

Phylogenetic Affinities of the Rare and Enigmatic Limb-Reduced *Anelytropsis* (Reptilia: Squamata) as Inferred with Mitochondrial 16S rRNA Sequence Data

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ABSTRACT.—The phylogenetic placement of the limbless lizard genus *Anelytropsis* within Squamata was investigated using partial mitochondrial 16S rRNA sequences (422 bp). A total of 30 species, representing most of the major currently recognized squamate clades, was included in the analysis. As in previous morphological studies, *Anelytropsis* was strongly supported in Bayesian (mixture and unpartitioned models) and maximum-likelihood analyses as the sister taxon of *Dibamus*. Thus, a monophyletic Dibamidae composed of these two genera is supported by molecular data for the first time. Furthermore, several relationships in the inferred tree, although weakly supported, were congruent with those found in previous molecular phylogenetic analyses. Among these, Gekkota and the Dibamidae were recovered as relatively basal groups within Squamata. A nonmonophyletic Scleroglossa and Lacertiformes (= Amphisbaenia + Lacertidae + Teiidae + Gymnophthalmidae) also were recovered. Although only weakly supported, a major difference from other recent molecular studies is the basal position of Serpentes.

The Dibamidae is a small, enigmatic group of essentially limbless squamate reptiles that encompasses two genera (*Dibamus* and *Anelytropsis*; Pough et al., 2004). The monotypic genus *Anelytropsis* is restricted to some areas of northeastern Mexico, and it is known in scientific collections only from a few specimens (Campbell, 1974; Greer, 1985). Long placed in its own monotypic family (Anelytropsidae), the phylogenetic affinity of *Anelytropsis* was not seriously investigated until Miller's (1966) and Etheridge's (1967) pioneer comparative studies of the inner ear and caudal vertebrae morphology, respectively. These authors suggested that *Anelytropsis* might be closely related to *Dibamus*, a group restricted to southeast Asia; however, it was only after Greer's (1985) morphological study that these two taxa were consistently placed as the only genera of the family Dibamidae.

The phylogenetic placement of Dibamidae within the Squamata, whether or not including *Anelytropsis*, has been the subject of intense debate. Previous morphological studies have been far from conclusive, suggesting that

dibamids might be closely related to several different major squamate clades such as snakes (Haas, 1973; Seen and Northcutt, 1973; Greer, 1985), scincids (Cope, 1900; Camp, 1923; Seen and Northcutt, 1973), geckos (Hebard and Charipper, 1955a,b; Underwood, 1957; McDowell, 1972), or amphisbaenians (Hallermann, 1998; Lee, 1998). Thus, these morphological studies have resulted in phylogenetic hypotheses for Dibamidae that are rather discordant or contradictory. Also, no previous morphological studies have included *Anelytropsis* and *Dibamus* as terminal taxa in an explicit phylogenetic analysis; thus, the monophyly of Dibamidae has never been rigorously tested.

The phylogenetic relationships of Squamata have been the subject of a number of recent studies based on morphological (e.g., Hallermann, 1998; Lee, 1998) and molecular (e.g., Saint et al., 1998; Harris et al., 1999, 2001; Harris, 2003) data. Historically, the phylogenetic placement of the major limbless clades (i.e., Serpentes, Amphisbaenia, Dibamidae) has been problematic and controversial (Estes et al., 1988; Kluge, 1989), but recent molecular systematic studies have seemingly resolved some of these issues in squamate phylogeny. Townsend et al. (2004) and Vidal and Hedges (2005) provide strong molecular support that amphisbaenians are closely related to lacertids and that snakes are

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nested within a clade containing Anguimorpha and Iguania (although the interrelationships among these three clades are unclear). These authors also provide seemingly strong evidence that Dibamidae is a relatively basal clade, with the preferred hypotheses of both of these molecular studies placing *Dibamus* as the sister taxon to all remaining squamates.

