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Molecular Phylogenetics of Squamata: The Position of Snakes, Amphisbaenians, and Dibamids, and the Root of the Squamate Tree

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Abstract.—Squamate reptiles (snakes, lizards, and amphisbaenians) serve as model systems for evolutionary studies of a variety of morphological and behavioral traits, and phylogeny is crucial to many generalizations derived from such studies. Specifically, the traditional dichotomy between Iguania (anoles, iguanas, chameleons, etc.) and Scleroglossa (skinks, geckos, snakes, etc.) has been correlated with major evolutionary shifts within Squamata. We present a molecular phylogenetic study of 69 squamate species using approximately 4600 (2876 parsimony-informative) base pairs (bp) of DNA sequence data from the nuclear genes *RAG-1* (∼2750 bp) and *c-mos* (∼360 bp) and the mitochondrial *ND2* region (∼1500 bp), sampling all major clades and most major subclades. Under our hypothesis, species previously placed in Iguania, Anguimorpha, and almost all recognized squamate families form strongly supported monophyletic groups. However, species previously placed in Scleroglossa, Varanoidea, and several other higher taxa do not form monophyletic groups. Iguania, the traditional sister group of Scleroglossa, is actually highly nested within Scleroglossa. This unconventional rooting does not seem to be due to long-branch attraction, base composition biases among taxa, or convergence caused by similar selective forces acting on nonsister taxa. Studies of functional tongue morphology and feeding mode have contrasted the similar states found in *Sphenodon* (the nearest outgroup to squamates) and Iguania with those of Scleroglossa, but our findings suggest that similar states in *Sphenodon* and Iguania result from homoplasy. Snakes, amphisbaenians, and dibamid lizards, limbless forms whose phylogenetic positions historically have been impossible to place with confidence, are not grouped together and appear to have evolved this condition independently. Amphisbaenians are the sister group of lacertids, and dibamid lizards diverged early in squamate evolutionary history. Snakes are grouped with iguanians, lacertiforms, and anguimorphs, but are not nested within anguimorphs. [Amphisbaenia; Dibamidae; DNA; Iguania; lizards; long-branch attraction; mitochondrial; nuclear; phylogeny; Scleroglossa; Serpentes, Squamata.]

Evolutionary biologists often seek generalities about evolutionary processes from detailed studies of particular model systems, and squamate reptiles have provided a large number of such systems (e.g., Huey et al., 1983; Vitt and Pianka, 1994). An accurate squamate phylogeny is crucial to studies of morphological, behavioral, and life-history variation because phylogeny is a key part of comparative methodology (Miles and Dunham, 1993). For example, herpetological studies of foraging mode and prey chemical discrimination (Cooper, 1995; Perry, 1999), demographic tactics (Clobert et al., 1998), and home-range variation (Perry and Garland, 2002) have all explicitly incorporated phylogeny into their testing framework to insure appropriate, independent comparisons. The evolution of squamate tongue morphology and chemoreception abilities is cited as a prime example of the importance of history in present-day distribution patterns and ecology (Schwenk and Wagner, 2001; Vitt et al., 2003). A subtle shift in prey-prehension technique is thought to have allowed major changes in tongue morphology and chemosensory abilities (Cooper, 1995; Schwenk, 1993; Schwenk and Wagner, 2001). This shift coincided with the Early Jurassic split between the two major squamate clades, Iguania and Scleroglossa, and allowed the scleroglossans to exploit a variety of new habitats and foraging modes unavailable to iguanians, such that scleroglossans now predominate on a global scale, over 200 million years (my) later (Schwenk and Wagner, 2001; Vitt et al., 2003). All of these inferences are heavily dependent on a correct rooting of the squamate tree, which itself is dependent on comparison of character states in outgroup taxa.

The nearest extant outgroup to squamates consists only of the superficially lizard-like tuataras from New Zealand, the only remaining members of a once much more widespread and diverse group (Evans et al., 2001; Reynoso, 2000). Squamata itself is a diverse assemblage including all reptiles commonly called lizards plus three limbless groups: snakes, amphisbaenians, and dibamid lizards. Table 1 gives a summary of current squamate classification and includes the higher taxon names used in this paper. Recent morphological studies (Estes et al., 1988; Lee, 1998; Lee and Caldwell, 2000; Reynoso, 1998) agree on some groupings of families into higher taxa (e.g., Anguimorpha, Iguania, Scleroglossa), and on the basal dichotomy between Iguania and Scleroglossa, but the phylogenetic relationships among many higher squamate taxa remain uncertain. Historically, the limbless clades have been particularly difficult to place based on morphology because the limbless condition eliminates many characters, although utilizing fossil taxa can sometimes help (e.g., Lee and Caldwell, 1998; Zaher and Rieppel, 1999). Furthermore, because limblessness is often associated with a fossorial lifestyle, cranial morphology in these animals is also often radically altered from that of nonburrowing squamates (Lee, 1998).

Snakes are by far the most ecologically diverse and familiar of these limbless groups, with over 2900 species occupying a variety of terrestrial, arboreal, fossorial, and aquatic habitats on all major land masses except

TABLE 1. Phylogenetic taxonomy of sequamates based on morphology.[a]

- SQUAMATA
  - Iguania
    - Iguanidae[b]
    - Acrodonta
      - Agamidae[b]
      - Chamaeleonidae
  - Scleroglossa
    - Incertae sedis: Dibamidae, Amphisbaenia, Serpentes
    - Gekkonidae
    - Autarchoglossa
      - Scincomorpha
        - Lacertoidea
          - Xantusiidae[c]
          - Lacertiformes
            - Lacertidae
            - Teioidea
              - Teiidae
              - Gymnophthalmidae
        - Scincoidea
          - Scincidae
          - Cordylidae
      - Anguimorpha
        - Anguidae
        - Xenosauridae
          - *Xenosaurus*
          - *Shinisaurus*
        - Varanoidea
          - Helodermatidae
          - Varanidae
            - *Lanthanotus*
            - *Varanus*

[a]Modified from Estes et al. (1998).
[b]Taxa of uncertain monophyly by morphological criteria (Estes et al., 1988; Frost and Etheridge, 1989).
[c]Note that the position of this family has been especially labile in morphological studies. See Vicario et al. (2003) for a review.

Antarctica (Pough et al., 2004). Amphisbaenians are a much more homogeneous, completely fossorial group with major radiations in South America and northern Africa (extending into southern Europe), and two small clades confined to North America. One of these, the Bipedidae, is unique among amphisbaenians in its retention of forelimbs. Dibamid lizards are a small, poorly known, completely fossorial group with a curiously disjunct distribution (Southeast Asia/Sunda Shelf and northeastern Mexico), which suggests that this group was previously more widespread. Although snakes (Rieppel, 1983; Rieppel, 1985), amphisbaenians (Gans, 1978), and dibamids (Greer, 1985) each exhibit characters that might place them phylogenetically outside all other squamates, most authors now agree that all three are probably nested within lizards. Estes et al. (1988) designated these three groups as "Scleroglossa incertae sedis," and many morphological studies (e.g., Greer, 1985; Hallermann, 1998; Lee, 1998; Lee and Caldwell, 2000; Reynoso, 1998; Rieppel, 1984; Wu et al., 1996) have specifically addressed placement of these taxa. A common feature of most recent morphological studies (e.g., Lee, 1998; Lee and Caldwell, 2000; Reynoso, 1998; Wu et al., 1996) is the grouping of at least two of these limbless groups in a clade.

Few molecular studies have addressed higher-level relationships within Squamata on a broad scale. Most studies concerned with suprafamilial relationships have had limited outgroup taxon sampling (e.g., Ast, 2001; Donnellan et al., 1999; Macey et al., 1997b, 1999, 2000; Odierna et al., 2002; Saint et al., 1998; Whiting et al., 2003) and were thus not designed to produce a comprehensive higher-level squamate phylogeny, although several specific points have been clarified through these molecular studies. Donellan et al. (1999) support Kluge's (1987) conclusion that the Australian Pygopodidae (another limbless group) is closely related to the Australian diplodactyline geckos. Ast (2001) reports monophyly of Varanoidea (Varanidae + Helodermatidae), but only relative to anguids and anniellids, because *Xenosaurus* and *Shinisaurus* were not included as outgroups. Whiting et al. (2003) find strong support for the New World Xantusiidae as the sister taxon of the African Cordylidae.

Harris (2003) and Harris et al. (2001; 1999) use sequence data from the nuclear proto-oncogene *c-mos* to investigate higher squamate relationships (ultimately 162 sequences representing all major squamate clades except Dibamidae were analyzed). Many of the higher-level relationships recovered in these studies conflict with those of morphological studies. However, the gene fragment used for these studies is only approximately 360 bp, and most basal relationships are weakly supported. Vidal and Hedges (2004) use this same *c-mos* fragment along with approximately 500 bp of the protein-coding nuclear gene *RAG-1* to investigate relationships among major snake taxa as well as the position of snakes within Squamata. This study, which includes representatives of all recognized squamate families, finds strong bootstrap support for snakes in a phylogenetic position outside of Anguimorpha, contradicting several morphological studies (e.g., Lee, 1998; Lee and Caldwell, 2000; McDowell and Bogert, 1954). However, most other basal squamate relationships are not well supported.

Here we present a phylogenetic study of Squamata using three independent molecular data sets. Along with mitochondrial data from the *ND2* region, we have utilized the same *c-mos* fragment as the previous studies, and we have collected data from almost the entire length of *RAG-1*.

MATERIALS AND METHODS

Taxon Sampling

Rhynchocephalia is traditionally considered the closest outgroup to Squamata, although some molecular evidence (Hedges and Poling, 1999) suggests the arrangement (Rhynchocephalia, (Testudines, Archosauria)). We have therefore included representatives from all three of these taxa as outgroups. Within the ingroup, all recognized major squamate clades (i.e., lizard families, amphisbaenians, dibamids, and snakes) are represented, as well as many major subclades. In diverse families (or other equivalent taxa), we attempted to sample species from both sides of the most basal divergence, as inferred from morphological studies and/or previous molecular

work, as follows: Agamidae (Frost and Etheridge, 1989; Macey et al., 2000), Chamaeleonidae (Klaver and Böhme, 1986; Townsend and Larson, 2002, unpublished data), Gekkonidae (Donnellan et al., 1999; Kluge, 1987), Amphisbaenia (Kearney, 2003), Scincidae (Greer, 1970; Whiting et al., 2003), Cordylidae (Lang, 1991; Odierna et al., 2002), Serpentes (Heise et al., 1995; Rieppel, 1988), and Anguidae (Gauthier, 1982; Macey et al., 1999). For families with uncertain intrafamilial relationships, e.g., Iguanidae (Frost and Etheridge, 1989), we tried to sample all major subclades to assure that the deepest divergence was spanned. A total of 69 ingroup species were sampled for *RAG-1* and the mitochondrial fragment, and 44 ingroup species (including all major clades) were sampled for *c-mos*. Almost all *RAG-1* and mitochondrial sequences were collected from the same individuals; however, many of the *c-mos* sequences are from previously published studies, and exact species matches were not always possible. For combined analyses in which species representing particular higher taxa were not the same across all data partitions, we have labeled the resulting trees with the name of the higher taxon. For example, draconine agamids are represented by *Calotes calotes* and *C. versicolor* in the *RAG-1* and *c-mos* data sets, respectively, and their concatenated sequences are labeled "Draconinae" in the combined analyses. See Appendix for museum and GenBank accession numbers for all specimens.

Laboratory Protocols

Genomic DNA was extracted from muscle, liver, or skin tissue (stored either frozen or in 70% to 95% ethanol) using DNEasy Tissue Extraction Kits (Qiagen, Inc.) and stored in AE buffer. Mitochondrial polymerase chain reaction (PCR) products were amplified from genomic DNA using an initial denaturation at 95°C for 2 min, then a denaturation at 95°C for 35 s, annealing at 50°C for 35 s, and extension at 70°C for 150 s with 4 s added to each successive extension cycle for 30 cycles. Nuclear genomic DNA was originally amplified using the touchdown protocol of Groth and Barrowclough (1999). All PCR products were purified on 1.3% low-melt agarose gels and reamplified under the same conditions used to amplify the mitochondrial genomic DNA, except that the annealing temperature was reduced to 45°C. Promega *Taq* polymerase (Promega, Inc., Madison, Wisconsin) was used for all amplifications. Reamplified products were purified on 0.8% high-melt agarose gels, and template extracted using Viogene Gel Extraction Kits (Viogene, Inc., Taipei, Taiwan) and sequenced in both directions using ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kits with AmpliTaq DNA Polymerase (Perkin Elmer, Norwalk, Connecticut) following the manufacturer's instructions. Sequencing products were analyzed with ABI 373 or 377 Automated Sequencers (Applied Biosystems, Foster City, California) or an MJ Research BaseStation (MJ Research, San Francisco, California). Multiple overlapping PCR fragments were sequenced for the mitochondrial and *RAG-1* partitions, which helped guard against PCR contamination problems. When certain taxa fell in unexpected places in some trees (e.g., *Heloderma)*, multiple individuals or closely related taxa were at least partially sequenced when possible to confirm our findings.

