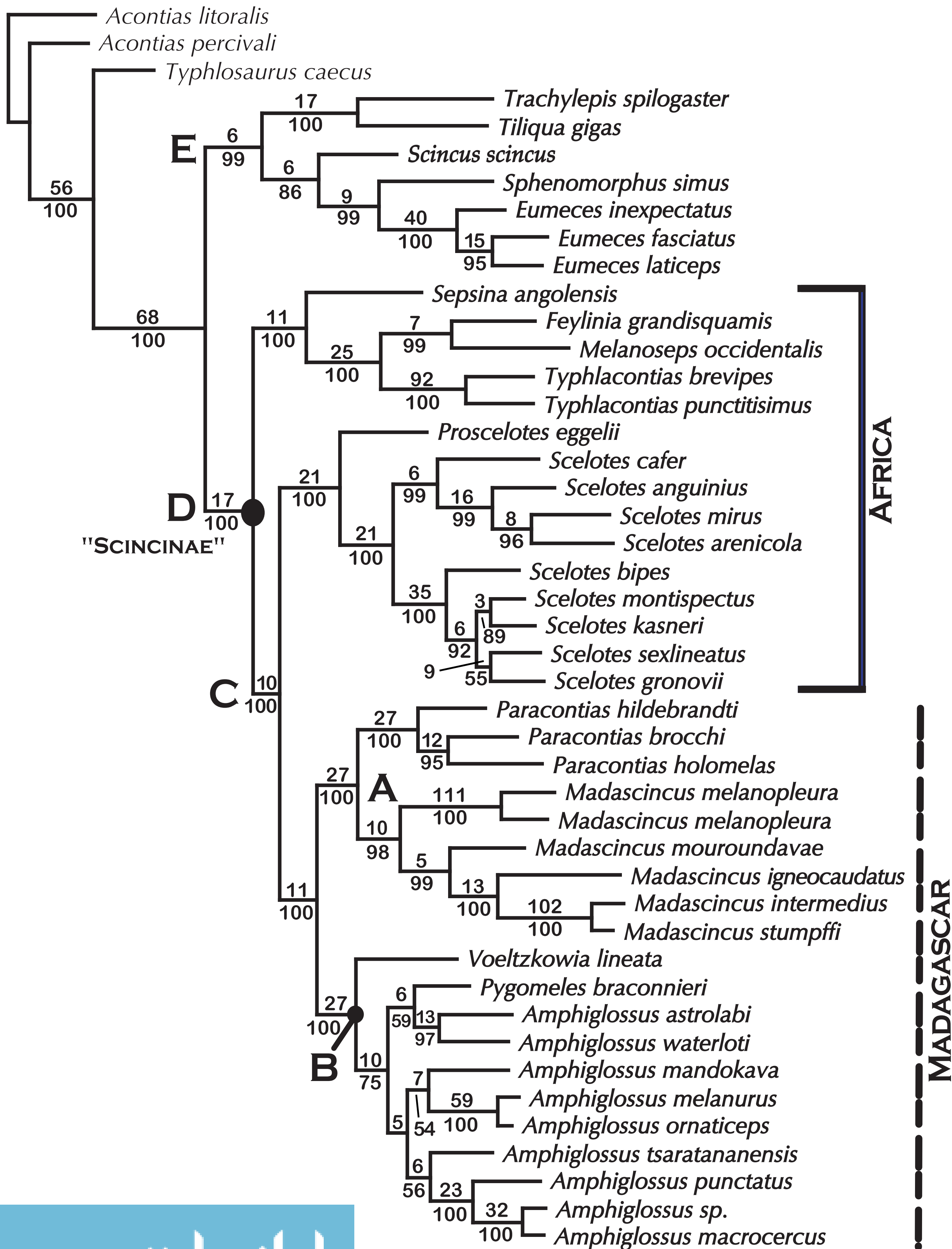

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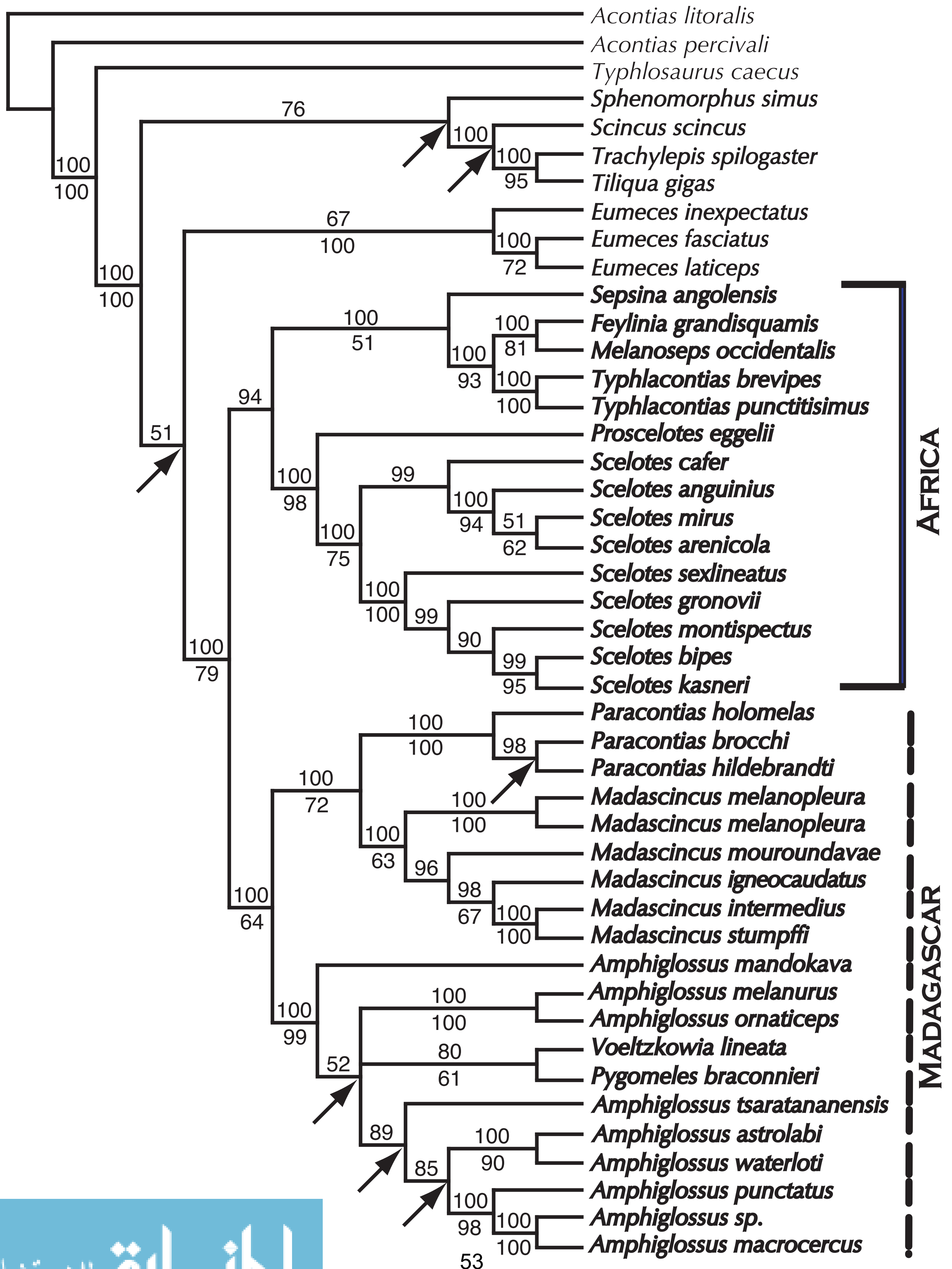