Although these recent molecular studies have included the Old World *Dibamus* as a representative of the Dibamidae, these studies did not address or test the monophyly of Dibamidae because of the exclusion of the New World *Anelytropsis* (i.e., the lack of tissue samples prevented the inclusion of *Anelytropsis*). In 1992, a specimen of *Anelytropsis papillosus* was collected 4 km southwest of Arroyo Blanco, Hidalgo, Mexico, and the specimen and a frozen tissue sample from it was deposited in the Museo de Zoología "Alfonso L. Herrera" of the Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC). Unfortunately, DNA extracted from this sample was seriously degraded. Although several attempts to amplify different regions of the mtDNA (i.e., 12S, ND1, ND2, ND4) were made, only a small fragment of mtDNA, corresponding to the 16S rRNA gene, was successfully amplified. Herein, we report the results of a phylogenetic analysis of Squamata based on sequences of this small region of the 16S rRNA gene to assess the phylogenetic position of *Anelytropsis* within Squamata and discuss the relationships of Dibamidae with respect to the other major squamate clades.

MATERIALS AND METHODS

Taxon Sampling.—A total of 30 taxa was examined. These taxa comprised species representative of the major clades of Squamata, including members of the limbless groups *Anelytropsis*, *Dibamus*, *Amphisbaenia*, and *Serpentes* and most of the currently recognized noniguanian lizard families. A member of the Rhynchocephalia (*Sphenodon punctatus*) was included as outgroup. Previous phylogenetic studies based on nuclear and mitochondrial DNA sequences consistently recovered Rhynchocephalia as sister group of Squamata (Saint et al., 1998; Townsend et al., 2004).

Two new sequences were generated for this study: DIBAMIDAE: *A. papillosus*, MZFC 5389, Mexico: Hidalgo: 4 km southwest of Arroyo Blanco; GenBank accession number DQ990973; and XENOSAURIDAE: *Xenosaurus platyceps*, MZFC uncataloged, collector number NM-135, Mexico: Tamulipas: 17.7 km southwest of Ciudad Victoria (23°36.832'N, 99°14.664'W), 1,929 m elevation, GenBank accession number

DQ990972. The remaining sequences used in this study were retrieved from GenBank (accession numbers in parentheses): AGAMIDAE: *Pogona vitticeps* (AB166795); AMPHISBAENIA: *Amphisbaena schmidti* (AY605475), *Bipes canaliculatus* (NC_006288), *Geocalamus acutus* (AY605476); ANGUIDAE: *Abronia graminea* (AB080273); CHAMAELONIDAE: *Chamaeleo africanus* (AF121955), *Furcifer labordi* (AF215264); CORDYLIDAE: *Cordylus warreni* (AB079613); DIBAMIDAE: *Dibamus* sp. (AY649149); GEKKONIDAE: *Eublepharis maculatus* (AB028762), *Rhacodactylus trachyrhynchus* (AF215258); GYMNOPHTHALMIDAE: *Gymnophthalmus speciosus* (AF101368); IGUANIDAE: *Crotaphytus collaris* (L41443), *Gambelia wislizenii* (AY217944), *Iguana iguana* (AJ278511), *Sceloporus virgatus* (AF440085); LACERTIDAE: *Acanthodactylus erythrurus* (AF206607), *Lacerta andreanskyi* (AF206603), *Podarcis hispanica* (DQ081104); SCINCIDAE: *Eumeces egregius* (AB016606); SERPENTES: *Dinodon semicarinatus* (AB008539), *Oviphis okinavensis* (AB175670), *Ramphotyphlops bicolor* (AY442836); SHINISAURIDAE: *Shinisaurus crocodilurus* (AB080274); TEIIDAE: *Ameiva plei* (AY359507); VARANIDAE: *Varanus komodoensis* (AB080275); XANTUSIIDAE: *Xantusia vigilis* (AY217993); RHYNCHOCEPHALIA: *Sphenodon punctatus* (L28076).