Primers used in this study can be found on the Systematic Biology website (http://systematicbiology.org/). Mitochondrial sequences cover an approximately 1500 bp region corresponding to positions 4419 to 5933 on the human mitochondrial genome (Anderson et al., 1981), including portions of *ND1* (subunit 1 of NADH dehydrogenase) and *COI* (subunit I of cytochrome *c* oxidase), the 8 tRNA genes for glutamine, isoleucine, methionine, tryptophan, alanine, asparagine, cysteine, and tyrosine, the entire *ND2* (subunit 2 of NADH dehydrogenase) gene, and the stem-and-loop structure representing the origin for light-strand replication (O_L). *RAG-1* sequences cover an approximately 2800-bp coding region corresponding to positions 84 to 3126 on the published chicken *RAG-1* gene (Carlson et al., 1991). *C-mos* sequences cover an approximately 374-bp coding region corresponding to positions 513 to 888 on the human *c-mos* gene (Watson et al., 1982). Forty-four mitochondrial and 35 *c-mos* sequences were obtained from GenBank (see Appendix), and 28 mitochondrial and 12 *c-mos* sequences were newly generated for this study, as were 71 of the 73 *RAG-1* sequences. All mitochondrial GenBank sequences were previously generated in our laboratory, and were checked against the corresponding sequences in their original alignments to verify their identity. When possible, *c-mos* sequences from taxa closely related to those used in our study were also downloaded from GenBank for comparison to help detect mislabeled sequences (no mistakes were found).

Alignments and Phylogenetic Analyses

Sequences were edited and assembled using SeqMan II (DNASTAR, Inc., Madison, Wisconsin). Alignments of protein-coding regions were performed on amino acid translations using Clustal X (Thompson et al., 1997) at a variety of gap-opening and gap-extension penalties. For pairwise aligments, gap-opening penalties were set to 10, 20, and 35 with respective gap-extension penalties of 0.1, 0.45, and 0.75. Corresponding multiple-alignment penalties were 10, 15, and 20 (gap-opening) and 0.1, 0.2, and 0.3 (gap-extension). Regions for which alignments differed between the three suites of settings were excluded from all analyses. Genes coding for tRNAs were aligned manually following the structural models of Kumazawa and Nishida (1993). Length-variable loops that could not be confidently aligned were excluded from all analyses. All gaps were treated as missing data. There are several opinions on combining data from different sources for phylogenetic analysis. One is that all available data should be included in any analysis (Kluge, 1989), and a second is that partitions should always be analyzed separately, with congruence between partitions inferred as strong support for particular relationships (Miyamoto and Fitch, 1995). Finally, a third alternative is to test for congruence between data partitions, then combine them if the test is passed (e.g., Bull et al., 1993; Farris et al., 1994;

Huelsenbeck and Bull, 1996; Rodrigo et al., 1993). However, it is difficult to take a strictly formulaic approach to this problem. For example, as discussed by Wiens (1998), significant global tests of data incongruence do not indicate whether the incongruence is spread throughout the data or is concentrated in specific parts, and therefore not performing combined analyses based solely on the results of these tests seems inappropriate. Furthermore, recently developed methods of mixed-model analysis (e.g., Nylander et al., 2004) may make this issue less relevant (barring horizontal transfer and other similar evolutionary events). Our strategy was to perform both separate and combined analyses, and, in the case of conflicting topologies, to examine characteristics (rates of evolution, relative branch lengths, etc.) of each data partition to find potential explanations for the conflict (see Results for discussion of specific cases).

To explore the possibility of heterogeneous selective pressures on the protein-coding nuclear genes (see Results), we used DnaSP (Rozas and Rozas, 1999) to calculate ratios of synonymous substitutions per synonymous site (K_s) to nonsynonymous substitutions per nonsynonymous site (K_a) for all possible pairwise taxon comparisons. Average K_a/K_s ratios were then calculated within each major clade as well as among clades. Average K_a/K_s ratios significantly greater than one (as determined by t-tests) indicate directional selection in at least some of the species/clades compared, whereas ratios significantly less than one indicate stabilizing or purifying selection (see Messier and Stewart, 1997 for a more detailed discussion).

The model of evolution and all maximum-likelihood (ML) parameters were estimated for each data set individually using hierarchical likelihood-ratio tests as implemented in Modeltest (Posada and Crandall, 1998). Maximum-likelihood analyses were conducted using the heuristic search option of PAUP* (Swofford, 1998) and a neighbor-joining tree as a starting tree for TBR branch swapping. Computational limitations precluded the use of nonparametric bootstrapping under the likelihood criterion.

Bayesian analyses were performed using MrBayes 3.0b3 (Ronquist and Huelsenbeck, 2003) under the same model used for the corresponding likelihood analyses. One major concern with combining separate data sets is that evolutionary models may differ substantially between them (see Huelsenbeck et al., 1996). Version 3 of MrBayes allows parameter values to be estimated separately (under potentially different evolutionary models) for different data partitions, and this has the potential to alleviate this problem (Nylander et al., 2004). However, as more parameters are estimated, the potential for loss of statistical power increases. The magnitude of this problem is not fully understood, but it seems likely that excessive partitioning of the data could create its own problems. We have therefore used multiple partitioning schemes in our combined analyses and compared their effects on topology and branch support (see Results). For all Bayesian analyses, four incrementally heated Markov chains were started from random trees and run for 4,000,000 generations each. The effect of heating the chains is to increase the probability of acceptance of new parameter-value propositions; this flattens the landscape somewhat, allowing the chains to cross valleys and to explore treespace more effectively. Chains were sampled every 1000 generations to ensure that the samples were independent. Through inspection of the likelihood scores and model parameters in the output file, we determined the number of generations required for stabilization and discarded all trees obtained prior to stabilization as burn-in. Two independent analyses were conducted for each data set, and their resulting topologies, posterior clade probabilities, and log-likelihood (lnL) values at stationarity were compared to prevent drawing the posterior distributions from local optima. Trees from the posterior distribution were imported into PAUP* (Swofford, 1998) and, after excluding the burn-in, a majority-rule consensus tree was constructed showing relative occurrences (i.e., the posterior probabilities) of all nodes in the tree.

Maximum-parsimony (MP) analyses were performed using PAUP* (Swofford, 1998) under the heuristic search option with 100 random-addition replicates. Nonparametric bootstrap resampling was applied to assess heuristic support for individual nodes (Felsenstein, 1985b) using 1000 bootstrap pseudoreplicates with 25 random additions per pseudoreplicate. Branch-support (decay) indices (Bremer, 1994) were calculated as heuristic support measures for all resolved internal branches of the tree using the "Decay Index PAUP File" feature of MacClade (Maddison and Maddison, 2000). DeBry (2001) showed that the variance among significant decay indices within a single tree can be large, and that rigorous interpretation of decay values must take branch lengths into account. However, in the absence of explicit statistical testing of each node, we feel that in many cases the decay index is still useful as a rough guide to relative levels of support (especially once bootstrap values reach their maximum of 100). As an indicator of relative homoplasy among data sets, retention indices (Farris, 1989) were also calculated.

Testing Alternative Topologies

Statistical support for individual nodes was assessed using two separate nonparametric tests, the parsimony-based Wilcoxon signed-ranks (Templeton) test (Felsenstein, 1985a; Templeton, 1983) and the likelihood-based SH test (Shimodaira and Hasegawa, 1999). Felsenstein (1985a) showed that one-tailed probabilities for the Templeton test are close to the exact probabilities and that use of two-tailed probabilities is always conservative. Consistent with these findings, the two-tailed version of this test is generally conservative relative to alternative tests that ask whether character data statistically discriminate alternative phylogenetic topologies (e.g., Lee, 2000; Townsend and Larson, 2002). Bonferroni corrections for multiple tests (Rice, 1989) are very conservative, and were not applied to this already conservative test. Goldman et al. (2000) commented that

the Templeton test is appropriate only when all trees being tested are specified a priori, because by using the best (MP) tree derived from the data at hand, the test is potentially biased to be less conservative. The magnitude of this potential bias is unknown.

Goldman et al. (2000) suggested using instead the SH test (Shimodaira and Hasegawa, 1999), which uses a resampling method to overcome this potential bias and also makes corrections for multiple comparisons. Theoretically, this test requires that all possible topologies be compared simultaneously, an obvious impossibility with data sets of more than a few taxa. Buckley et al. (2001) suggested restricting the set of possible topologies to only those reasonably likely to be the true topology, but even this is impractical with most data sets. In testing particular nodes, we conducted both of these tests as follows. First, constraint trees containing only a single resolved node were constructed using MacClade (Maddison and Maddison, 2000). Next, for the Templeton test, the shortest trees *not* containing this node were found using PAUP* (Swofford, 1998), and these trees were then compared to the shortest unconstrained tree using the "Tree Scores" option of PAUP* (Swofford, 1998). An analogous procedure (using likelihoods instead of tree lengths) was followed for the SH test. This use of the SH test reduces to an appropriately "centered" KH test (Goldman et al., 2000). Because neither test was performed under technically perfect conditions, borderline-significant results should be interpreted with caution.

Our *RAG-1* tree roots at one of two relatively long branches (MP roots it at *Dibamus*, ML at Gekkonidae; see Figure 1 and TreeBASE website). To test the hypothesis that long-branch attraction (LBA; Felsenstein, 1978) might cause an aberrant rooting, we followed Wiens and Hollingsworth's (2000) implementation of the parametric bootstrapping method of Huelsenbeck (1997). We first rerooted our *RAG-1* ML topology at Iguania (the morphological root; see Results) and reoptimized all branch lengths and other model parameters on this tree. Next, we used Seq-Gen (Rambaut and Grassly, 1997) to simulate 100 data sets on this topology, with sequence length equal to that of the *RAG-1* data set. MP and ML analyses were performed on each of these data sets, and a tally was kept for each optimality criterion of the number of correct and incorrect rootings (relative to the simulated topology), as well as the number of times the tree rooted specifically at either geckos or *Dibamus*. As a general rule with this type of test, if parsimony tends to root the tree incorrectly but likelihood does not, this suggests that LBA is a potential problem. In this specific case, if either analysis tends to recover a tree rooted at geckos and/or *Dibamus*, the original rooting from the real *RAG-1* data would be highly suspect. Because unconstrained ML analyses were not computationally feasible, intrafamilial relationships were constrained to match those from the original *RAG-1* ML analysis, but interfamilial relationships were free to vary.

Results from the mitochondrial analysis suggest that LBA might occur between two specific clades at the end of long internal branches. We therefore performed a similar study with the mitochondrial data and topology, this time separating the two suspicious long branches in the model tree used for the simulations. In this case, if parsimony tends incorrectly to join the long branches while likelihood does not, this result suggests that LBA is a problem.