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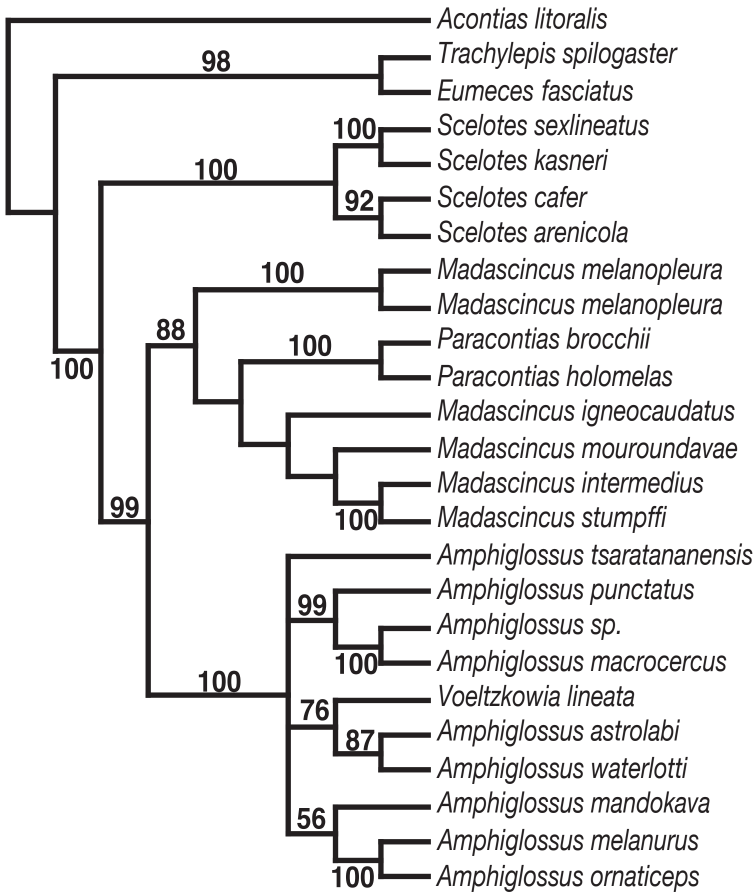

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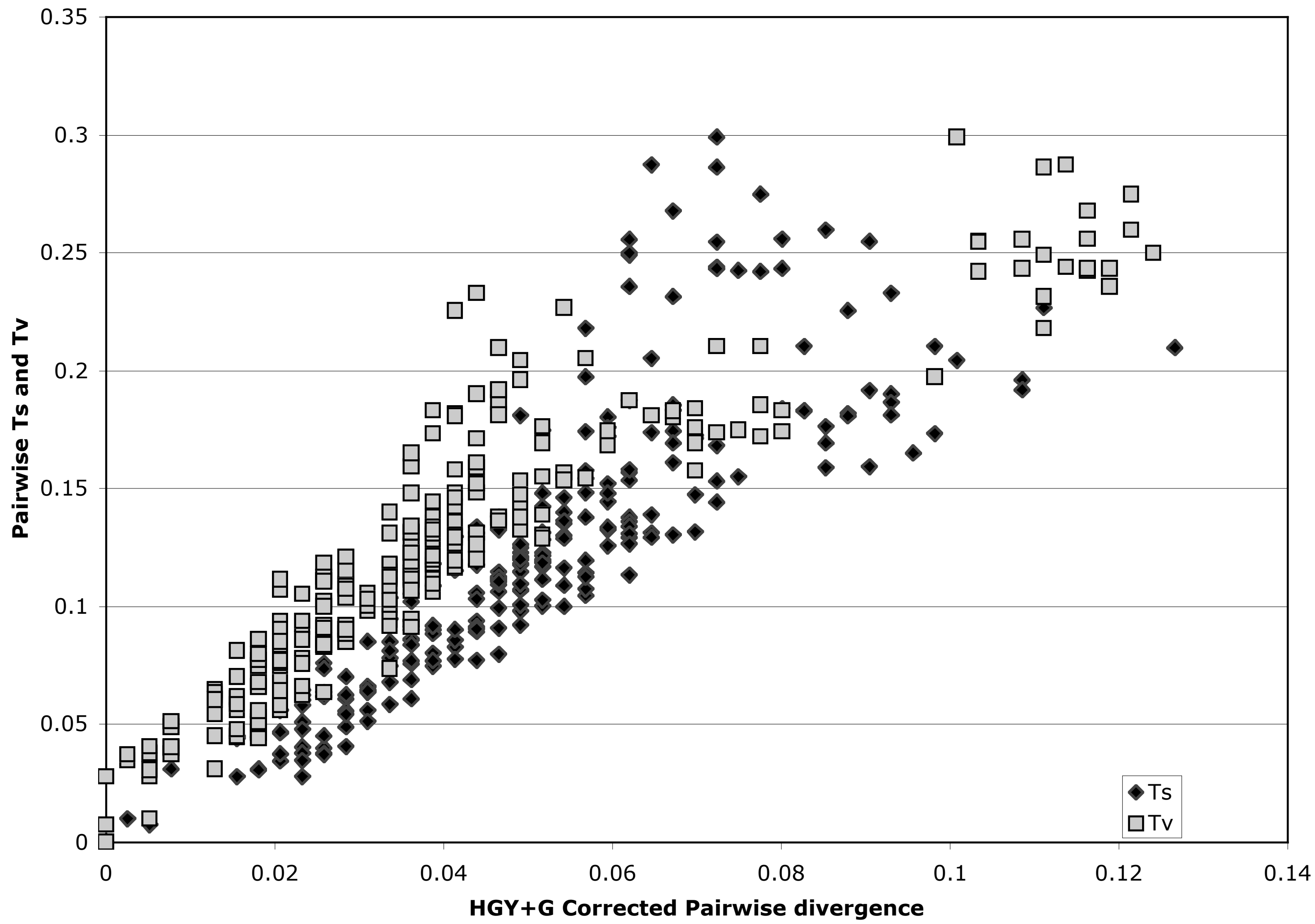

Figure 4

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RUNNING TITLE: ML ALIGNMENT AND SOUTH AMERICAN *MABUYA*

Title: Maximum Likelihood Alignment in South American *Mabuya*: testing for and dating a second colonization.

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ABSTRACT:

The rapid increase in the ability to generate molecular data, and the focus on model based methods for tree reconstruction have greatly advanced the use of phylogenetics in many fields. The recent flurry of new analytical techniques has been focused almost solely on tree reconstruction, whereas alignment has been largely neglected. In this paper we use six genes and a diverse sampling of South American lizards of the genus *Mabuya* to compare maximum likelihood alignments with more standard alignment methods. Relationships within South American *Mabuya* are inferred using partitioned Bayesian analysis with independent models and parameter settings for each gene region. We test the hypothesis of a second colonization of the New World by African *Mabuya* using parametric bootstrapping, and use both maximum likelihood and Bayesian methods to date divergence times within *Mabuya*.