PCR and DNA Sequencing.—The DNA sequence data examined in this study consisted of a portion (~500 bp) of the mitochondrial 16S rRNA gene. This gene has been successfully employed in various studies at different taxonomic levels within Squamata (e.g., Wiens et al., 1999; Pang et al., 2003; Whiting et al., 2003; Lin et al., 2003). Although this fragment of the 16S rRNA gene has been used previously in a combined (= morphology + DNA) phylogenetic analysis of higher-level squamate phylogeny (Lee et al., 2004), it has never been used solely to reconstruct squamate relationships.

For the sequences generated for this study, genomic DNA was extracted following the phenol-chloroform DNA extraction protocol described by Hillis et al. (1996). Although several primers were tried to amplify a larger fragment of the 16S rRNA gene from the *Anelytropsis* sample, these attempts failed, presumably because of the highly degraded condition of the DNA extracted. PCRs were carried out in a 25 µl final volume employing 1 µl of template DNA, 0.2 µl of each primer (1 µM), 1 µl of dNTPs (2 µM), 2.5 µl of 10 X PCR buffer, 1.5 µl of MgCl₂, 0.15 µl of *Taq* polymerase (5 U/µl), and 18.45 µl of H₂O. The primers used for amplification of the examined mtDNA fragment were those designed by Reeder (2003; fwd: 16AR2: 5'-CCCGMCTGTTTACCAAAAACA-3') and Reeder (1995; rev: 16d: 5'-CTCCGG TCTGAACCTCAGATCACGTAG-3'). PCR con-

ditions consisted of an initial cycle at 95°C for 5 min, followed by 38 cycles of denaturation at 94°C for 30 sec, annealing at 53°C for 30 sec, and extension at 72°C for 1 min, and a final extension step at 72°C for 5 min. PCR products were purified by gel filtration using Sephadex G50 columns and subsequently sequenced using the Big Dye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems) and an ABI 3100 Genetic Analyzer sequencer.

Sequence alignment was performed manually using the 16S rRNA secondary structure of *Sceloporus* in Wiens and Reeder (1997). Regions including indels were characterized according to the three categories proposed by Gillespie (2004). A total of 10 ambiguously aligned regions were excluded from subsequent analyses. Of these, six regions were of ambiguous alignment with no identifiable base pairing (RAAs); two were RAAs with adjacent regions of expansion and contraction (RECs); and the remaining two corresponded to regions of slipped-strand compensation (RSCs). The 16S rRNA matrix and the maximum-likelihood topology inferred from it can be retrieved from the TreeBase web page (Study accession number S1991, Matrix accession number M3705).

Phylogenetic Analysis.—The aligned DNA sequences were analyzed using both Bayesian and maximum-likelihood (ML) methods of phylogenetic inference. For the Bayesian analyses, two approaches were undertaken. One was a standard unpartitioned analysis conducted using MrBayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003), using the GTR + I + Γ model. This model was chosen on the basis of the Akaike information criterion (AIC) using MrModeltest v. 2.2 (Nylander, 2004). Two independent analyses were run, each consisting of 4.0×10^6 generations with a random starting tree, default uniform priors and four Markov chains (with default heating values) sampled every 100 generations. Stationary of the log-likelihood ($-\ln L$) values was determined visually, and the postburn-in tree samples of each analysis were used to construct a 50% majority-rule consensus tree. Given that the consensus topologies derived from the independent analyses were identical, their postburn-in sampled trees were pooled to create a single 50% majority-rule consensus tree.