The most consistent well-supported difference between our topology and the topologies found in all recent morphological studies concerns the placement of the squamate root. If the morphological rooting is affected by misleading convergence between two or more taxa, the characters supporting this rooting might be found to be concentrated in one anatomical area. This would not necessarily be true for all scenarios but if, for example, there were convergence in feeding morphology between sphenodontids and iguanians (and iguanians did not actually branch off early in squamate history), we might expect that skull and jaw characters might be overrepresented in the list of characters supporting a rooting at Iguania. Likewise, if convergence is a problem with the molecular rooting, we might expect its supporting characters to be concentrated in one particular genic functional domain. To identify the source of conflict on this point, we performed separate tests using Lee and Caldwell's (2000) morphological data (including fossils) and our *RAG-1* data. First, using Lee and Caldwell's (2000) taxa, we constructed a tree congruent with our *RAG-1* ML topology. We then made a second topology by rerooting this tree at Iguania (in accordance with morphological hypotheses). After excluding 27 characters identified by Lee (1998) as potentially correlated to a fossorial existence (this exclusion does not reduce support for the morphological rooting), we mapped Lee and Caldwell's (2000) morphological characters onto each of these trees and identified those that required more steps on the tree with the molecular rooting. Using the anatomical divisions given in Lee and Caldwell (2000), we then performed chi-square tests to determine if one or more anatomical regions were overrepresented in the list of characters opposing the molecular rooting (see Harshman et al., 2003, for a similar use of this test). Performing an exactly analogous test on the molecular data is difficult, because some functional domains of the gene are known to be more variable than others (Willett et al., 1997), and the variable regions might be expected to contain proportionately more informative characters than the conserved regions. As a proxy, we divided the *RAG-1* gene into two regions, the more highly variable 5′ one-third of the gene that codes for protein-binding sites, and the more highly conserved 3′ two-thirds of the gene responsible for target-site recognition and DNA binding (reviewed in Willett et al., 1997). These regions were analyzed separately to check for congruence among their respective topologies.

RESULTS

Phylogenetic Results from Nuclear Genes

All complete, aligned data files (with excluded positions marked as such), along with trees from all

individual data sets, are available on the TreeBASE website (http://www.treebase.org/treebase/). The *RAG-1* MP, ML, and Bayesian topologies are all very similar, and all nodes receiving high heuristic support from the parsimony analysis (bootstrap >90%) also have posterior probabilities >95% in the Bayesian analysis (Fig. 1). The *c-mos* ML and Bayesian topologies are very similar, and both analyses recover all moderately to highly supported nodes (bootstrap >80%) from the *c-mos* parsimony analysis (Fig. 2). Although the *c-mos* data set contains fewer species, all major clades from the *RAG-1* analysis are still represented. The topology of the *c-mos* MP strict consensus tree is largely compatible with the *RAG-1* topology, although many deeper relationships are not resolved (Fig. 2).

The model parameters estimated for the *RAG-1* and *c-mos* genes are similar (although Modeltest chose the simpler HKY model for *c-mos*, probably due to the relatively short length of this data set), and the two genes have very similar levels of divergence among squamates (Table 2, Figs. 1 and 2). Sequence data from these two genes were combined and analyzed as a single nuclear data set (Fig. 3). For clarity, results of these combined analyses are detailed here, with references to individual analyses (Figs. 1 and 2; see TreeBASE website for individual MP and ML/Bayesian topologies) as necessary. Parsimony, Bayesian, and likelihood topologies from the combined *RAG-1* and *c-mos* data are largely congruent with each other as well as with corresponding trees from each data set analyzed singly. *RAG-1*/*c-mos* parsimony and Bayesian support values are at least as high as those from the *RAG-1* data alone, and often substantially higher (Figs. 1 and 3). Combined Bayesian analyses were run both with parameter values estimated separately for the *RAG-1* (GTR+I+G) and *c-mos* (HKY+I+G) partitions (Fig. 3) and also as a single data partition under a GTR+I+G model (results not shown). Topologies were identical between these analyses, and posterior probabilities were very similar as well.

In all analyses, when more than one subclade is represented, monophyly of almost all recognized families is recovered with strong support (the one exception is a paraphyletic Agamidae found in ML and Bayesian analyses) (Fig. 3). Note that because Estes et al. (1988) defined their taxa so that taxon names would remain stable, it is technically impossible that Scincidae, for example, could be nonmonophyletic (i.e., only its taxon composition can change). For brevity, however, we will make reference to monophyly, paraphyly, etc. of these taxa throughout this paper, with the understanding that we are actually referring to the groups placed in these taxa by Estes et al. (1988) at the time of their definition. Traditional suprafamilial groups recovered with strong support in the *RAG-1*/*c-mos* analyses include Acrodonta, Iguania, Anguimorpha (also characterized by a one-codon insertion at positions 128 to 130 in the aligned *RAG-1* data set), and Teioidea. Interestingly, several nontraditional relationships are also recovered with strong support.

Tests of Phylogenetic Rooting

Most striking is the absence of a monophyletic Scleroglossa as the sister taxon of Iguania (Fig. 3). Instead, the deepest divergence is between *Dibamus* and all other squamates, and Iguania occupies a highly nested position in the tree. Both a paraphyletic Scleroglossa and highly nested Iguania are contradicted by substantial morphological evidence (e.g., Lee and Caldwell, 2000). Furthermore, this specific conflict between molecules and morphology can be resolved by simply rerooting our nuclear topology at Iguania. We therefore considered four scenarios that might have led to an incorrect rooting caused by misleading sequence convergence between nonsister taxa in the nuclear analyses: heterogeneous

TABLE 2. Properties of character variation for all protein coding genes (analyzed by first, second, and third codon positions), plus tRNA genes of the mitochondrial genome.

Data set	Parsimony-informative sites	Nucleotide bias by codon position				Alpha[a]	RI[b]	Average genetic distance[c]
		%A	%C	%G	%T			
RAG-1 (2765)	1489					1.711	0.602	0.213 (0.149)
1st position	367	0.31	0.20	0.30	0.19			
2nd position	256	0.35	0.20	0.18	0.27			
3rd position	866	0.27	0.22	0.22	0.29			
c-mos (359)	212					2.816	0.578	0.238 (0.169)
1st position	64	0.30	0.20	0.30	0.20			
2nd position	42	0.28	0.20	0.21	0.31			
3rd position	114	0.23	0.24	0.19	0.34			
MtDNA (1497)	1175					0.565	0.352	1.034 (0.329)
1st position	277	0.38	0.27	0.15	0.20			
2nd position	222	0.15	0.35	0.11	0.39			
3rd position	349	0.49	0.31	0.05	0.15			
tRNA loops	54							
tRNA stems	275							

[a]Gamma-distribution shape parameter describing rate heterogeneity estimated by maximum likelihood.

[b]Retention Index (Farris, 1989).

[c]Average pairwise maximum likelihood–corrected percent sequence divergence between all ingroup taxa, calculated for each data set under its own evolutionary model and set of parameter values (found in corresponding figure legends). Average uncorrected percent sequence divergences are in parentheses.

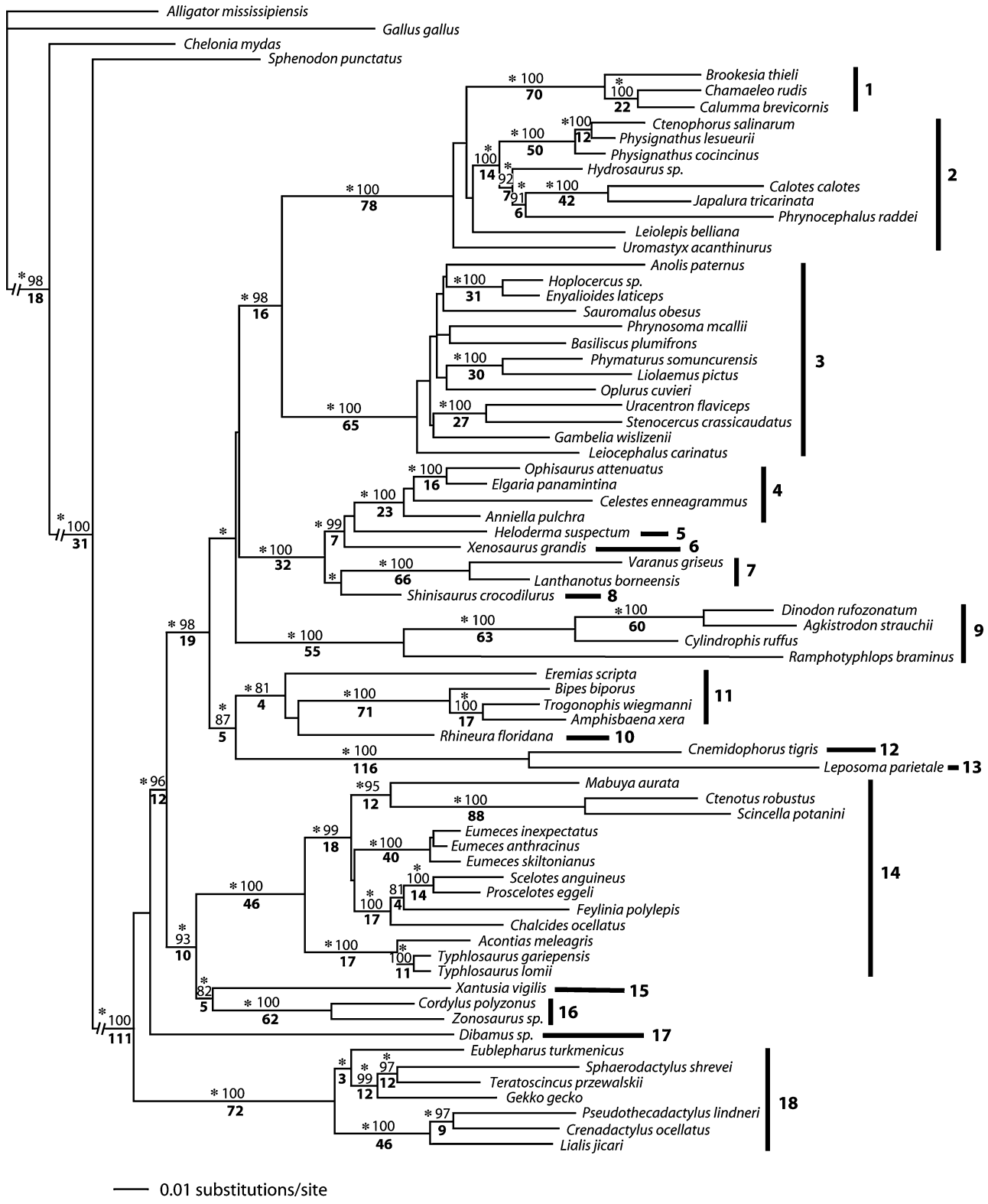

FIGURE 1. *RAG-1* data, ML phylogram (GTR+I+G model; −lnL = 50519.59; A = 0.3007, C = 0.2242, G = 0.2254, T = 0.2497; AC = 1.3332, AG = 4.7011, AT = 0.9186, CG = 0.8644, CT = 5.7274, GT = 1.0; I = 0.3352; G = 1.7108). Asterisks indicate branches that receive a posterior probability of 95% or greater in the Bayesian analysis. MP bootstrap proportions >70% (above branches) and decay indices (below branches) are provided for all nodes congruent between analyses based on the two optimality criteria. Numbers to the right denote major taxonomic units as follows: 1. Chamaeleonidae; 2. Agamidae; 3. Iguanidae; 4. Anguidae; 5. Helodermatidae; 6. Xenosauridae; 7. Varanidae; 8. Shinisauridae; 9. Serpentes; 10. Lacertidae; 11. Amphisbaenia; 12. Teiidae; 13. Gymnophthalmidae; 14. Scincidae; 15. Xantusiidae; 16. Cordylidae; 17. Dibamidae; 18. Gekkonidae. Outgroup branches with hatch marks have been shortened.