Our results show that the consistent use of model based methods in both alignment and tree reconstruction lead to more optimal topologies than the use of independent criterion in alignment and tree reconstruction. We find South American *Mabuya* to be nonmonophyletic, and parametric bootstrap analysis confirms the significance of two independent colonizations from Africa. Relationships within South American *Mabuya* are very complex and found to be in need of taxonomic revision, specifically the species complexes of *M. heathi* and *M. agilis*, and *M. bistriata* (sensu Rodrigues 2000).

Key Words: Maximum Likelihood Alignment, Alignment, Direct Optimization, *Mabuya*

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The family Scincidae (skinks), one of the largest families of squamates, is thought to have originated in Africa and then diversified and spread through Asia and Australia to their current worldwide distribution (Greer, 1970b). In South America skinks are represented solely by the genus *Mabuya*, individuals of which occur on the mainland as well as many islands. As a whole the genus *Mabuya* is a very large (>100 spp.) and distributed over a wide range. There are currently 18-20 recognized species of *Mabuya* in the NewWorld, although the nomenclature and taxonomic status of many species is chaotic and in need of revision (Rodrigues, 2000). South American *Mabuya* are unusual in that while inhabiting the lowland tropics, all mainland species are viviparous. The first new placental morphotype (Type IV) in over half a decade was recently described in order to accommodate the specializations seen in the chorioallantoic placenta of New World *Mabuya* (Blackburn and Vitt, 2002). Virtually all nutrients required for placental development are provided through maternal-fetal nutrient transfer that appears to be convergent with that of Eutherian mammals. Viviparity is one of the characters that shows great variation in the genus *Mabuya*, there are viviparous species in Africa, and a few in Asia, but the majority of the group is oviparous.

Mabuya is hypothesized to have originated in South America via a single rafting event from Africa in the Miocene (Horton, 1973). The one possible exception is *M. atlantica* which is endemic to the small volcanic island group of Fernando de Noronha, 375 km off the coast of Brazil (Almeida, 2000). Previous workers have suggested that *M. atlantica* is more closely related to African species of *Mabuya* than those on mainland South America, and that consequently this species arrived via a second independent colonization event. Morphological characters; including presacral vertebrae counts,

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keeled dorsal scales, coloration, and oviparity, have been presented to support the close relationship between African species of *Mabuya* and *M. atlantica* (Greer et al., 2000; Horton, 1973; Travassos, 1948). Muasfeld et al. (Mausfeld et al., 2002) sequenced 12S rDNA and 16S rDNA for 21 taxa, finding support for a second colonization event, and spilitting *Mabuya* into four genera based on geographic distribution. While we do not doubt that paraphyly of *Mabuya* is a real possibility (Greer, 1970a; Greer, 1977; Greer, 1979; Honda et al., 1999), we argue that there has been no study to date with sufficient sampling to test and appropriately redefine *Mabuya,* the advocates of this splitting failed to include the critical genera *Dasia* and *Apterygodon,* and for these reasons this reclassification is premature. With the exception of the South American species, none of the geographical groups have morphological synapomorphies with which to define them. We therefore regard *Mabuya* as a single genus within this paper. Because these authors restricted themselves to a relatively sparse sampling of taxa and relied solely on mitochondrial markers, we argue that the intriguing second colonization hypothesis is in need of further confirmation via independent genetic markers and more extensive analyses with recently developed analytical tools.

Phylogenetic inference has become a powerful tool for establishing and testing specific evolutionary hypotheses. With our increased ability to generate DNA sequence data has come the desire to refine analytical methodologies such that they more accurately reflect molecular evolution. For instance, it is now feasible to analyze relatively large datasets with model based methods via Bayesian techniques, and recent methods allow different models to be applied to different data partitions (Huelsenbeck and Ronquist, 2001), in an attempt to more accurately reflect molecular evolutionary

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processes. While great emphasis has been placed on the tree reconstruction phase of phylogenetic analysis, the development of analytical techniques which incorporate specific models of DNA evolution into the alignment of the data have received much less attention. Given that some commonly used markers (e.g., ribosomal DNA and nuclear intron sequences) have proven difficult to align, multiple alignment can influence a phylogenetic topology more so than the specific models of sequence evolution used during tree reconstruction (Ogden and Whiting, 2003). Moreover, if a specific model of nucleotide substitution is used in reconstructing the phylogeny for a given dataset, then to be logically consistent, the identical model should be used in producing the multiple alignment on which the tree is based (Wheeler, 2004). This underscores the fact that multiple sequence alignment, like tree reconstruction, is an inference, and that the same level of sophistication should be brought to the alignment process as to the tree reconstruction process.

One methodology that attempts logical consistency throughout the entire analytical procedure is Direct Optimization (DO, formally Optimization Alignment, (Wheeler, 2004; Wheeler, 1996). DO obviates the need to reconstruct a multiple sequence alignment by dynamically optimizing alignment simultaneously with tree reconstruction. Thus one can reconstruct a tree without postulating a specific multiple alignment, and consistently apply a single criterion throughout the entire analytical procedure. While a theoretical justification for this methodology is beyond the scope of this paper (but see; (Wheeler, 1996), it is important to note that once an optimal topology is found, an implied alignment can be generated from that topology and subjected to more traditional methods of analysis. DO as implemented in the computer program POY

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(Gladstein and Wheeler, 2003) can perform analyses using complex models of sequence evolution under a likelihood framework, allowing the same model of sequence evolution to be applied during alignment as during tree reconstruction. Consequently POY can be used as a tool to generate implied alignments, using complex models of sequence evolution under the likelihood optimality criterion, which can then be used like a multiple alignment for further analyses. For parsimony it has been demonstrated that an implied alignment from POY will produce trees which are significantly shorter than those which can be produced under other methods of multiple sequence alignment (Ogden and Whiting, 2003; Wheeler, 2003). However, what has yet to be demonstrated is whether an implied alignment generated under likelihood models will lead to topologies that are significantly more optimal than can be found using standard methods of multiple sequence alignment. In this paper we have sequenced seven genetic markers for a wide sampling of South American spp. of *Mabuya* and extensive outgroups. Sequences were aligned in POY using likelihood models of sequence evolution as well as commonly used alignment programs that do not employ likelihood models. Topologies were reconstructed from both classes of alignments in order to determine whether a likelihood alignment leads to more optimal likelihood solutions than those obtained via non-likelihood alignment techniques.

Once the optimal topology for these data is obtained, it is possible to rigorously test the hypothesis of two colonization events of South America, and further investigate this hypothesis by estimating dates for these events. In the nearly 40 years since its proposal, the molecular clock (Zuckerland and Pauling, 1965) has been influential in many fields primarily because it provides a means for dating specific evolutionary events.

