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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.

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

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	–

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	

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-

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

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

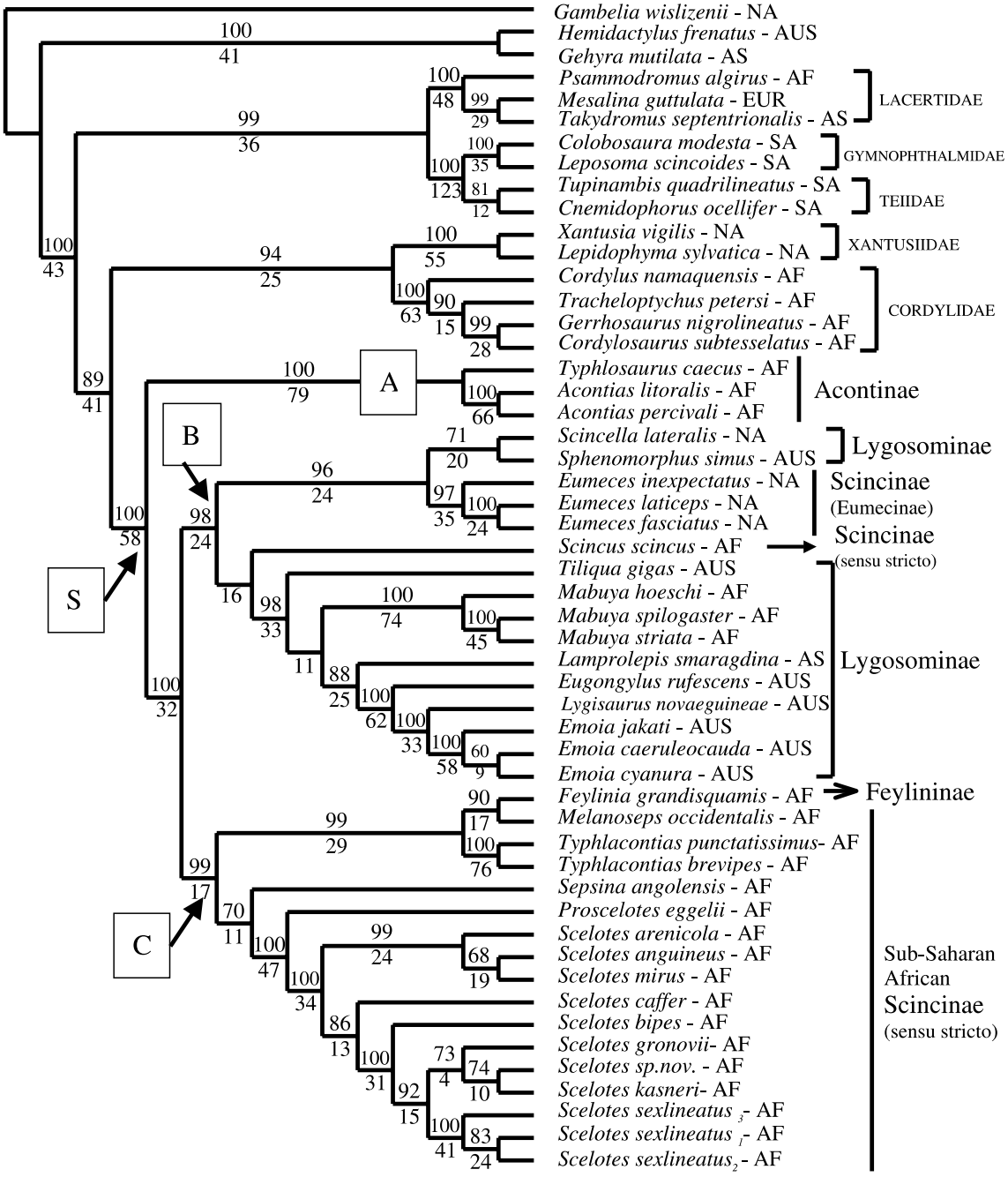

Fig. 1. Optimization alignment (parameter set 1:1:1) and Parsimony (gaps coded as 5th state) topology, cost 13,029. Numbers above branches are bootstrap support (values below 50% are not shown); numbers below branches are Bremer support values. Clade S, Scincidae; clade A, Acontinae; clade B, Lygosominae + *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

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).

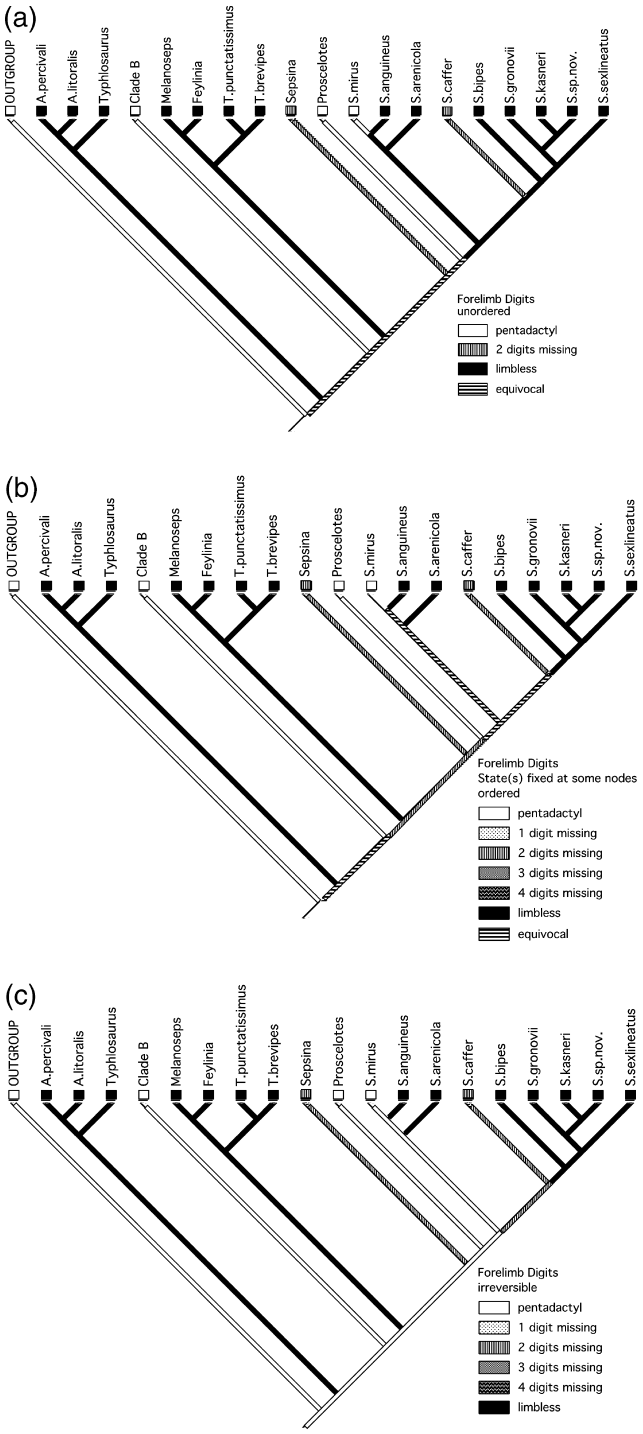

Fig. 2. Parsimony reconstructions for forelimb digit character: 0—pentadactyl, 1—1 digit missing, 2—2 digits missing, etc. *Note*. in order to simplify figures the entire tree has not been shown. (a) Unordered character reconstruction with a total cost of 7, showing support for limb gain (*S. mirus*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

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

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

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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