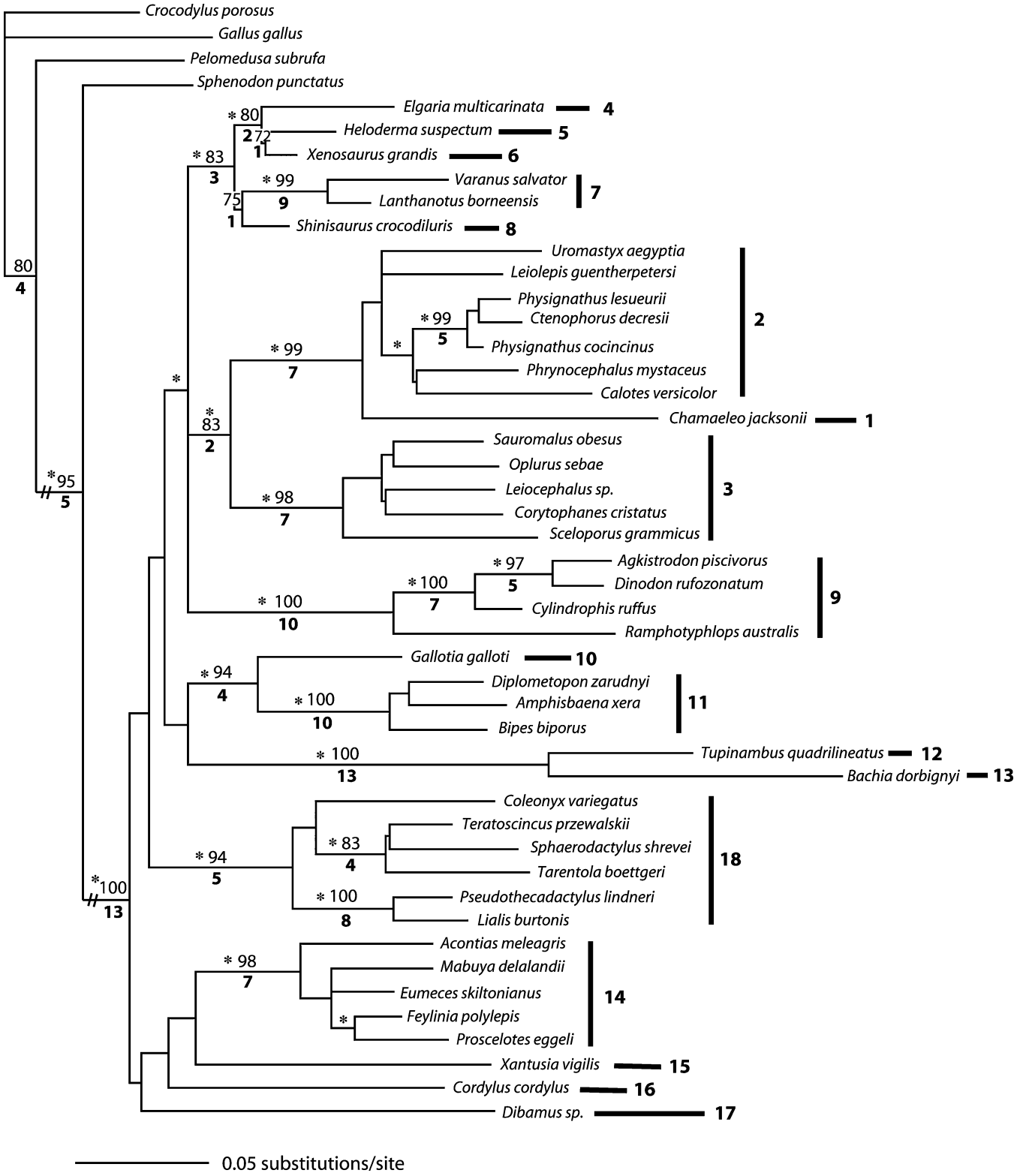

FIGURE 2. *C-mos* data, ML phylogram (HKY+I+G model, −lnL = 5431.52; A = 0.2712, C = 0.2527, G = 0.2231, T = 0.2530; Ti/Tv = 2.4357; I = 0.3084; G = 3.5785). Asterisks indicate branches that receive a posterior probability of 95% or greater in the Bayesian analysis. MP bootstrap proportions >70% (above branches) and decay indices (below branches) are provided for all nodes congruent between analyses based on the two optimality criteria. Annotations and numbering as in Figure 1.

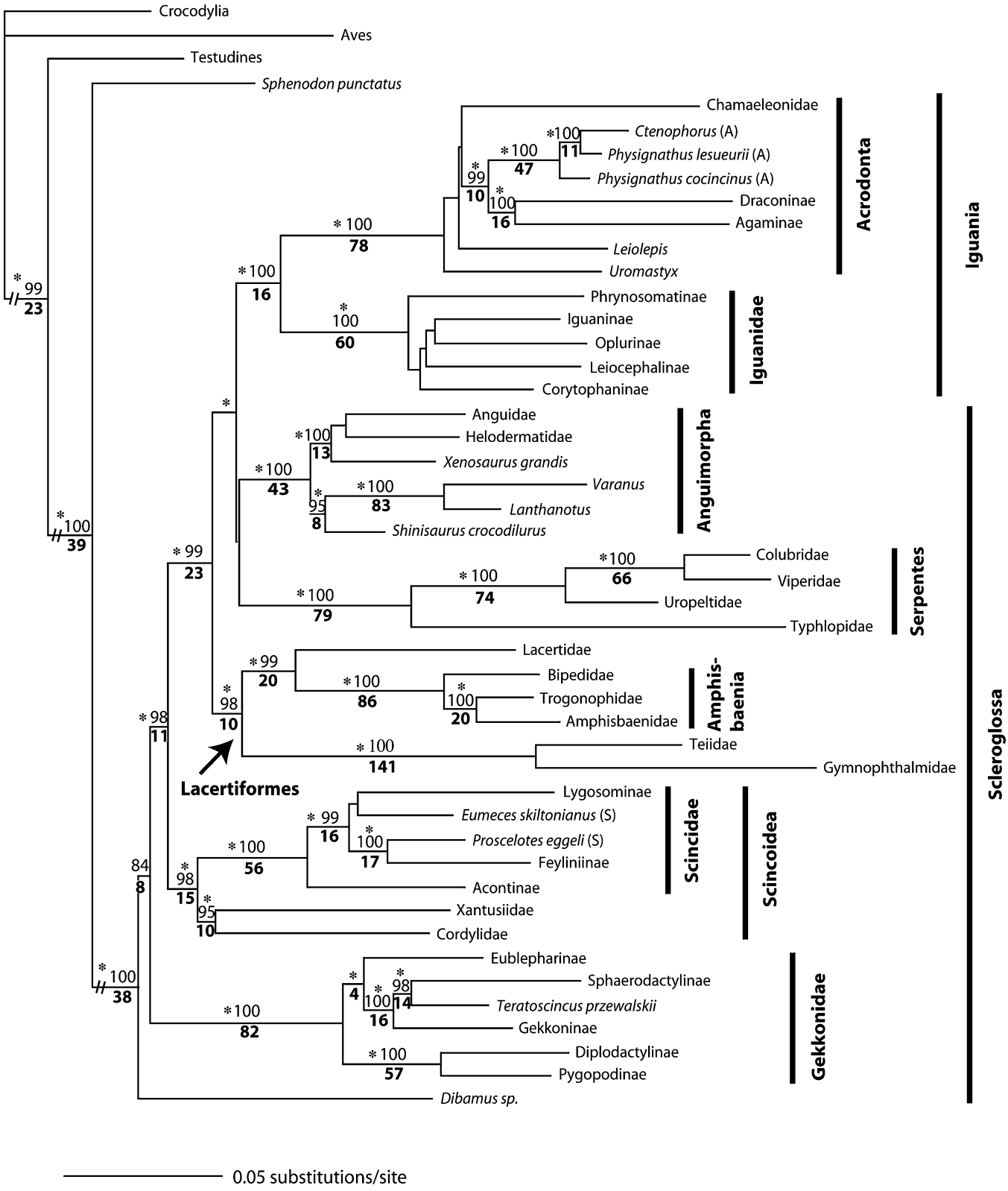

FIGURE 3. Combined *RAG-1* and *c-mos* data, ML phylogram (GTR+I+G model; −lnL = 44562.45; A = 0.3022, C = 0.2212, G = 0.2243, T = 0.2523; AC = 1.4034, AG = 4.9417, AT = 0.9147, CG = 0.9256, CT = 5.9064, GT = 1.0; I = 0.3543; G = 2.0844). Asterisks indicate branches that receive a posterior probability of 95% or greater in the Bayesian analysis. MP bootstrap proportions >70% (above branches) and decay indices (below branches) are provided for all nodes congruent between analyses based on the two optimality criteria. A = Amphibolurinae; S = Scincinae. Other annotations as in Figure 1.

base composition among taxa, heterogeneous selection pressures, phylogenetic randomization of outgroup sequences with respect to ingroup sequences, and long-branch attraction.

Many authors have documented the potentially misleading effects of heterogeneous base composition among taxa in phylogenetic studies (e.g., Lockhart et al., 1994; Steel et al., 1993; Tarrio et al., 2000; Tarrio et al., 2001). Chi-square tests for homogeneity of base frequencies on the *RAG-1* data show that only third positions are significantly heterogeneous ($P < 0.001$). We therefore used Lockhart et al.'s (1994) LogDet transformation (with an invariant sites parameter) to correct for base frequency heterogeneity (see Tarrio et al., 2001 for comments on this test) in a minimum evolution (ME) analysis of the full *RAG-1* data set. This analysis still finds Gekkonidae as the sister taxon of all other squamates (a Scincoidea-*Dibamus* clade is the next to diverge), and Iguania is still highly nested. Furthermore, this same basic topology is also found from MP and ML analyses using only first and second codon positions.

Harris (2003) found substantial heterogeneity in *c-mos* third codon positions among squamates, specifically high GC content among some teiids (the most basal ingroup taxon in his analysis) and his outgroup taxa. In our study, base frequencies at third positions of *c-mos* codons are not significantly heterogeneous ($P = 0.1664$ for all taxa, $P = 1.000$ for ingroup only). However, third-position GC content of our outgroup taxa (average 63.2%) is markedly higher than that of the ingroup taxa (average 41.5%), and Tarrio et al. (2000) suggested that this situation could cause incorrect rooting. However, ME analysis of LogDet-corrected *c-mos* data places Gekkonidae near the base of the tree and finds a highly nested Iguania, as do MP and ML analyses using only first and second codon positions. All of these topologies are similar to those derived from MP and ML analyses of the full *c-mos* data set (Fig. 2).

Heterogeneous selection pressure affecting the genes used for phylogenetic inference is another potentially confounding factor. If two or more nonsister lineages undergo a period of similar selection that is divergent with respect to the remaining lineages, then parallel or convergent amino acid replacements (in the case of a protein-coding gene) in these nonsister lineages could be problematic for phylogenetic analyses. However, K_a/K_s values are remarkably uniform within and among clades for both the *RAG-1* and *c-mos* genes. The test clades used for each gene include Iguanidae, Acrodonta, Gekkonidae, Serpentes, Anguimorpha, Lacertiformes (including Amphisbaenia), and Scincoidea (including Xantusiidae). For both *RAG-1* and *c-mos*, K_a/K_s values for both within-clade comparisons (*RAG-1* average 0.13 [0.12–0.15]; *c-mos* average 0.30 [0.23–0.54]) and between-clade comparisons (*RAG-1* average 0.11 [0.10–0.12]; *c-mos* average 0.24 [0.16–0.33]) are significantly different from 1. For both data sets, all except within-clade snake comparisons are highly significant even after Bonferroni correction for multiple tests (Rice, 1989). These results indicate that both genes are under strong stabilizing selection, both within and among clades. K_a/K_s values significantly greater than 1 between particular test clades or groups of test clades would indicate a shift in selective regimes, even if all within-clade values remained small (Messier and Stewart, 1997), and this would be a cause for concern. However, because all values were small, no evidence exists for heterogeneous selection that might mislead results of the nuclear analyses by causing convergence in protein structure between nonsister taxa.

Graham et al. (2002) showed that if the nearest outgroups are extremely divergent such that phylogenetic information is essentially randomized with respect to the ingroup taxa, trees will tend to root incorrectly on long terminal ingroup branches (this is an extreme example of LBA). However, even with uninformative characters excluded, average uncorrected *Sphenodon*-ingroup distance is only 0.358, which is well below the 0.75 expected from random DNA data. Average ML distances between ingroup taxa and turtles and crocodylids, respectively, are generally less than 5% higher than *Sphenodon*-ingroup distances (birds are somewhat more divergent). Analyses repeated with all possible outgroup combinations always give the same ingroup topology, with support values very similar to those obtained in the original analysis.

Finally, both *Dibamus* and Gekkonidae sit at the ends of relatively long branches in the *RAG-1* analysis (Fig. 1). Therefore, even though the outgroup sequences are definitely not random with respect to ingroup sequences, LBA needs to be considered as a potential cause of our unconventional rooting. Although support is high for the *RAG-1* branching order, LBA is related to the phenomenon of statistical inconsistency, and higher support values for an incorrect topology are predicted from theory as the amount of data increases (Felsenstein, 1978). One simple test for LBA is to remove the suspicious long branches and to see if the remaining topology is stable. We therefore sequentially removed (without replacement) the sister taxa of the remaining squamates from the *RAG-1* data set (i.e., first Gekkonidae, then *Dibamus*, etc.; see Fig. 1) and then analyzed each of these modified data sets with both parsimony and likelihood. In each of these analyses, Iguania remained the most nested group. We also tried excluding Acrodonta from all these analyses, thus making the branch to Iguanidae even longer, but the topology remained congruent with those from the original analyses.