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As more studies have investigated the rate of molecular evolution it has become clear that this rate varies across lineages and gene regions, causing most datasets to violate the ultrametric assumptions imposed by the molecular clock. In an effort to overcome rate heterogeneity across the topology two classes of methods have been proposed; the first is to remove the taxa or genes that violate the clock and use the pruned tree to calculate divergence times (Hedges et al., 1996; Li and Tanimura, 1987; Takezaki et al., 1995), while more recent methods attempt to relax the molecular clock and accommodate heterogeneity across the topology while estimating divergence dates (Huelsenbeck et al., 2000; Kishino et al., 2001; Sanderson, 2002; Yoder and Yang, 2000). All methods that incorporate variation in the rate of molecular evolution are based on the premise that closely related lineages have similar rates, thereby minimizing local rate changes on the tree. One method replaces the global molecular clock with multiple "local clocks", thereby allowing rates to vary across the tree, while forcing defined groups or classes to have a constant rate (Yoder and Yang, 2000). Other methods eliminate both global and local clocks, and apply a likelihood penalty if rates among closely related taxa vary too much (Sanderson, 1997; Sanderson, 2002). Bayesian methods have been developed which attempt to model the rate of molecular evolution across the tree, thereby estimating rate variation and divergence times simultaneously (Huelsenbeck et al., 2000; Kishino et al., 2001; Thorne et al., 1998). A few of these models have recently been extended for use with multilocus datasets; by assuming variation in divergence times among genes is small, one can take advantage of the increased data and different evolutionary rates among genes to obtain more reliable divergence dates (Thorne and Kishino, 2002; Yang

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and Yoder, 2003). In this paper we apply both ML and Bayesian methods designed for multilocus datasets to estimate divergence times within South American Mabuya.

MATERIALS AND METHODS

Sampling

We sequenced 84 individuals of *Mabuya* representing 35 species from South and West Africa (11 spp.), Asia (5 spp.), Madagascar (7 spp.), Fernando de Noronha (1 sp.), and South America (11 spp.). Based on prior study (Whiting et al., 2003), outgroup taxa were chosen from Scincidae including *Eumeces*, *Tiliqua*, *Lamprolepis*, and *Typhlosaurus*, and all trees were rooted to *Acontias* (For detailed specimen information see appendix 1). Molecular data collected included ~5000 base pairs of DNA sequence from 12S rDNA, 16S rDNA, and cytochrome b (cytb) mitochondrial genes, the nuclear proto-oncogene C-mos, and nuclear introns from the alpha-Enolase (Enol), Glyceraldehyde-3-phosphate dehydrogenase (Gapdh), and Myosin Heavy Chain 2 (MYH2) genes. GenBank Accession numbers will be added upon acceptance.

DNA was extracted from liver or muscle tissue preserved in 95-100% ethanol using the Qiagen DNeasy kit (Valencia, CA). DNA templates and controls were amplified using standard PCR techniques in 50 μl reactions, and products were visualized via 2% agarose gel electrophoresis. Primers and protocols for the amplification of 16S, 12S, Enol, C-mos, cytb, and Gapdh are detailed elsewhere (Whiting et al., 2003; Whiting et al., 2004). MYH2 was amplified with the primers MYH2-F (5'GAACACCAGCCTC ATCAACC 3') and MYH2-R (5'TGGTGTCCTGCTCCTTCTTC3') (Dolman and Phillips, 2004; Lyons et al., 1999; Lyons et al., 1997), using amplitaq gold® (Perkin

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Elmer), and the following cycling profile: 95˚(10:00); 94˚(0:30), 62˚(0:45), 72˚(0:45) x 35 cycles; 72˚(5:00). Target products were purified using the Montage™ PCR_{96} Filter Plate and Kit (Millipore Co.) and sequenced using the Perkin Elmer Big Dye® version 3 cycle sequencing kit. Sequencing reactions were purified using Sephadex® in MultiScreen ™ Durapore PVDF plates (Millipore Co.). Purified sequencing reactions were analyzed on either an ABI 3100, or ABI 3730 automated sequencer. To insure the accuracy of sequences, negative controls were included in every reaction, complementary strands were sequenced, and sequences were manually checked using the original chromatograph data in the program Sequencher® 4.0 (GeneCodes Co.).

Alignment

The protein coding genes, C-mos and cytb, were aligned according to conservation of the amino acid reading frame in Sequencher 4.1 and input to POY 3.11 as "prealigned". The combined data were analyzed in POY under s1, s1g, s2g, s3g, and s6g submodels which are comparable to 1, 2, 3, and 6 parameter models of evolution with gaps treated as a fifth state, and all parameters estimated from the data. We performed 5 replicates of sequence addition and the –ln L scores of the trees resulting from each model were compared using likelihood ratio tests to determine the appropriate model of evolution for the data. Once the appropriate model was determined, 100 replicates of random sequence addition were performed on an IBM 1320 Linux cluster supercomputer. The best tree resulting from the 100 replicates was used as the starting tree for an additional 10 swapping runs to ensure the optimal tree was found. The implied alignment

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corresponding to the best –ln L score was used for all further analyses. Alignment was also performed in ClustalX (Thompson et al., 1997) using the default parameters.

Tree Reconstruction

The aligned data were partitioned by gene region, and Modeltest (Posada and Crandall, 1998) was used to determine the appropriate model of sequence evolution for each gene. A partitioned Bayesian analysis was performed in MrBayes 3.0 (Huelsenbeck and Ronquist, 2001) under the chosen models, with 2,500,000 generations, four chains (three heated, one cold), trees sampled every 1000 generations, and all parameters allowed to vary between partitions. Three individual analyses were run to ensure consistency, and "burn-in" was determined as the point at which likelihood scores plateaued and stabilized. Trees prior to stationarity were discarded, and a 50% majority rule consensus was taken of all remaining data points to obtain the final topology. In order to remove any bias resulting from the use of different models in tree reconstruction, additional Bayesian analyses were performed using a single model (GTR + G + I) for both the POY and ClustalX alignments.

Hypothesis Testing

In order to test the hypothesis of two colonization events of South America from Africa, we used PAUP* (Swofford, 2002) to search for the best tree given the constraint of a single colonization (=null hypothesis), and compared that to the optimal tree found under two colonizations (= alternative hypothesis) using the Shimodira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) with Rell optimization and 10000 bootstrap

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replicates. To further test this hypothesis, we performed parametric bootstrapping (Huelsenbeck et al., 1996b; Swofford et al., 1996) by simulating 1000 datasets in Seq-Gen 1.2.7 (Rambaut and Grassly, 1997) using the single colonization tree, and the model and parameters estimated for the combined dataset. The length differences between the constraint tree and the optimal tree for each simulated dataset were used to generate the expected distribution of length differences under the null hypothesis. The observed length difference from the original dataset was then compared to the null distribution to determine the significance of the empirical results.

Divergence Time Estimation

All divergence time estimation methods that do not invoke a molecular clock require the use of calibration dates. The oldest known undisputable skink fossil (*Eumeces sp*. – middle Oligocene) (Estes, 1983), and the cessation of volcanic activity on the islands of Fernando de Noronha (3.3-1.7 MYA) (Almeida, 2000) were used as calibration points in all divergence time analyses. Due to uncertainty in the exact placement of the fossil within our topology, calibration points were conservatively placed at the stem nodes rather than the crown nodes.

Yang and Yoder's (Yang and Yoder, 2003) maximum likelihood method using local clocks was implemented in PAML, and all analyses were run under both the molecular clock and the local clock methods. Each gene partition was analyzed separately, as well as a combined analysis of all data. Calibrated nodes must be set as absolute dates rather than maximum or minimum ages, therefore the average date in the range was designated for the node (28.5 and 2.5 MYA respectively). Four rate classes

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were defined, the first for the Acontine root, one for Asian *Mabuya* and remaining outgroups, one for African/Malagasy *Mabuya*, and the last for the South American *Mabuya*. For all analyses the REV model was used with all parameters being estimated, and in combined analyses each gene was allowed to have different parameters.