In addition to the unpartitioned Bayesian analysis, analyses were performed using a phylogenetic mixture model that accommodates pattern-heterogeneity in the DNA sequence data (Pagel and Meade, 2004). Unlike partitioned Bayesian analyses (e.g., Nylander et al., 2004; Brandley et al., 2005), mixture model analyses take pattern-heterogeneity (i.e., different nucleotide substitution patterns exhibited

by different subsets of the data) into account without designating data partitions a priori. These analyses were performed using the computer program BayesPhylogenies (Pagel and Meade, 2004) under the GTR+ Γ model of sequence evolution (the most general model that can be implemented in BayesPhylogenies). Because partitions or subsets of the data are not identified a priori, it is not possible to test various partition-specific models of evolution as generally done in partitioned-Bayesian analyses. Thus, it is recommended that the most general model (i.e., GTR + Γ) be used because any potentially simpler models for a posteriori discovered subsets of data will be special cases of the more general model (M. Pagel and A. Meade, unpubl.).

Using BayesPhylogenies, we conducted a total of 24 independent MCMC runs of various nQ and nQ + Γ phylogenetic mixture models (each particular model run twice to ensure convergence on same posterior distribution), with “n” varying between one and six independent rate matrices (Qs) and Γ representing a gamma-rates model (as generally implemented in other model based phylogenetic analyses to accommodate rate-heterogeneity) with four discrete rate categories. Each MCMC run consisted of 3.0×10^6 generations with a random starting tree, default uniform priors, and four Markov chains (with default heating values) sampled every 1,000 generations. As in the unpartitioned Bayesian analyses, stationary was determined visually by plotting $-\ln L$ against generation time, and the postburn-in tree samples of each analysis were used to construct a 50% majority-rule consensus tree. As recommended by Pagel and Meade (2004), three different criteria were evaluated to select the most appropriate mixture model and preferred analysis (for recent example, also see Torres-Carvajal et al., 2006): (1) $2\ln B$ factors ($2\ln B = 6-10$ as strong evidence and $2\ln B > 10$ as very strong evidence against an alternative hypothesis; Kass and Raftery, 1995); (2) variability of estimated tree length and rate parameters; and (3) relative matrix weights. The Bayes factor is commonly used in model selection; however, Bayes factors may be overestimated when the number of independent nucleotide sites is far fewer than the total number of sites in a data set (Pagel and Meade, 2004, 2005), thus, necessitating the additional criteria in model selection. And finally, in the unpartitioned and mixture model Bayesian analyses, clades were considered strongly supported if their posterior probabilities were ≥ 0.95 (Wilcox et al., 2002; Alfaro et al., 2003; Huelsenbeck and Rannala, 2004).

The ML analyses were performed in PAUP* (v. 4.0b10; D. L. Swofford, Sinauer Associates,

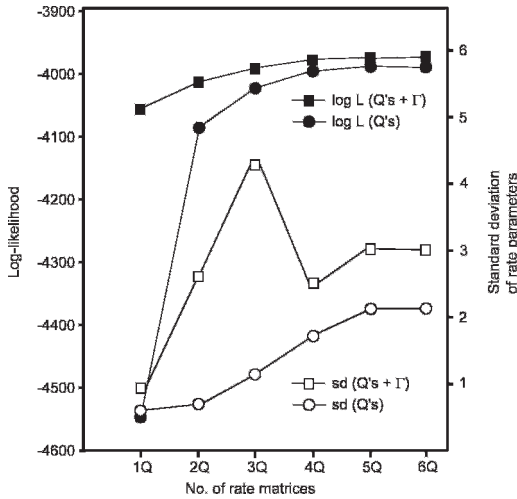


FIG. 1. Plot of the harmonic means of log-likelihoods (left y-axis) and means of the rate parameter standard deviations (right y-axis) of various phylogenetic mixture models with and without gamma rate heterogeneity (Γ).