Simulation studies likewise suggest that LBA is not responsible for our rooting. Analysis of simulated data sets modeled on the *RAG-1* ML tree rerooted at Iguania never produced a rooting at Gekkonidae and/or *Dibamus* (Fig. 4A); this was true even when the branches to Iguania and *Dibamus* were artificially shortened and lengthened, respectively, to accentuate any tendency toward rooting at *Dibamus* (Fig. 4B). In all of these simulations, parsimony failed to recover the modeled topology more often than likelihood; however, this is because parsimony often incorrectly rooted the tree at Serpentes, another clade subtended by a relatively long branch (Fig. 1).

Analyses performed to localize the signal for the molecular and morphological rootings in the *RAG-1* and

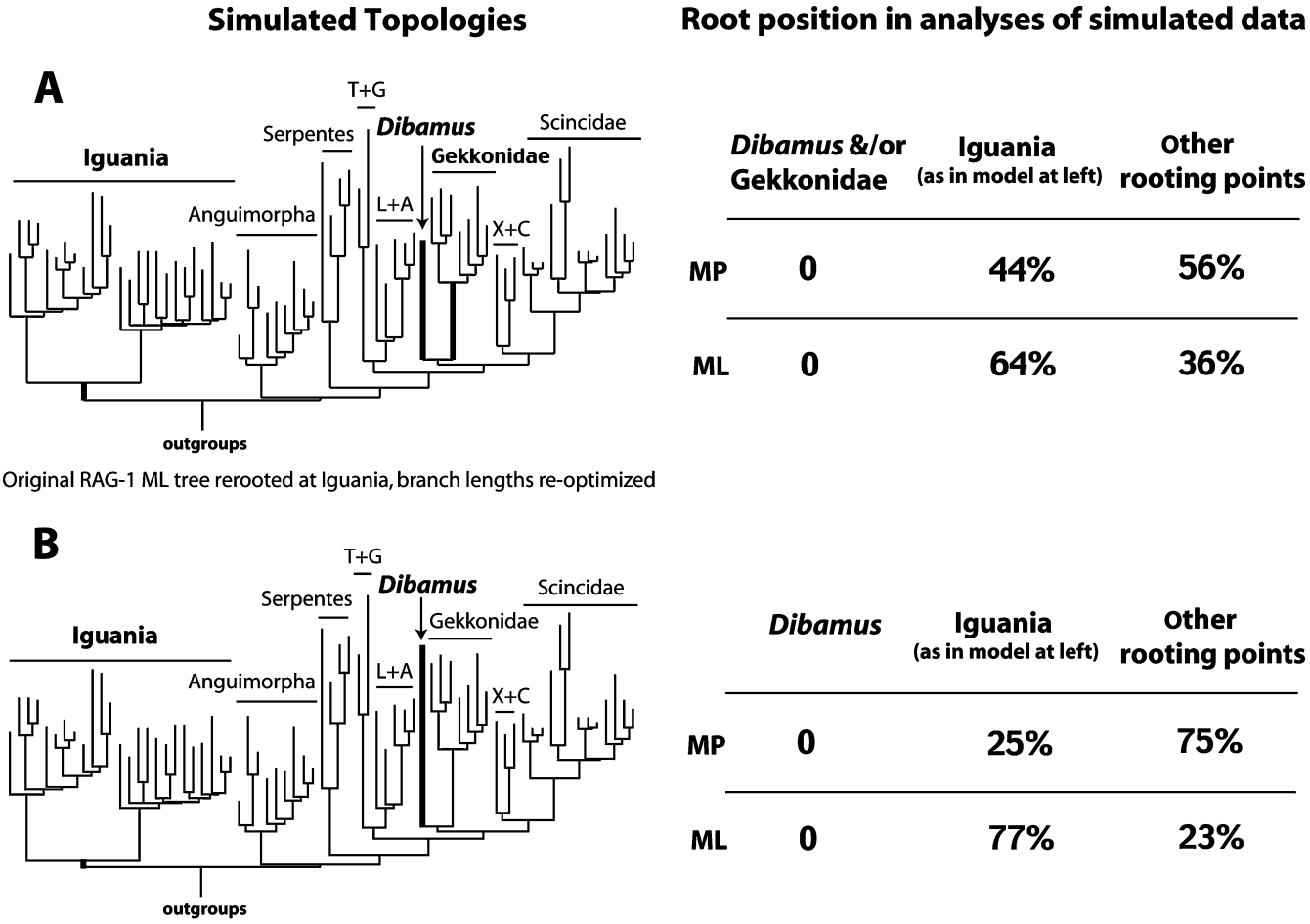

FIGURE 4. Results from two sets of 100 parametric simulations designed to detect long-branch attraction that, if present, might have caused an incorrect rooting at geckos or *Dibamus* in the *RAG-1* analyses. T+G = Teiidae/Gymnophthalmidae; L+A = Lacertidae/Amphisbaenia; X+C = Xantusiidae/Cordylidae. (A) In the model tree for the first set, branch lengths are simply reoptimized on the *RAG-1* ML topology rerooted at Iguania. (B) The second model tree is identical, except that the branch leading to Iguania is multiplied by 0.33, and the branch to *Dibamus* is multiplied by 1.5 (see text). For clarity, outgroups are not shown. For both MP and ML analyses, the table shows the percentage of times the tree correctly rooted at Iguania as well as the number of incorrect rootings, both at the potential problem taxa and at various other points in the tree. In both sets of simulations, incorrect rootings at Serpentes in the MP analyses accounted for most of the difference in accuracy compared to the ML analyses. As a control, a third set of 100 simulated data sets was constructed using as a model the exact *RAG-1* ML topology, which is rooted at geckos (Fig. 1). For these data sets, MP and ML recover the modeled topology 57 and 87 times, respectively. Thirty-four of the 43 incorrect topologies recovered under MP root the tree at *Dibamus*.

morphological (Lee and Caldwell, 2000) data, respectively, found that support for the alternative rootings is not concentrated in one particular subset of either data set. Chi-square tests show that the morphological characters favoring the morphological rooting are randomly distributed among anatomical regions (Table 3), and separate analyses performed on sequence from the two broad functional domains of the *RAG-1* gene likewise both recover the molecular rooting found with the full *RAG-1* data.

Phylogenetic Positions of Limbless Taxa

Snakes, amphisbaenians, and *Dibamus* each are placed in separate parts of the tree, and alternative hypotheses placing any two of these as sister taxa are statistically rejected (Table 4). Analyses based on the two different optimality criteria disagree on the exact placement of snakes, although it is clear that they are nested well within squamates (Fig. 3). Parsimony places snakes as the sister taxon of Lacertiformes (including amphisbaenians) with weak support (bootstrap of 52). However, likelihood recovers a clade containing snakes, anguimorphs, and iguanians, and this arrangement is strongly supported by Bayesian results (Fig. 3). A sister-taxon relationship between snakes and Varanidae is statistically rejected (Table 4).

Inclusion of amphisbaenians within the traditional Lacertiformes (Lacertidae + Teioidea) is statistically supported (Table 4), and heuristic support is strong for a sister-taxon relationship between lacertids and

TABLE 3. Chi-square tests of the random distribution among character partitions of morphological characters[a] favoring the morphological rooting (at Iguania) of the molecular topology[b] over the molecular rooting (at *Dibamus*) of the molecular topology. SS = 7.54; $P = 0.479$.

Partition	Observed	Expected
Skull roof	9	7.55
Braincase and assoc. structures	6	3.49
Palate and assoc. structures	5	3.02
Lower jaw	3	4.53
Dentition	0	2.21
Axial skeleton	2	3.72
Shoulder girdle/forelimb	2	2.21
Pelvic girdle/hindlimb	2	1.39
Miscellaneous osteological	1	1.80

[a]Characters and character partitions taken from Lee and Caldwell (2000).
[b] *RAG-1* and *c-mos* data (Fig. 3).

amphisbaenians (Fig. 3). This latter relationship may be supported by a structural character as well. *Gallotia* (a lacertid) has a seven-codon deletion at *c-mos* positions 220 to 240, and all sampled amphisbaenians share an overlapping eight-codon deletion at positions 217 to 240, suggesting that the original deletion was simply extended by one codon in amphisbaenians. Harris et al. (1999) reported a seven-codon deletion in this general region for two gekkonines. Although the alignment in this area is not completely unambiguous, alignments made with Clustal X (Thompson et al., 1997) at a variety of gap penalties (see Materials and Methods) suggest that the lacertid and gekkonine deletions do not involve the same codon positions. Furthermore, forcing the gekkonine and lacertid deletions to coincide requires two separate, smaller amphisbaenian deletions instead of the one found at all Clustal gap-penalty settings used.

TABLE 4. Results of Wilcoxon signed-ranks (Templeton) and SH tests of topology.

Alternative hypotheses tested[a]	Templeton[b]	SH
RAG-1 and *c-mos*		
Monophyly of Scleroglossa	0.0002*	0.000*
Nonmonophyly of Iguania	0.028*	0.000*
Monophyly of (Serpentes + Dibamidae)	0.0001*	0.000*
Monophyly of (Serpentes + Amphisbaenia)	0.007*	0.000*
Monophyly of (Dibamidae + Amphisbaenia)	<0.0001*	0.000*
Monophyly of (Serpentes + (*Varanus*/*Lanthanotus*)	0.0001*	0.000*
Monophyly of (Lacertidae + Teioidea), excluding Amphisbaenia	0.044*	0.003*
Nonmonophyly of (Lacertidae + Amphisbaenia)	0.066	0.003*
Monophyly of Varanoidea (*Heloderma* + *Varanus*/*Lanthanotus*)	0.0088*	0.000*
Monophyly of (*Xenosaurus* + *Shinisaurus*)	0.029*	0.000*
Monophyly of (Scincidae + Cordylidae), excluding Xantusiidae	0.188	0.110
Acontinae not the sister taxon of other Scincidae	0.095	0.008*
mtDNA		
Monophyly of Scleroglossa	0.289	0.057
Nonmonophyly of (Serpentes + Acrodonta)	0.591	0.272
Monophyly of (Serpentes + *Varanus* + *Lanthanotus*)	0.052	0.001*
Nonmonophyly of (*Sphaerodactylus* + *Gecko*)	0.747	0.167
RAG-1, *c-mos*, and mtDNA		
Nonmonophyly of (Lacertidae + Amphisbaenia)	0.0001*	0.000*
Nonmonophyly of Agamidae	0.316	0.221
Nonmonophyly of (Xantusiidae + Cordylidae)	0.062	0.001*
Nonmonophyly of (Trogonophidae + Amphisbaenidae)	0.003*	0.001*

[a]A significant result means that the stated alternative hypothesis is rejected. Asterisks indicate significance at the 0.05 level.
[b]One-tailed probabilities are shown. Doubling these values will give the more conservative two-tailed probabilities.

Relationships within Amphisbaenia are strongly supported. The amphisbaenian family Rhineuridae is not represented in the combined *RAG-1* and *c-mos* data set because of problems amplifying the *c-mos* fragment. However, a Templeton test performed on the *RAG-1* data alone provides strong support ($P < 0.0001$) for monophyly of the other three amphisbaenian families to the exclusion of *Rhineura* (Townsend, 2002). Furthermore, in the combined analysis, Trogonophidae and Amphisbaenidae form a well supported clade exclusive of Bipedidae (Fig. 3).

In MP, ML, and Bayesian analyses, *Dibamus* is the sister taxon of a clade containing all other squamates, and geckos are the second group to diverge from the ancestral squamate lineage. Both parsimony and Bayesian measures strongly support grouping the remaining squamates to the exclusion of geckos and *Dibamus* (Fig. 3).

Other Well-Supported Clades from the Nuclear Analyses

Strong support is found for Xantusiidae as the sister taxon of Cordylidae, and for the placement of Helodermatidae within a *Xenosaurus*-Anguidae clade to the exclusion of Varanidae, which is often considered the sister group of helodermatids (Fig. 3). *Shinisaurus* and Varanidae form a clade, and a sister-taxon relationship between *Xenosaurus* and *Shinisaurus* (the traditional Xenosauridae) is statistically rejected (Table 4).

Within Scincidae, phylogenetic positions of the two limbless subfamilies are well supported. Acontinae is the sister group to a clade containing all other skinks, and Feylininae is closely related to African scincines (actually nested within this group; see Fig. 1). Monophyly of African and North American scincines is not supported (Fig. 3).