To implement Thorne's multilocus Bayesian method (Thorne and Kishino, 2002), the best model and all parameters were estimated using *baseml*. Model information was then converted to the appropriate format using *pamltomodelinfo*, and utilized by the *estbranches* program to determine branch lengths. The output from *estbranches* for all genes was used in combination with the Bayesian topology to calculate divergence times in the program *multidivtime*. For *multidivtime*, the same calibration points as listed above were used, but the *Eumeces* fossil was set as a minimum age, while the geological data for Fernando de Noronha was set as a maximum age. The burn-in was set to 100,000 and the number of samples after burn-in was also 100,000 with 100 generations between each sample. The *a priori* expected number of time units (rttm) was set to 28.5 (taken from the fossil *Eumeces* date), and the standard deviation for this prior (rttmsd) was set to 20. As suggested, the mean for the prior distribution of rate at the root (rtrate) was calculated as the mean of the summed branch lengths from root to tip divided by rttm, and the standard deviation for this prior (rtratesd) was set equal to rtrate. The Brownian motion prior (brownmean), and its standard deviation (brownsd) were both set to 0.07 (brownmean*rttm = 2). The parameters minab, newk, othk, and thek were left at the default settings, and the absolute oldest age for the root was set at 100MYA (bigtime).

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Results and Discussion

Alignment and Tree Reconstruction

The likelihood ratio test identified the s6g model (= modified GTR+gaps) as the best justified model under POY, and this model was used to generate the alignment for all subsequent analyses. The implied alignment from the POY topology (-lnL=54049.92) consisted of 5073 characters 1703 of which are parsimony informative. The ClustalX alignment resulted in 5003 characters with 1902 parsimony informative sites. The models and parameters chosen by Modeltest for each partition in the POY and ClustalX alignments are shown in table 1.

The partitioned Bayesian analyses reached stationarity after 100,000 generations, and after discarding the first 100 trees (burn-in) from each of the three separate runs the average –ln L score for all remaining trees for the POY alignment was –50585.455 (SD 278.1), while the ClustalX alignment average was –61118.714 (SD 294.25). Similarly, when the GTR + G + I model is used for all data partitions, the average –ln L score after burnin for the POY alignment was 51021.270 (SD 280.94), while the average –ln L score for the same analyses using the ClustalX alignment was 61581.638 (SD 368.199). These results suggest that a likelihood alignment generated under Direct Optimization results in tree topologies that are more optimal than those found under ClustalX, and underscore the importance of being analytically consistent in applying the same optimality criterion throughout the alignment and tree reconstruction process. This result for likelihood parallels that found for parsimony, in that POY results in significantly more optimal topologies than ClustalX (Ogden and Whiting, 2003; Wheeler, 2003). Intuitively, this is in large part because ClustalX generates an alignment from a single “guide tree” found

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under a simple clustering algorithm, while POY uses heuristic methods to evaluate millions of guide trees using more sophisticated criteria before selecting an optimal alignment (Wheeler, 2003). It should be recognized that POY is not intended to be a multiple alignment program in the traditional sense, and that the implied alignment represents the optimization of sequence data for a given (optimal) topology. Wheeler (Wheeler, 2003; Wheeler, 2004) recommends that the topology from POY should be favored rather than using the implied alignment for further tree reconstruction, since this will result in the globally most optimal solution and be logically consistent. Nonetheless, POY provides an estimate for a likelihood alignment, and this likelihood alignment produces likelihood topologies that are significantly more optimal than those generated via standard multiple alignment methods. In combination with similar results found under other optimality criterion, these results support the conclusion that direct optimization produces more optimal results regardless of the optimality criterion or model used.

South American Mabuya

The partitioned Bayesian analysis resulted in a topology strongly supporting two colonization events into South America (figure 2). The genus *Mabuya* is supported as monophyletic, with the Asian species basal to an Afro/Malagasy/Fernando de Noronha clade, and a South American mainland clade (similar to previous studies; (Mausfeld et al., 2002). These results suggest that while the colonization of Fernando de Noronha was certainly from an African form, the colonizing species of mainland South America could have originated in either Asia or Africa. Within the Afro/Malagasy/Fernando de

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Noronha clade, our data support the monophyly of the Malagasy species, in contrast to previous findings (Mausfeld et al., 2002) which suggested paraphyly. *Mabuya atlantica* is recovered as sister group to two *Mabuya* species widely distributed throughout west and southern Africa, while the Malagasy and southern African species are sister taxa. Within the South American clade, *M. frenata* is basal to two remaining clades. The first is composed of a large species complex consisting of a minimum of three species, to which either the name *M. bistriata* (sensu Rodrigues 2000) or *M. nigropunctata* (sensu Avila-Peres, 1995) has been applied. Nested within the *M. bistriata* clade is *M. carvalhoi*, a recently described and distinct morphological species, this result is considered tentative until further sampling of *M. carvalhoi* can be added. The second clade consists of *M. guaporicola*, *M. ficta*, and *M. dorsivitatta* all as distinct well supported species, *M. agmosticha* and *M. macrorhyncha* are confirmed as closest relatives (Rodrigues, 2000), and are related to another complex consisting of *M. agilis* and *M. heathi*. Obviously there is still much taxonomic work and revision to be done on South American *Mabuya*, but that is outside of the scope of this paper.

The results from both the SH test and the parametric bootstrap both significantly reject the null hypothesis of a single colonization of South America, $p=0.019$ and $p>0.01$ respectively. The difference between the optimal tree and the single colonization tree in the original dataset was 50, whereas the largest difference between trees in the simulated datasets was 24 (see figure 3). The parametric bootstrap is thought to be the most rigorous and appropriate test for an *a priori* hypothesis of relationships (Huelsenbeck et al., 1996a; Swofford et al., 1996). These results, along with morphological characters,

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combine to strongly support the hypothesis of two colonization events of the New World by *Mabuya*.

Fernando de Noronha is currently inhabited by only two lizard species, *Mabuya atlantica* and *Tupinambis merianae*, which is known to have been introduced to the islands in the second half of the 20th century. While our results strongly reject the hypothesis that *Mabuya atlantica* could have been introduced from Brazil, the archipelago of Fernando de Noronha was visited by sailors and traders, and the possibility of human introduction from Africa can not be addressed by these analyses. The Italian explorer Americo Vespucius visited Fernando de Noronha in August of 1503, and the following is an excerpt from a letter to Piero Soderini detailing his fourth voyage to America: " This island we found uninhabited. It had plenty of fresh-water, and an abundance of trees filled with countless numbers of land and marine birds, which were so simple, that they suffered themselves to be taken with the hand. We took so many of them that we loaded a boat with them. We saw no other animals, except some very large rats and lizards with two tails, and some snakes" (Lester, 1886). The 'large rats' mentioned by Vespucius were the extinct *Noronhomys vespucii*, while the 'snakes' were most likely *Amphisbaena ridleyi*, and the 'lizards with two tails' were probably specimens of *Mabuya atlantica* with aberrantly regenerated tails. This visit of Vespucius was just shortly after Columbus' discovery of the Americas, and provides strong evidence that *Mabuya atlantica* was present on Fernando de Noronha before the arrival of humans, thereby supporting the hypothesis of two independent colonization events via rafting to the New World.