Sunderland, MA, 2000) under the GTR + I + Γ model of nucleotide evolution (base frequencies: A = 0.35, C = 0.22, G = 0.20, T = 0.23; Shape = 0.4273; Pinvar = 0.2907), with this model being chosen on the basis of the AIC in Modeltest v. 3.7 (Posada and Crandall, 1998). The analysis consisted of a heuristic search with stepwise addition (100 random addition sequences) and tree bisection and reconnection (TBR) branch swapping. Nodal support was estimated via a nonparametric bootstrap analysis (Felsenstein, 1985) with 100 full heuristic pseudo-replicates (two random addition sequence replicates per bootstrap pseudo-replicate and TBR branch swapping). Clades with bootstrap proportions $\geq 70\%$ were considered strongly supported (Hillis and Bull, 1993; but see their caveats).

A Bayesian approach to hypothesis testing (e.g., Buckley et al., 2002; Reeder, 2003; Brandley et al., 2005) was used to test the following alternative phylogenetic placements of Dibamidae (= *Dibamus* + *Anelytropis*; a strongly

supported clade in all above phylogenetic analyses; see Results): (1) sister group of amphisbaenians; (2) sister to snakes; (3) sister to gekkotans; and (4) forms a clade with amphisbaenians and snakes.

RESULTS

The data set consisted of a total of 422 unambiguously aligned positions, 201 of which were variable and 152 parsimoniously informative. The Bayesian analyses under the mixture model approach resulted in differences in topologies and estimated parameters depending on which models were used (i.e., 1 – 6Q and 1 – 6Q + Γ). Significant rate-heterogeneity was present in the data, as indicated by the mean $-\ln L$ of the 1Q + Γ model being about 514 units better than the mean $-\ln L$ of the one rate model (1Q; Fig. 1). Given this, mixture models that did not explicitly accommodate rate-heterogeneity with Γ (i.e., 1 – 6Q) were not considered for final model selection, even though $-\ln L$ scores improved with the incorporation of additional pattern-heterogeneity matrices (i.e., 2 – 6Q; but $-\ln L$ scores were still all worse than comparable analyses incorporating Γ ; see Fig. 1).

Use of the 2lnB suggested that successive incorporation of additional pattern-heterogeneity matrices (i.e., 1 – 4Q + Γ) were strongly supported and represent better models of evolution for the mitochondrial rDNA (i.e., all 2lnB > 10; Table 1). Even the incorporation of a fifth and sixth rate matrix (i.e., 5 – 6Q + Γ) appeared to have strong support (i.e., 2lnB = 6–10). However, examination of the averages of the tree length and rate parameter standard deviations showed a marked increase in variability as increasing numbers of rate matrices were incorporated into the mixture model. The Bayes factor may strongly suggest that the 6Q + Γ model best explains the evolution of the data (although marginally), the increasing averages of the parameter standard deviations indicated that parameters were increasingly being poorly estimated as the number of rate matrices increased (Fig. 1). Also, when the relative

TABLE 1. Bayes factors estimates and relative matrix weights used in the selection of phylogenetic mixture models incorporating Γ .

Model	Harmonic mean $-\ln L$	Bayes factor estimate 2lnB	Relative matrix weights
1Q + Γ	-4,055.682	n/a	1.0
2Q + Γ	-4,014.317	82.7	0.39, 0.61
3Q + Γ	-3,991.013	46.6	0.24, 0.53, 0.23
4Q + Γ	-3,978.054	25.9	0.18, 0.27, 0.32, 0.24
5Q + Γ	-3,974.598	6.9	0.17, 0.27, 0.28, 0.09, 0.19
6Q + Γ	-3,971.690	5.8	0.06, 0.09, 0.08, 0.27, 0.28, 0.22

matrix weights were evaluated, it was evident that the incorporation of five or more rate matrices was only adding rate matrices that received little weight (i.e., ≤ 0.09 ; Table 1). Given that there was very strong 2lnB support for the simpler 4Q + Γ model and all of its matrices received relatively high matrix weights (i.e., ≥ 0.18 ; Table 1), the results of the Bayesian analysis using this mixture model were preferred.