Relationships within Gekkonidae are well supported. Pygopodinae is the sister taxon of Diplodactylinae (Fig. 3), and this relationship is further supported by a shared one-codon deletion in the *RAG-1* data set at positions 125 to 127. *Teratoscincus* and Sphaerodactylinae form the sister group of Gekkoninae with high bootstrap and Bayesian support (Fig. 3). MP recovers Eublepharinae as the sister taxon of (*Teratoscincus* + Sphaerodactylinae + Gekkoninae), but the bootstrap value is <70%. However, ML and Bayesian analyses recover this same relationship, and Bayesian

support is high (Fig. 3). Furthermore, independent support for this arrangement comes from a shared four-codon deletion at positions 95 to 106 in the *RAG-1* data set. No other sampled gekkonids have any deleted bases in this region, and the surrounding amino acid sequence is conserved across geckos, making alignment unambiguous.

Mitochondrial-DNA Analyses

The mtDNA MP strict consensus of four trees is unresolved at many deeper nodes, but resolved portions of the tree are largely compatible with the mtDNA ML topology (Fig. 5). The Bayesian consensus topology is similar to the ML topology, and both analyses recover all moderately to highly supported nodes (bootstrap >80%) from the parsimony analysis, with one exception within geckos (see below).

Chamaeleonidae, Agamidae, Acrodonta, Iguanidae, Anguimorpha, Serpentes, Scincidae, Amphisbaenia, and Teioidea all receive moderate to high parsimony bootstrap support, and all of these clades except Agamidae receive high Bayesian support (Fig. 5). Not all relationships within Amphisbaenia could be evaluated because mtDNA sequence could not be obtained from *Rhineura.* However, relationships among the remaining amphisbaenian families are strongly supported, and mirror exactly the results of the nuclear analysis (Fig. 3). Furthermore, the lacertid-amphisbaenian clade identified in *RAG-1* and *c-mos* analyses is once again recovered with moderate parsimony and high Bayesian support (Fig. 5).

Within Gekkonidae, the mtDNA ML and Bayesian analyses find the same topology as all nuclear analyses, except that Eublepharinae (represented by *Eublepharus turkmenicus*) is the sister taxon of all other gekkonids. The mtDNA MP tree differs in finding moderate bootstrap support (81) for a sister-taxon relationship between Gekkoninae and Sphaerodactylinae, although this result is not supported by statistical tests (see Table 4). Interestingly, in the ML analysis the branches leading to gekkonines and sphaerodactylines are each roughly twice as long as the branch leading to *Teratoscincus* (Fig. 5). This result suggests that LBA may account for the mitochondrial parsimony gekkonine/sphaerodactyline clade, which is at odds with all other analyses in this study. Mitochondrial data agree with the nuclear data on the nesting of feylinine skinks within African scincines, and a sister-taxon relationship between xantusiids and cordylids.

In disagreement with the nuclear analyses, moderate (parsimony) to strong (Bayesian) support is found for a snake-acrodont clade (Fig. 5), although this result is not supported by the nonparametric statistical tests (Table 4). The branches subtending each of snakes and acrodonts are much longer than most other branches of similar depth in the tree (Fig. 5). Interestingly, both of these taxa have gene rearrangements associated with unusual positions of mitochondrial replication origins (Kumazawa and Nishida, 1995; Macey et al., 1997a), a situation that might be related to an increased rate of molecular evolution. The long snake and acrodont branches, combined with strong support for a monophyletic Iguania from the nuclear data (Fig. 3, Table 4), suggests that LBA might be causing this very unorthodox arrangement.

Results from parametric bootstrapping simulations support the LBA hypothesis (Fig. 6). When snakes and anguimorphs are constrained to be sister taxa (a more traditional scenario) in 100 simulated data sets, equal-weights parsimony correctly recovers this clade in only 25% of replicates, whereas in 62% of replicates parsimony incorrectly recovers a snake-acrodont clade, as in the original analysis of the real mitochondrial data. In contrast, ML recovers the correct snake-anguimorph clade 78% of the time, and incorrectly recovers a snake-acrodont clade only 14% of the time.

In a second, more extreme deviation from the original mitochondrial-based topology, 100 data sets are simulated in which acrodonts and iguanids form a monophyletic Iguania as the sister taxon of a clade containing snakes and anguimorphs, a topology compatible with all well-supported nodes from the nuclear analysis (Fig. 3). Results from this analysis further support a role for LBA in the mitochondrial results. Equal-weights parsimony recovers the correct topology only 12% of the time, and 46% of the analyses incorrectly place snakes as the sister taxon of acrodonts. Meanwhile, likelihood recovers the correct topology 56% of the time, and incorrectly recovers a snake-acrodont clade in only 5% of the simulation replicates.

Average ML-corrected distances between ingroup taxa are nearly five times as large for the mitochondrial data as they are for the *RAG-1* data (Table 2). Likewise, although the mitochondrial data set has only slightly more than half the number of characters of the *RAG-1* data set (and only one less species) (Table 2), the mitochondrial MP tree is approximately 45% longer than the corresponding *RAG-1* tree (17237 and 9900 steps, respectively). Thus, the mitochondrial data are more likely to show saturation at more basal nodes, perhaps explaining the lack of resolution and poor support at deeper levels (Fig. 5). For this reason, nodes at the deepest levels of the squamate tree are probably best assessed from the nuclear data alone. However, the high levels of homoplasy in the mitochondrial data should not affect all levels of the tree equally; indeed, at more shallow levels, there is strong support for many clades. Although we know that the nuclear and mitochondrial data sets conflict in some areas (e.g., the placement of snakes), there is no reason to assume that these partitions are wholly incongruent, and we therefore combine all data sets for a final analysis. Bayesian runs were performed using one, two (nuclear and mitochondrial), and three (*RAG-1*, *c-mos*, and mitochondrial; Fig. 7) data partitions. Topologies were identical and support values were very similar for all partitioning schemes.

Monophyly of Agamidae has moderate (parsimony) to strong (Bayesian) support (Fig. 7), but is not supported by nonparametric tests (Table 4). Support is statistically significant (Table 4) for lacertid-amphisbaenian,

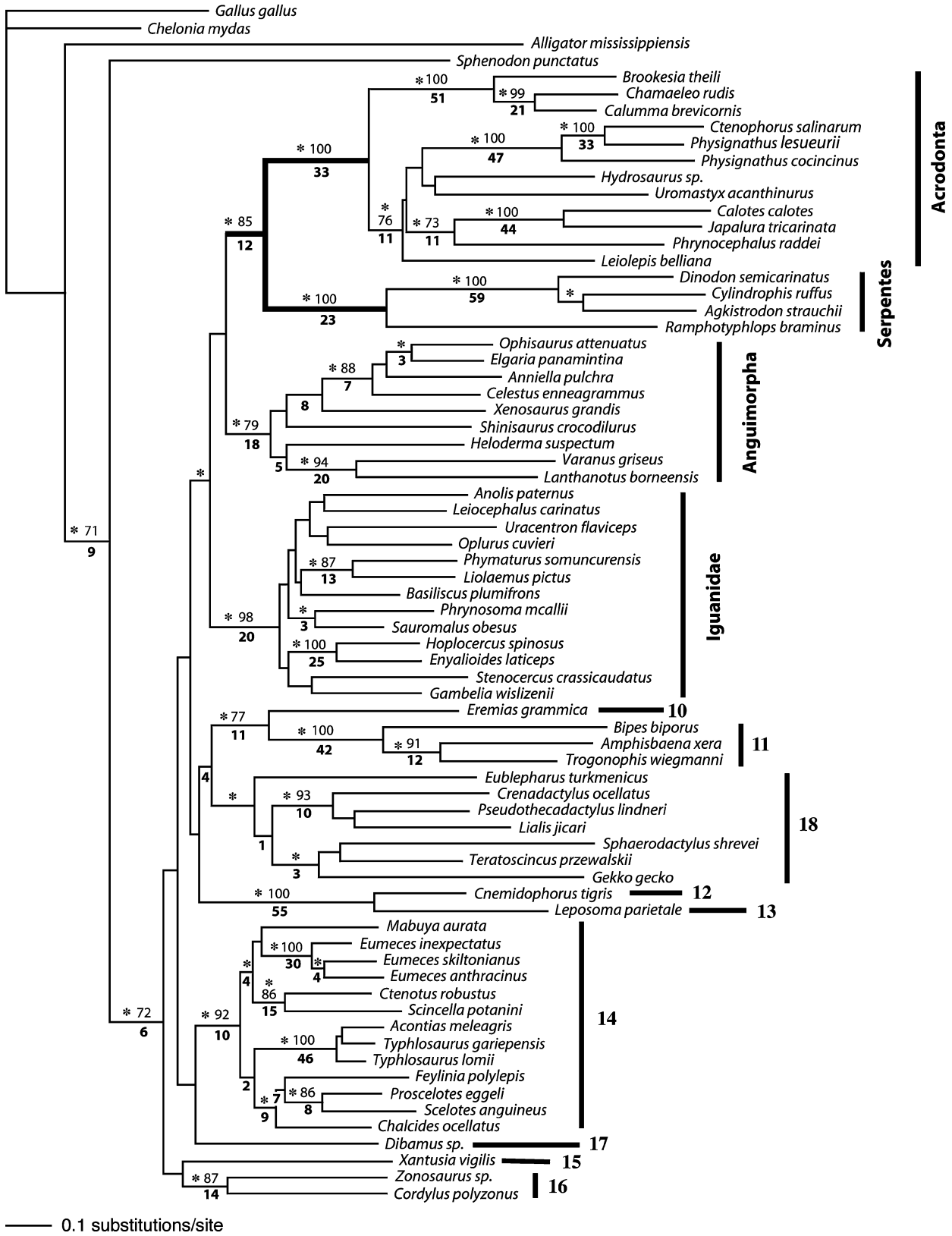

FIGURE 5. Mitochondrial data, ML phylogram (GTR+I+G model; −lnL = 63989.06; A = 0.4150, C = 0.3394, G = 0.0593, T = 0.1863; AC = 0.3588, AG = 2.5905, AT = 0.4988, CG = 0.3158, CT = 2.4827, GT = 1.0; I = 0.0951, G = 0.5652). Asterisks indicate branches that receive a posterior probability of 95% or greater in the Bayesian analysis. MP bootstrap proportions >70% (above branches) and decay indices (below branches) are provided for all nodes congruent between analyses based on the two optimality criteria. To facilitate discussion of potential LBA (see text), long internal branches leading to snakes and acrodonts are in bold, and pertinent higher taxa are labeled. Numbering as in Figure 1.