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Divergence Time Estimates

Divergence time estimates for each gene partition calculated in PAML were averaged to give an idea of the range of dates found by single gene analyses in comparison to the combined analyses (table 2). The colonization of mainland South America must have occurred on the branch between nodes 96 and 121 on figure 2, while the branch between nodes 112 and119 represents the colonization of Fernando de Noronha. Node 112 was used as a calibration point, and therefore in PAML analyses this node was fixed at a time of 2.5 MYA. For node 119, the average of individual gene analyses with the clock enforced is 0.46 MYA (SD=0.904) and the combined analysis under the clock gives a date of 1.4 MYA. The average date of node 119 for individual genes analyzed under the local clock method is 0.42 MYA (SD=0.9194), while the combined local clock analysis results in a date of 2.4 MYA. For mainland South America, the average date for all individual gene analyses with the molecular clock enforced is between 9.6 MYA (SD=2.617) and 4.6 MYA (SD=1.346) for nodes 96 and 121 respectively, while the date for the global analysis of all genes under the molecular clock is between 6.8 and 3.1 MYA. When the molecular clock is relaxed and local clocks are used, the average date from individual gene analyses is 4.99 MYA (SD=2.526) and 2.4 MYA (SD=1.447) for nodes 96 and 121 respectively, while the combined local clock analysis results in a date of between 11.03 and 7.1 MYA.

When the molecular clock is enforced, the Bayesian method results in a date of 2.1 MYA (see table3) for the colonization of Fernando de Noronha, and a date of 58.2 MYA for the mainland South American colonization. When the assumptions of the molecular clock are removed and rates and times are estimated simultaneously the date

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for the colonization of Fernando de Noronha is 3.3 MYA (SD=0.002), while the mainland colonization is dated at 66.8 MYA (SD=0.5).

The comparison of results from the ML and Bayesian methods emphasizes the disparity often found when more than one method of divergence estimation is used. Previous studies have shown that there are large amounts of error associated with all divergence estimators, and the dependability of results is directly correlated with the number and quality of calibration points. The further you are from a calibration node the greater the error associated with the divergence estimate, as shown in this analysis by the large difference in the dates for node 96 (11.3 MYA and 66.8 MYA). Due to the paucity of the fossil record for skinks divergence dates estimated under any method would be questionable, but the one firm conclusion supported by all analyses is that the colonization of mainland South America preceded the colonization of Fernando de Noronha.

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Table 1: Modeltest results for each gene from the POY implied alignment and the ClustalX alignment.

	16S	**12S**	**Cytb**	**C-mos**	**Enol**	**Gapdh**	**MYH2**
POY implied alignment	TrN+I+G	TrN+I+G	TVM+I+G	HKY+G	HKY+G	HKY+G	HKY+G
ClustalX alignment	TrN+I+G	TrN+I+G	TVM+I+G	HKY+G	HKY+G	K81uf+G	K81uf+G

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Table 2: Maximum Likelihood divergence time estimates for selected nodes under the molecular clock (m.clock) and local clock (local) methods as implemented in PAML (Yang and Yoder, 2003). Values were computed for individual gene partitions and the average and standard deviation over all partitions are represented here, as well as the dates from the combined analysis of all data. All dates are MYA, and node numbers are listed on figure 2.

	Average m. clock	**SD m.clock**	*Combined* **m. clock**	**Average local**	**SD local**	*Combined* **local**
Node 92	28.5	0	28.5	28.5	0	28.5
Node 93	16.1432857	5.18854312	16.9275	19.0172143	2.38322025	18.95
Node 94	13.7931429	3.65749301	11.1645	16.363	3.10974534	13.3045
Node 95	11.1511429	2.65915608	10.6945	12.2907857	3.71212447	11.4375
Node 96	9.5805	2.61684941	6.756	4.98628571	2.52597925	11.302
Node 97	6.30957143	1.17710738	5.1955	3.447	2.1399096	8.541
Node 98	6.21007143	1.18974382	3.834	3.35128571	2.18907927	7.2195
Node 112	2.5	0	2.5	2.5	0	2.5
Node 113	2.40985714	0.14981592	1.8075	2.34478571	0.2199242	2.3295
Node 119	0.462	0.90390081	1.421	0.4195	0.91938322	2.3575
Node 121	4.57878571	1.34627792	3.0735	2.4465	1.44702744	7.058
Node 122	2.64435714	0.86765017	2.7605	1.52785714	0.53568039	5.7745
Node 129	4.38157143	1.19488219	1.997	2.38207143	1.49316742	5.7285
Node 130	4.03821429	1.33540848	1.9685	2.05892857	0.94212974	2.7985

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Node 131	3.83678571	1.52037605	1.549	1.86335714	0.74470233	2.0915
Node 133	3.30828571	1.15728086	1.1785	1.62892857	0.60525865	1.515
Node 138	3.80321429	1.18412288	1.855	1.97757143	0.9891582	1.259
Node 139	1.06671429	0.62047837	1.436	0.44585714	0.32269758	0.7675
Node 147	3.04364286	0.92234455	1.332	1.79278571	1.64197876	3.8135

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Table 3: Bayesian divergence time estimates for selected nodes computed with and without the molecular clock enforced in the program *multidivtime* (Thorne and Kishino, 2002). All dates are MYA, and node numbers are listed on figure 2.

	Molecular clock	**No Clock**	**SD No clock**
Node 92	58.16175	69.44241	0.05113
Node 93	58.16117	68.84610	0.05069
Node 94	58.16059	68.50724	0.05044
Node 95	58.16001	68.13830	0.05017
Node 96	58.15942	68.00379	0.05007
Node 97	58.15884	67.73900	0.04988
Node 98	58.15303	67.73223	0.04987
Node 112	2.09095	3.29754	0.00243
Node 113	2.09075	3.29721	0.00243
Node 119	2.09093	3.28103	0.00242
Node 121	58.15884	66.78992	0.04918
Node 122	58.15826	66.65918	0.04908
Node 129	31.01663	66.76895	0.04916
Node 130	31.01632	66.69263	0.04911
Node 131	31.01600	66.65999	0.04908
Node 133	31.01569	66.56284	0.04901

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Node 138	31.01600	66.53605	0.04899
Node 139	31.01569	66.13084	0.04869
Node 147	31.01632	66.59856	0.04904

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FIGURE LEGENDS

Figure 1: Map showing the position of Fernando de Noronha in relation to South America.

Figure 2: Partitioned Bayesian analysis of the POY ML implied alignment, 50% majority rule consensus. Nodes with posterior probabilities below 0.90 are marked <, while 0.90-0.95 are marked *, and all unmarked nodes have values of 0.95-1. Node numbers are marked with # and correspond to divergence times (tables 2 and 3). Nodes used for calibration in divergence estimation are marked with C.

Figure 3: Parametric bootstrap distribution of tree length differences between constrained and optimal topologies constructed from 1000 simulated datasets. Empirical length difference is denoted with the arrow.