The 50% majority-rule consensus tree from the preferred mixed-model Bayesian analysis (i.e., 4Q + Γ) depicted two basal clades (Fig. 2): one strongly supported clade representing Serpentes (mixed-model posterior probability [mmPP] = 0.99; unpartitioned posterior probability [PP] = 0.99; bootstrap proportion [BP] = 81) and a larger weakly supported clade containing all remaining squamates (mmPP = 0.61; PP = 0.60). This latter clade supports a clade with the gekkotans (mmPP = 0.60; PP = 0.59) as the sister group to the rest, followed in ascendant order by *Xantusia*, a weakly supported clade containing the scincid *Eumeces* and the dibamids (*Anelytropsis* and *Dibamus*), and a more exclusive clade (= Clade A) with all the remaining squamate taxa. Although dibamid monophyly was strongly supported (mmPP = 0.99; PP = 0.98; BS = 74), these other basal relationships (including the placement of Dibamidae) were all weakly supported by the mtDNA data. Clade A was composed of two weakly supported main subclades. One of these subclades contains the members of Iguania (whose monophyly was weakly supported) and Anguimorpha (i.e., Varanidae, Shinisauridae, Anguinae, and Xenosauridae); however, anguimorph monophyly was not supported by the mtDNA data. Within this specific subclade, only the acrodont and chamaeleon clades were strongly supported in all analyses. The ML analysis suggested strong support (i.e., BS > 70%) for shinisaur + anguid + xenosaur and anguid + xenosaur clades, but the Bayesian analyses provided weak support or marginally strong support (at best) for these relationships. The other main subclade included the cordylid and lacertiforms (i.e., amphisbaenians, lacertids, teiids, and gymnophthalmids). Within this subclade, all the basal relationships were weakly supported, but the mtDNA data did provide strong support for amphisbaenians (mmPP and PP = 0.99; BS = 87), lacertids (mmPP = 0.97; PP = 0.98; BS = 77), and teioids (mmPP and PP = 1.0; BS = 100).

The ML analysis (GTR +I+ Γ ; six optimal trees; $-\ln L = 4005.09$) and the unpartitioned Bayesian analysis (GTR + I + Γ ; posterior distribution harmonic mean $-\ln L = 4,044.411$) resulted in topologies that were essentially

the same to each other. The only differences being weakly supported alternate relationships among the three lacertids (i.e., ML - *Acanthodactylus* + *Lacerta*; BA - *Acanthodactylus* + *Podarcis*) and alternate placements of *Gambelia* (i.e., ML - *Gambelia* + *Crotaphytus*; BA - *Gambelia* + Acrodonta). There were more topological differences between these two analyses and the preferred tree from the mixture model Bayesian analysis, but these only involved weakly supported alternative relationships (e.g., *Xantusia* was sister taxon to Lacertiformes; *Varanus* was sister taxon to a *Xantusia* + Lacertiformes clade, *Eumeces* was sister taxon to a clade containing all squamates to the exclusion of dibamids, gekkotans and snakes; pleurodont paraphyly).

Although many of the higher-level squamate relationships inferred by the mtDNA data were weakly supported, the Bayesian approach for hypothesis testing supported the preferred Bayesian phylogeny (Fig. 2) as a significantly better explanation of the data than the following alternative phylogenetic hypotheses: dibamids + amphisbaenians, dibamids + gekkonids, and dibamids + amphisbaenians + snakes. The alternative hypothesis that constrained dibamids + snakes could not be statistically rejected; however, this specific conclusion was a result of a single tree in the 95% credible set (consisting of 475 trees) grouping dibamids with snakes.

DISCUSSION

Greer (1985) regarded *Anelytropsis* and *Dibamus* as each other's closest relatives on the basis that they share a large number of derived character states, two of which are unique in squamates: a complex construction of the bony secondary palate; and the presence of transverse plicae across the entire dorsal surface of the tongue. Despite the small size of the mitochondrial DNA fragment analyzed herein, and the reduced number of strongly supported higher-level squamate relationships, our results significantly support and confirm the close relationship between *Anelytropsis* and *Dibamus*. Thus, the recognition of a monophyletic Dibamidae composed of these two genera is supported by our data.