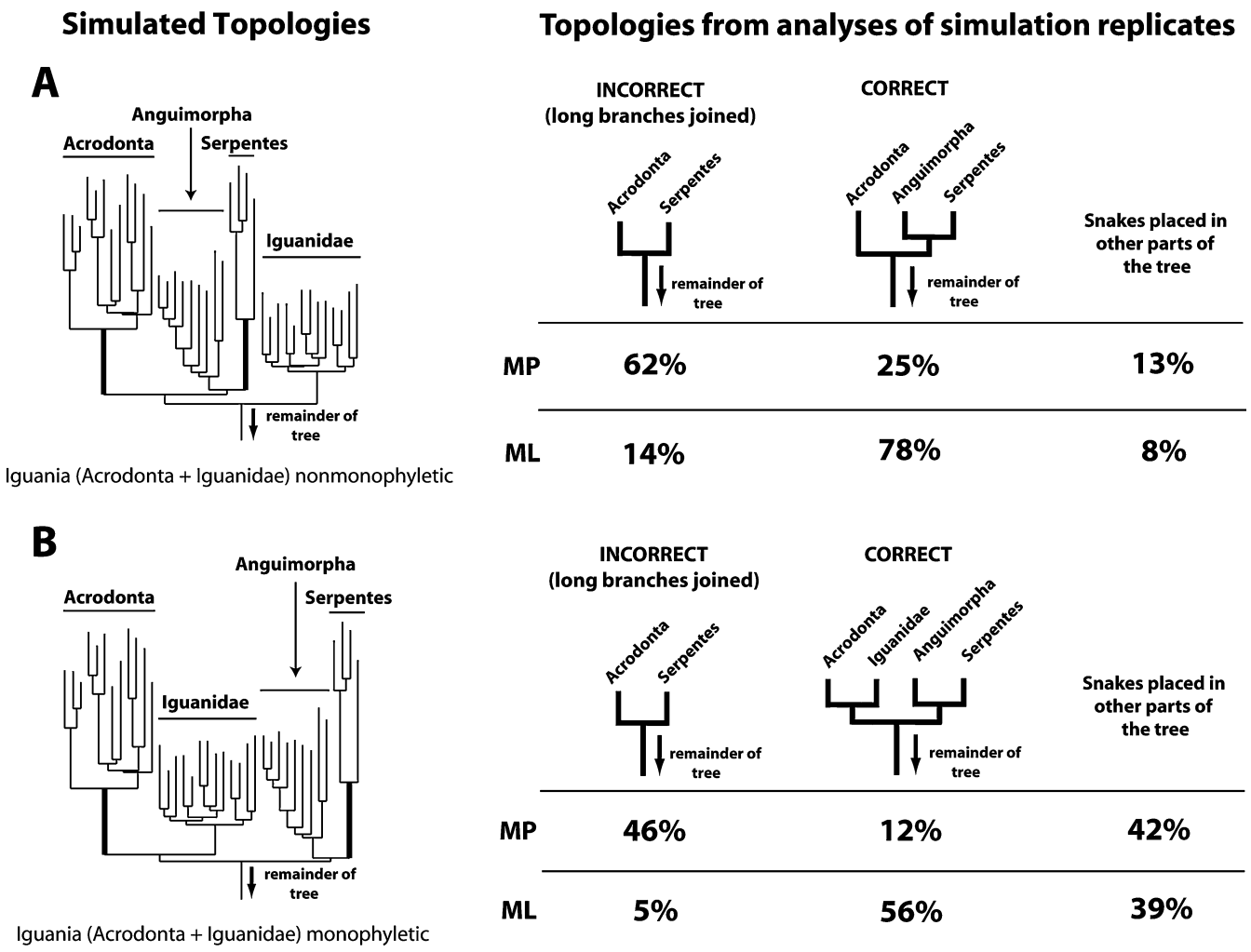

FIGURE 6. Results from two sets of 100 parametric simulations designed to detect potential long-branch attraction in the mitochondrial analysis. For all simulations, the long-branch taxa (snakes and acrodonts) are separated in the model trees by making snakes the sister taxon of anguimorphs. (A) In the model tree for the first set (top), Iguania is not monophyletic. (B) In the model tree for the second set (bottom), Iguania is monophyletic (see text). For clarity, only the relevant portions of the model trees are shown; the remainder of each tree is identical to the original mitochondrial ML topology of Figure 5. The tables show the number of times incorrect and correct topologies are recovered under MP and ML, respectively. As a control, 100 simulated data sets were constructed using as a model the exact ML topology of Figure 5, in which snakes and acrodonts are sister taxa. For these control data sets, MP and ML each recover the model topology in 98% of the analyses.

trogonophid-amphisbaenid, and xantusiid-cordylid (SH test only) clades. As in the mitochondrial analysis, MP places snakes as the sister taxon of acrodonts (Fig. 7A). Interestingly, the ML analysis, which should be more resistant to LBA, instead places snakes as the sister group of (Anguimorpha + Iguania) (Fig. 7B). Monophyly of Iguania (Acrodonta + Iguanidae) is supported by a posterior probability >95% in the Bayesian analysis, which agrees with well supported MP and Bayesian results from the nuclear analyses (Fig. 3).

Figure 8 summarizes molecular support for higher-level phylogenetic relationships within Squamata. In this figure, we have not constructed a consensus of all trees recovered from the three data sets used in this study. Rather, we present all nodes receiving both strong (>95%) bootstrap and Bayesian support from either the combined nuclear or the mitochondrial data set, and which are not contradicted with similar levels of support in the other data set. All nodes not meeting the above criteria are collapsed, thus giving a conservative estimate of well-supported squamate relationships.

DISCUSSION

Paraphyly of Scleroglossa and Evolution of Squamate Feeding

The most important discrepancy between our results and the morphological hypotheses is our strong statistical rejection of the hypothesis that taxa traditionally included in Scleroglossa form a monophyletic group. This grouping (though not the taxon name) dates to Camp's (1923) study, and it is supported by numerous osteological and soft-tissue characters (Estes et al., 1988; Schwenk, 1988 and subsequent authors), as well as behavioral

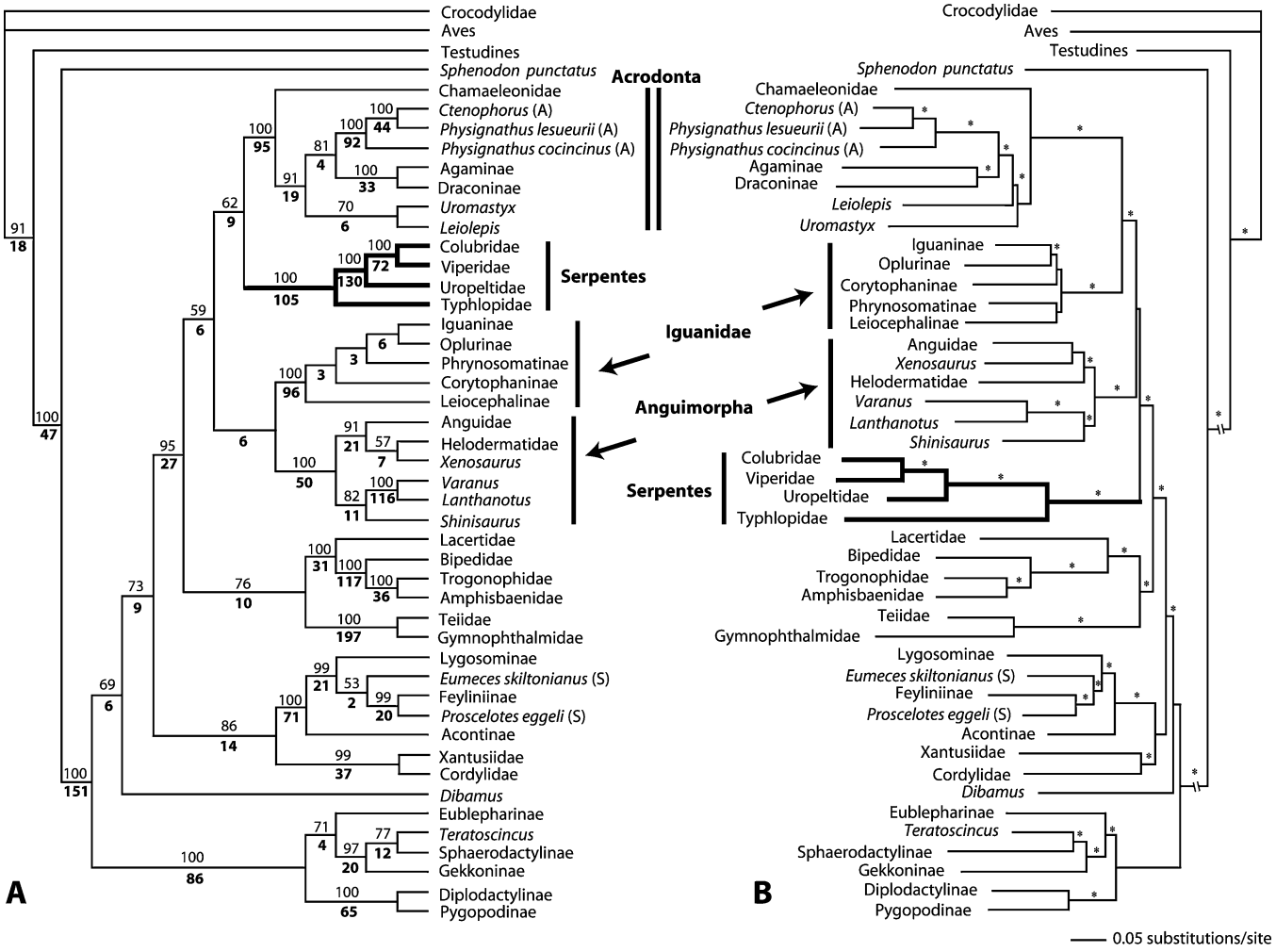

FIGURE 7. Results of combined *RAG-1*/*c-mos*/mtDNA analyses. To highlight its change in position under the two different optimality criteria, the snake clade is in bold. Other relevant higher taxa are labeled. A = Amphibolurinae; S = Scincinae. (A) Strict consensus of four most parsimonious trees (L = 22234, RI = 0.415). MP bootstrap proportions >70% are shown above branches and decay indices are in bold below branches. (B) ML phylogram (GTR+I+G model; −lnL = 93061.53458; A = 0.3456, C = 0.2690, G = 0.1506, T = 0.2348; AC = 1.7122, AG = 3.6083, AT = 0.9770, CG = 0.6230, CT = 5.0995, GT = 1.0; I = 0.2362, G = 0.7308). Asterisks indicate branches that receive a posterior probability of 95% or greater in the Bayesian analysis.

characters related to prey prehension (Schwenk and Throckmorton, 1989). The basal branches of the ingroup are all relatively small for all genes. However, although we do not specifically test the cost of moving the root across each of these branches individually, our results clearly reject its placement at Iguania, as congruence with the morphology would require.

Our results suggest reinterpretation of studies that have used comparative methodology to contrast Scleroglossa and Iguania. For example, Schwenk (1993) found a fundamental difference in tongue morphology and prey-prehension technique between iguanian (lingual prehension) and scleroglossan (jaw prehension) lizards. Schwenk (1986) reported that the tongue of *Sphenodon* (a lingual feeder) shares many features with iguanid lizards, including muscle-fiber architecture and hyobranchial-foretongue coupling. Based on these similarities, along with independent evidence for a basal dichotomy within squamates between Iguania and Scleroglossa (Estes et al., 1988), Schwenk (1986) concluded that *Sphenodon* and iguanians exhibit the ancestral squamate (and lepidosaurian) condition. This inference of the ancestral condition is problematic, however, because jaw prehension is widespread in the closest outgroups to lepidosaurs (birds, turtles, and crocodilians). Schwenk (1989) cites examples of lingual prehension in some of these groups as evidence that it is the ancestral state; however, given the difficulty in comparing the highly modified feeding apparatus between these distantly related groups (Schwenk, 1988), this conclusion may be unwarranted.

Under Schwenk's (1986) scenario, the common ancestor to Scleroglossa evolved a fundamentally different feeding system and associated tongue morphology. Nonherbivorous iguanians are generally considered ambush predators with little ability to detect chemical cues from