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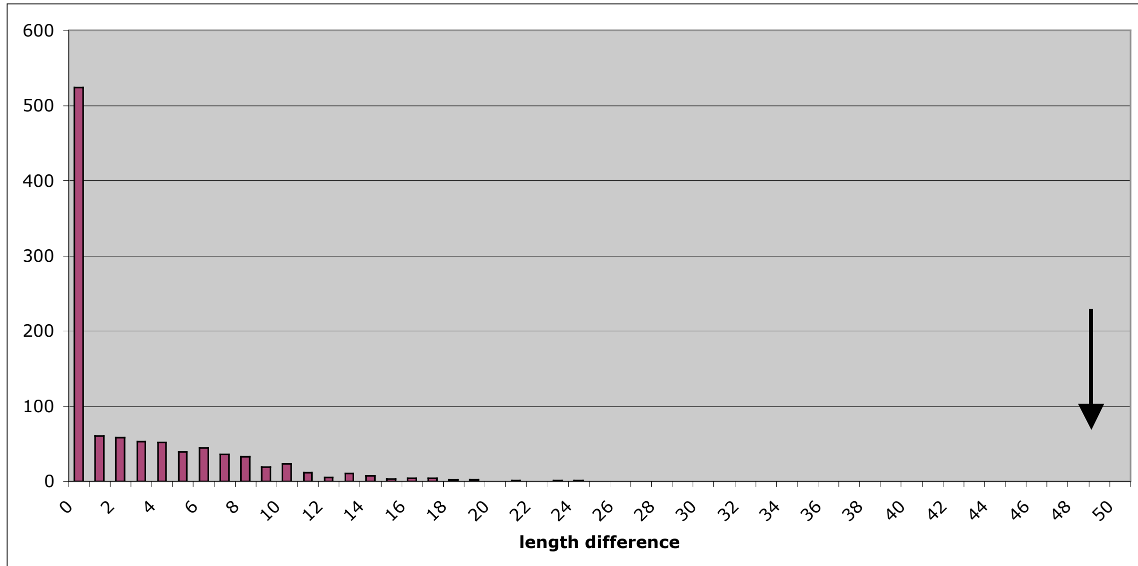
FIGURE 3:



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APPENDIX 1: Sampling Table showing collection numbers and localities for all specimens used, as well as the identification label used in figure 2.

Genus	Species	Locality	Collection #	Paper ID	Lab ID Number
Acontias	percivali	Unknown	YPM 12687	Acontias	A2
Eumeces	fasciatus	Florida; Holmes Co., Ponce de Leon Springs.	BYU46698	Eumeces fasciatus	S10
Eumeces	laticeps	Florida; Duval Co., Little Talbot Island.	BYU46336	Eumeces laticeps	S08
Lamprolepis	smaragdina	Unknown	BYU47331	Lamprolepis	L107
Mabuya	acutilabris	Namibia: Kunene region; Khorixas Dist.; Torra Bay rd, 48 Km W of Kamanjab	CAS 214651	acutilabris	L090
Mabuya	agilis	Brazil: ES, UHE Rosal	01207MRT	agilis-ES1	L006
Mabuya	agilis	Brazil: ES, UHE Rosal	MRT 1206	agilis-ES2	L019
Mabuya	agilis	Brazil: BA, Jacobina	LG 464	agilis-BA	L080
Mabuya	agilis	Brazil: TO, Peixe	MRT 3951	agilis-TO	L123
Mabuya	agmosticha	Brazil: AL, Xingó	LG901	agmosticha-AL1	L014

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Mabuya	agmosticha	Brazil: AL, Xingó	LG902	agmosticha-AL2	L015
Mabuya	atlantica	Brazil: PE, Fernando de Noronha	MRT4429	atlantica-PE1	L021
Mabuya	atlantica	Brazil: PE, Fernando de Noronha	MRT4427	atlantica-PE2	L022
Mabuya	atlantica	Brazil: PE, Fernando de Noronha	MRT4428	atlantica-PE3	L023
Mabuya	aureopunctata	Madagascar: Toliara; Amoasary,Vohidava near Bekinana	RAN 56250	aureopunctata	L116
Mabuya	binotata	Namibia: Kunene Reg.; Kamanjab District, 38.4 Km W. of Kamanjab	AMB 6997	binotata	L110
Mabuya	bistriata	Brazil: AC; Ca 5 km N. Porto Walker, inland from the Rio Juruá, 8o15'31.2"S 72o46'37"W	LSUMZ H-13610	bistriata-AC1	L099
Mabuya	bistriata	Brazil: AC; Ca 5 km N. Porto Walker, inland from the Rio Juruá, 8o15'31.2"S 72o46'37"W	LSUMZ H-13900	bistriata-AC2	L100
Mabuya	bistriata	Brazil: AM; 3o30.9'S 59o54.2'W	LSUMZ H-16489	bistriata-AM1	L102
Mabuya	bistriata	Brazil: AM; 3o30.9'S 59o54.2'W	LSUMZ H-16490	bistriata-AM2	L103
Mabuya	bistriata	Brazil: AP, Igarapé Camaipí	MRT 6300	bistriata-AP1	L127
Mabuya	bistriata	Brazil: AP, Igarapé Camaipí	MRT 6303	bistriata-AP2	L128

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Mabuya	bistriata	Brazil: CE, Mulungú	MRT 154	bistriata-CE1	L063
Mabuya	bistriata	Brazil: CE, Pacoti	MRT 097	bistriata-CE2	L061
Mabuya	bistriata	Brazil: GO, Niquelândia	LG 1085	bistriata-GO	L073.1
Mabuya	bistriata	Brazil: MT, Aripuanã	967956	bistriata-MT1	L008
Mabuya	bistriata	Brazil: MT, Aripuanã	967904	bistriata-MT2	L009
Mabuya	bistriata	Brazil: MT, UHE Manso	LG 1558	bistriata-MT3	L077.1
Mabuya	bistriata	Brazil: MT, UHE Manso	LG 1561	bistriata-MT4	L078.1
Mabuya	bistriata	Brazil: PA, Alter de Chão	MRT 916872	bistriata-PA1	L084
Mabuya	bistriata	Brazil: PA, Vai-Quem-Quer	LG 756	bistriata-PA2	L062
Mabuya	bistriata	Brazil: PA; Agropecuária Treviso LTDA, 101 km. S., 18 km E. Santarém, 3o9'2.4" 54o50'32.9"	LSUMZ H-14358	bistriata-PA3	L101
Mabuya	bistriata	Brazil: PI, Uruçuí-Una	MRT 2502	bistriata-PI	L072.1
Mabuya	bistriata	Brazil: RO; Rio Formoso, Parque Estadual Guajará-Mirim, approx. 90 km N. Nova Mamoré, 10o 19'S 64o 33'W	LSUMZ H-17864	bistriata-RO1	L104
Mabuya	bistriata	Brazil: RO; Rio Formoso, Parque Estadual Guajará-Mirim, approx. 90 km N. Nova Mamoré, 10o 19'S 64o 33'W	LSUMZ H-17865	bistriata-RO2	L105
Mabuya	bistriata	Brazil: RR; Fazenda Nova Esperança, 41 Km W. BR-174 on	LSUMZ H-	bistriata-RR1	L095