In our present study, the basal placement of Serpentes is not congruent with the two most recent and comprehensive molecular phylogenetic analyses of Squamata (Townsend et al., 2004 [4,600 bp of mtDNA and nuclear DNA]; Vidal and Hedges, 2005 [6,192 bp of nuclear DNA]), which both strongly supported a much more nested position of snakes within Squamata. However, the relatively basal position of dibamids is consistent with both of these

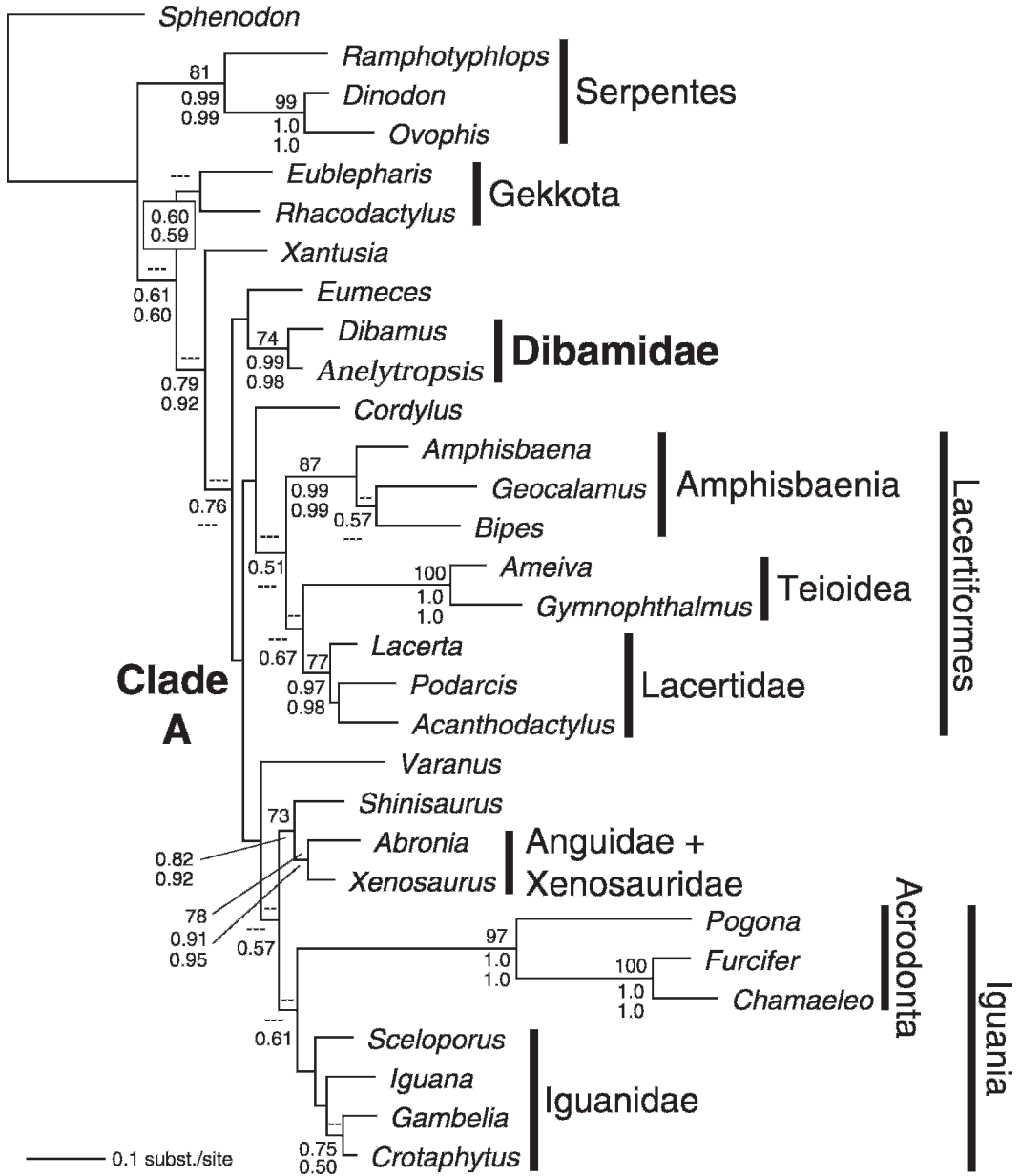


FIG. 2. The 50% majority-rule consensus tree (including compatible groups) obtained from the preferred mixture model (i.e., 4Q + I) Bayesian analysis of the 16S rRNA gene data. Numbers below branches represent estimated posterior probabilities (top: derived from mixture model analysis [mmPP]; bottom: derived from unpartitioned analysis [PP]). Numbers above branches represent estimated bootstrap proportions. Only support values greater than 0.50 (posterior probabilities) or 50% (bootstrap proportions) are shown. The major squamate clades that are named are those congruent with Townsend et al. (2004).

previous studies. Based on combined mitochondrial and nuclear DNA evidence, Townsend et al. (2004) placed gekkotans as the sister taxon to remaining squamates (with dibamids being the next most basal group), but these relative relationships between gekkotans and dibamids

to the rest of Squamata was weakly supported by their combined molecular data (although their data did strongly support these relatively basal positions of dibamids and gekkotans). Vidal and Hedges (2005) provided seemingly strong support for dibamids being the sister

taxon to the remaining squamates, with gekkotans being the next most basal clade (congruent with the nuclear DNA-only analysis of Townsend et al., 2004). Our study provides additional evidence toward the idea that dibamids are an old and relatively basal group of squamate reptiles.