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		BR-210 (appx. 10 km E. Rio Ajarani)	12369		
Mabuya	bistriata	Brazil: RR; Fazenda Nova Esperança, 41 Km W. BR-174 on BR-210 (appx. 10 km E. Rio Ajarani)	LSUMZ H-12311	bistriata-RR2	L098
Mabuya	boettgeri	Madagascar: Antananarivo; Ambatolampy, Ankaratra Manjakatompo	UMMZ 208917	boettgeri	L117
Mabuya	capensis	South Africa, Northern Cape Prov., Richtersveld Nat. Park, 28.11'02"S, 17.02'14"E, 580 m Elev	AMB 4765	capensis	L087
Mabuya	carvalhoi	Brazil: RR; 1 km W. BR-174 and 1-5 km. N. BR-210	LSUMZ H-12420	carvalhoi	L097
Mabuya	cumingi	Philippines: Luzon; Kalinga Prov, Municipality of Balbalan, Barangay Balbalasang	FMNH 259457	cumingi	L154
Mabuya	dorsivitatta	Brazil: SP, São Paulo	LG 1089	dorsivittata-SP3	L082
Mabuya	dorsivitatta	ARGENTINA. Cordoba Province. Rio Cuarto Department. Rio Cuarto city between Malvinas and Carretero bridges	5000-Luciano	dorsivittata-ARG	L167
Mabuya	dorsivittata	Brazil: SP, Ribeirão Grande	LG1273	dorsivittata-SP1	L010
Mabuya	dorsivittata	Brazil: SP, Ribeirão Grande	LG1274	dorsivittata-SP2	L011
Mabuya	dumasi	Madagascar: Mahajanga; Antsalova, Bemaraha reserve	RAN 54509	dumasi	L118

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		Ambalarano Tsingy			
Mabuya	elegans	Madagascar: Toliara; Amoasary,Vohidava near Bekinana	RAN 56248	elegans	L119
Mabuya	ficta	Brazil: AC, Estirão do Panela, PNSD	MBS 001	ficta-AC	L126
Mabuya	ficta	Brazil: AM; Rio Ituxi at the Madeirera Scheffer, 8o 20'47.0", 65o42'57.9"	LSUMZ H-14104	ficta-AM	L096
Mabuya	frenata	Brazil: GO, Santa Rita do Araguaia	LG861	frenata-GO1	L125
Mabuya	frenata	Brazil: MS, Corumbá	LG 1042	frenata-MS	L071.1
Mabuya	frenata	Brazil: PI, Serra das Confusões	SC 47	frenata-PI1	L066
Mabuya	frenata	Brazil: TO, Parque Nacional do Araguaia	PNA 77	frenata-TO1	L070.1
Mabuya	frenata	Brazil: TO, UHE Lajeado	MRT 08714	frenata-TO2	L124
Mabuya	frenata	Brazil: GO, Emas	L001	frenata-GO2	L064.1
Mabuya	frenata	Brazil: PI, Serra das Confusões	SC 28	frenata-PI2	L079.1
Mabuya	frenata	Brazil: MT, Gaúcha do Norte	LG 1247	frenata-MT	L069.1
Mabuya	gravenhorsti	Madagascar: Namoroka Reserve	RAX 00265	gravenhorsti	L120
Mabuya	guaporicola	Brazil: MT, UHE Manso	LG 1574	guaporicola-MT	L075.1
Mabuya	guaporicola	Brazil: TO, Parque Nacional do Araguaia	PNA185	guaporicola-TO	L016
Mabuya	heathi	Brazil: BA, Alagoado	907011	heathi-BA1	L017

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Mabuya	heathi	Brazil: BA, Jacobina	907101	heathi-BA2	L018
Mabuya	heathi	Brazil: BA, Mocambo do Vento	MRT 3671	heathi-BA3	L068
Mabuya	heathi	Brazil: PI, Serra das Confusões	SC 21	heathi-PI	L076.1
Mabuya	hoeschi	Namibia: Kunene region; Khorixas Dist.; Sesfontein rd., 52 km N. of Palmweg.	AMB 5947	hoeschi	L002
Mabuya	homalocephala	South Africa: Western Cape Prov.; ~4.6 km N of Grootbaai, Bloubergstrand on Melkbos rd.	AMB 7072	homocephala	L109
Mabuya	longicaudata	Lao PDR: Khammouane Prov.; Boualapha Dist., Hin namno NBCA	FMNH 255526	longicaudata	L156
Mabuya	macrorhyncha	Brazil: SP, Itanhaém, Ilha da Queimada Grande	LG1103	macrorhyncha-SP1	L013
Mabuya	macrorhyncha	Brazil: SP, Itanhaém, Ilha da Queimada Grande	LG 1102	macrorhyncha-SP2	L067
Mabuya	macularia	Lao PDR: Champassak Prov.; Pakxong Dist. , Dong Hua Sao NBCA	FMNH 258873	macularia	L153
Mabuya	madagascarensi s	Madagascar: Antananarivo; Ambatolampy, Ankaratra Ambohimirandrana	UMMZ 209103	madagascarensis	L121
Mabuya	multifasciata	Lao PDR: Champassak Prov.; Mounlapamok Dist., Dong Khanthung NBCA	FMNH 255530	multifasciata	L155

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Mabuya	occidentalis	South Africa, Northern Cape Prov., Farm Avonschijn 26.51'46"S, 21.06'24"E, 850 m Elev	AMB 6253	occidentalis	L091
Mabuya	perotetii	Ghana; USFWS	FMNH 262231	perotetii-1	L160
Mabuya	perotetii	Ghana; USFWS	FMNH 262230	perotetii-2	L161
Mabuya	perotetii	Ghana; USFWS	FMNH 262229	perotetii-3	L162
Mabuya	perotetii	Ghana; USFWS	FMNH 262228	perotetii-4	L163
Mabuya	perotetii	Ghana; USFWS	FMNH 262227	perotetii-5	L164
Mabuya	quinquetaeniata	Ghana; USFWS	FMNH 262232	quinquetaeniata-1	L159
Mabuya	quinquetaeniata	Ghana; USFWS	FMNH 262236	quinquetaeniata-2	L165
Mabuya	quinquetaeniata	Ghana; USFWS	FMNH 262235	quinquetaeniata-3	L166
Mabuya	quinquetaeniata	Unknown	BYU 47330	quinquetaeniata-4	L092
Mabuya	quinquetaeniata	Unknown	BYU 47350	quinquetaeniata-5	L108
Mabuya	rudis	Malaysia: Sabah; Sipitang Dist.	FMNH 239732	rudis	L152
Mabuya	spilogaster	Namibia: Erongo Region; Karibib Dist.; Usakos-Hentiesbaai rd., 10km E. of Spitzkop turnoff	AMB 5893	spilogaster	L001
Mabuya	striata	Namibia: Kunene Region; Opuwo Dist.; Opuwo rd., 87.6 km N. of Palmweg-Sesfontein rd.	AMB 5959	striata	L003

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Mabuya	variegata	South Africa: Western Cape Prov.; McDongall Bay	AMB 4505	variegata	L089
Mabuya	vato	Madagascar: Toliara; Amoasary,Vohidava near Bekinana	RAN 56249	vato	L122
Tiliqua	gigas	Papua New Guinea: Gulf Province; Kakoro Village, Lakekamu Basin.	BYU46821	Tiliqua	L035
Typhlosauru s	caecus	South Africa: Northern Cape Province; 9.9 Km S. of Lambertsbaai	AMB 6817	Typhlosaurus	A3

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