Although many of the other higher-level squamate relationships inferred in our study were weakly supported, our analyses did allow us to exclude some previous hypotheses of dibamid affinities. Bayesian hypothesis testing suggested that the alternative dibamids + amphisbaenians, dibamids + gekkotans, and dibamids + amphisbaenians + snakes relationships were significantly worse explanations of our mtDNA data. Our mtDNA data could not reject the alternative dibamids + snakes hypothesis. However, this specific result may be spurious since our inability to reject this alternate hypothesis is because of a single tree in the 95% credible set that supported such a relationship; also, a dibamids + snakes relationship does appear to be strongly rejected by Townsend et al. (2004) and Vidal and Hedges (2005), who both provide strong support for snakes being nested within a clade containing iguanians and anguimorphs. These two previous studies also appear to strongly reject a sister relationship between dibamids and gekkotans; however, it is possible to root their preferred phylogenetic hypotheses that would result in a dibamid + gekkotan clade. Thus, in future molecular phylogenetic studies of squamates, further attention should be placed on the root of Squamata, which is critical to these recently proposed hypotheses involving a relatively basal placement of dibamids.

It is worth noting that our limited phylogenetic analysis using 16S rDNA does corroborate some other squamate relationships inferred by Townsend et al. (2004) and Vidal and Hedges (2005). First, it is evident that Squamata should not be divided into two basal taxa, namely Iguania and Scleroglossa. Our study also corroborates (although weakly) a close phylogenetic affinity between amphisbaenians and lacertiform lizards (i.e., lacertids, gymnophthalmids, and teiids), as well as strongly supporting the traditional Teiioidea (i.e., Teiidae + Gymnophthalmidae). Also, there is support in the mtDNA data to group the anguimorphs with iguanians; although our mtDNA did not support anguimorph monophyly. A close relationship between anguimorphs and iguanians and between xenosaurs and anguids are relationships that are strongly supported by Townsend et al. (2004) and Vidal and Hedges (2005); thus, our new data provide additional support to these ideas.

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