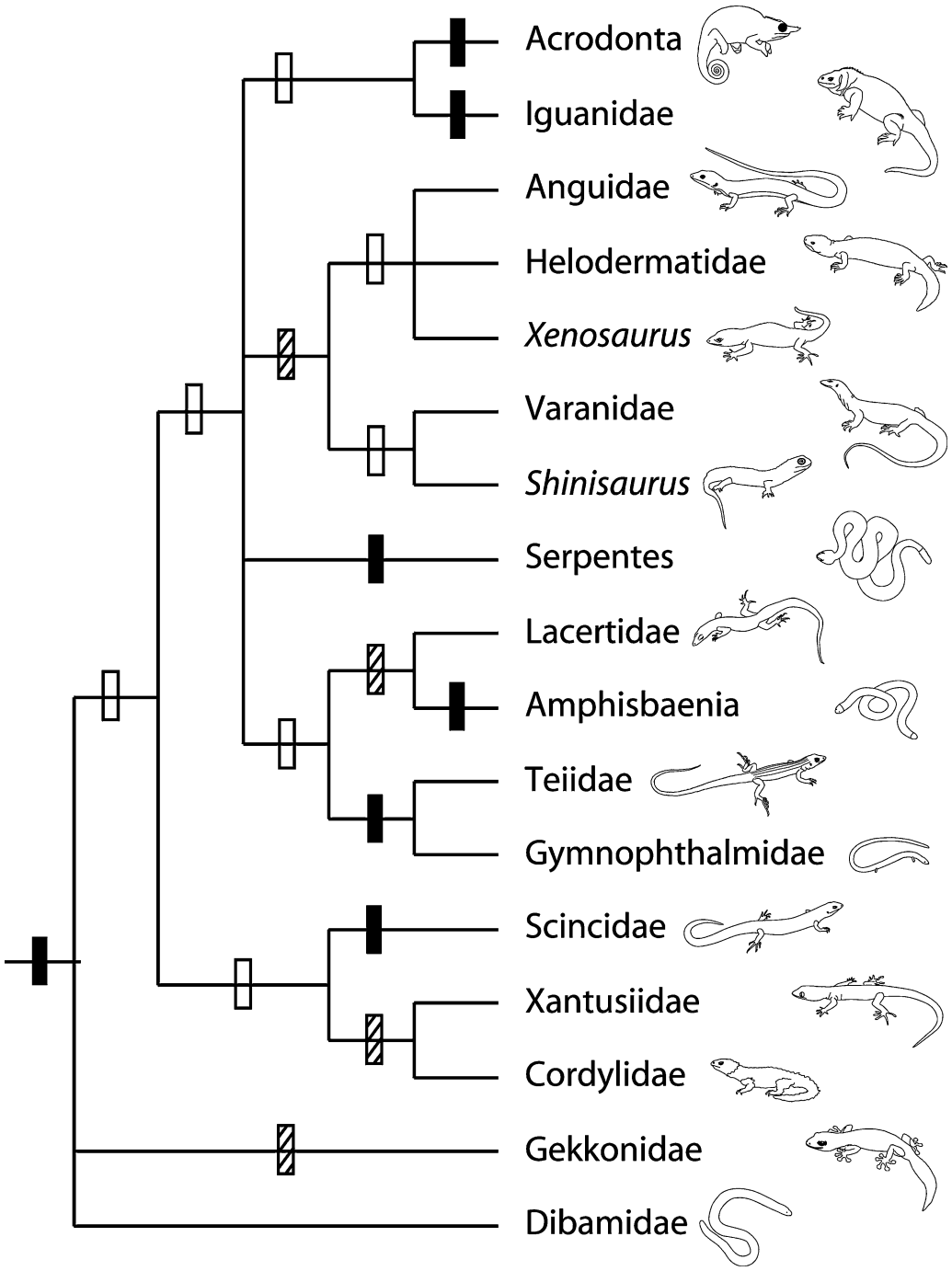

FIGURE 8. Summary of higher-level squamate phylogenetic relationships well supported by molecular data. Branches with any type of bar are supported by MP bootstraps and Bayesian posterior probabilities ≥95% in the combined nuclear analysis. Solid bars denote branches also supported by bootstraps and posterior probabilities ≥95% in the mitochondrial analysis. Hatched bars denote branches supported by posterior probabilities (but not bootstraps) ≥95% in the mitochondrial analysis. Open bars denote branches not congruent with any mitochondrial topology, but which are also not strongly contradicted (by the above support criteria) in the mitochondrial analyses. Note that *Rhineura* (Amphisbaenia) was not included in the analyses from which this figure was derived.

prey items (e.g., Cooper, 1995), whereas scleroglossans are often actively foraging lizards that tongue-flick to collect chemical cues from prey items (although several exceptions exist; see Perry, 1999). Release of the tongue from its prey-prehension duties is thought to have allowed this new role to evolve, whereas the functional constraints imposed by lingual prey-prehension presumably have prevented most iguanians and *Sphenodon* from developing olfactory capabilities to the same extent (Schwenk, 1993).

However, even if lingual prehension is assumed to be the ancestral lepidosaurian condition, it is possible

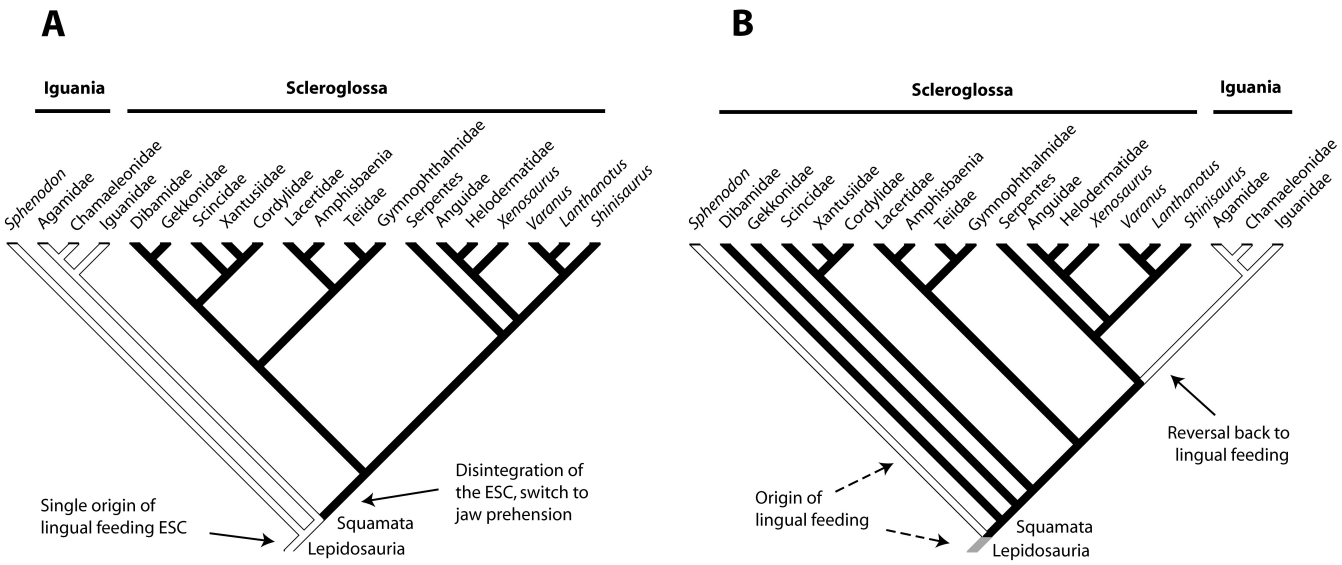

FIGURE 9. Evolution of feeding mode in lepidosaurs. White branches indicate lingual prehension, black branches jaw prehension, and the gray branch is equivocal. (A) Under a traditional monophyletic Scleroglossa, produced here by simply rerooting our nuclear topology, lingual-feeding arose once in a common ancestor of *Sphenodon* and squamates, and was lost in an ancestor to Scleroglossa. Note that we have adopted Schwenk's (1986) assumption that lingual prehension is ancestral for lepidosaurs (but see text). (B) Under our nuclear topology, feeding mode is more labile, with lingual feeding arising at least twice, once either in the lineage leading to *Sphenodon* or in a common ancestor of *Sphenodon* and squamates (allowing uncertainty in the outgroup designations), and once in an ancestor to Iguania. Dotted lines indicate ambiguity in the position of the first acquisition of lingual feeding. Data from Schwenk (2000).

that the similar feeding behavior and tongue morphology of *Sphenodon* and iguanians represent homoplasy rather than homology. As mentioned by Schwenk (1986), several authors (e.g., Gans, 1983; see also Wu, 1994) have noted that *Sphenodon* is not a basal but rather a highly nested taxon within Rhynchocephalia, a once widespread group that included a diversity of body plans and lifestyles, including long-legged terrestrial forms, long-bodied obligately aquatic forms, and specialized herbivores (Evans et al., 2001; Reynoso, 2000). Although *Sphenodon* is almost certainly the closest living relative to squamates, considering its character states ancestral for Squamata, especially when the characters involve largely soft-tissue anatomy and behavior, is problematic.

Schwenk and Wagner (2001) used suites of characters associated with both lingual-prehension and jaw-prehension modes of feeding to illustrate their evolutionarily stable configuration (ESC) concept, arguing that the phylogenetic stability of lingual feeding across a variety of habitats and lifestyles is evidence of a complex, integrated system. Internal selection for maintenance of the entire functional system results in only rare transitions from one system to another. In the example discussed here, the strong interdependence among components of the lingual-prehension feeding mode is thought to have led to its persistence in virtually all iguanians, regardless of habitat, diet, or other ecological variation. Only when the components of this system were somehow decoupled in the common ancestor to scleroglossans could jaw prehension and its associated olfactory and behavioral traits evolve.

Under our phylogenetic hypothesis, iguanians and *Sphenodon* (or some possibly distant ancestor to *Sphenodon*) are inferred to have acquired lingual prey-prehension techniques independently (Fig. 9). Because food prehension techniques, tongue musculature, and chemosensory ability are unknown for rhynchocephalians other than *Sphenodon*, this scenario is only slightly less parsimonious than the traditional view. Although similarities in muscle fiber and connective-tissue architecture between *Sphenodon* and iguanians may be explained most parsimoniously by symplesiomorphy (Schwenk, 1986), if lepidosaurian feeding systems truly are highly integrated and constrained, tongue morphology could evolve to be markedly similar in unrelated groups adopting the same feeding mode. Indeed, the apparent lability of feeding mode and tongue morphology is exemplified in the recovery (with each of the three gene regions) of an iguanian-anguimorph-snake clade, a group that represents the extremes of these traits within squamates.

Phylogenetics of Limblessness in Squamates

Our nuclear data statistically support separate origins for all major limbless groups, in contrast to most recent morphology-based inferences. In addition to phylogenetic analyses of base substitutions, almost identical multicodon deletions in the *c-mos* gene of amphisbaenians and *Gallotia* (a lacertid) provide further evidence that amphisbaenians are not closely related to either snakes or dibamids. Convergence or parallelism has likely misled morphological studies that find close relationships between two or more of these limbless groups.

Some morphological data support our findings with respect to each of the major limbless groups. For example, Greer (1985) cited several characters that would place dibamids phylogenetically outside squamates (although he found evidence for their placement in other parts of the tree as well). A dibamid-gekkonid relationship has been proposed several times (e.g., Underwood, 1957; Wu et al., 1996), most recently by Underwood and Lee (2000), who found these two taxa unique among squamates in their possession of paired egg teeth in addition to other potential gekkonid/dibamid synapomorphies. Our results are equivocal on the exact relationship of dibamids to gekkonids, except to indicate early divergence from the ancestral squamate lineage (see Figs. 1 and 3). Because of this phylogenetic position, paired egg teeth, along with most of the other similarities (Underwood and Lee, 2000), are compatible with our results whether they are viewed as synapomorphies of a dibamid-gekkonid clade or as pleisiomorphies of Squamata. This evidence is compatible with a morphological rooting of our nuclear topology (Fig. 9A).

The exact position of snakes is not resolved by our data, although support for a nested position within squamates is strong. Many workers (e.g., Lee, 1998; Lee and Caldwell, 2000; McDowell and Bogert, 1954; Reynoso, 1998) have suggested that snakes are closely related to anguimorph lizards. This general relationship is compatible with our data, although only Reynoso (1998) found snakes to be the sister taxon of Anguimorpha. The other studies place snakes *within* Anguimorpha as the sister taxon of Varanidae and/or Lanthanotidae, a phylogenetic position clearly rejected by our data. (See Vidal and Hedges [2004] for similar results using different taxon sampling and a subset of the characters used in our study.) Our simulations suggest that the moderately strong support for a snake/acrodont clade in the mtDNA analyses is caused by a long-branch problem. Wiens and Hollingsworth (2000) suggest that one criterion for demonstrating LBA in an empirical study should be strong external evidence that the inferred relationship is wrong, which we have in statistical support for Iguania in the nuclear analyses, as well as published morphological studies (e.g., Estes et al., 1988). Huelsenbeck (1997) proposes two other criteria, which are that the branches should be shown to be long enough to attract each other (shown in our simulations), and also, that another method less sensitive to LBA (e.g., ML) should not place the two long-branch taxa together. This second criterion is not fully satisfied here, because ML analysis of the mitochondrial data alone also recovers a snake-acrodont clade. However, ML is not immune to LBA (Huelsenbeck, 1995), and the fact that Huelsenbeck's criterion is satisfied in the combined nuclear and mitochondrial analyses strongly suggests LBA problems with the mitochondrial data.

Finally, placement of amphisbaenians near lacertids is not without precedent. Several authors have suggested a close relationship with various scincomorph taxa (e.g., Bogert, 1964; Böhme, 1981; Wu et al., 1996) or with Scincomorpha as a whole (Schwenk, 1988). Our findings regarding relationships among amphisbaenian families indicate that limblessness has evolved multiple times in this group. Rhineuridae, a limbless taxon, was the first amphisbaenian lineage to diverge. Next was Bipedidae, which have forelimbs, branching from the lineage that eventually split to form Trogonophidae and Amphisbaenidae, both of which completely lack external limbs. This result suggests either that limbs were independently lost by both the Rhineuridae and Trogonophidae/Amphisbaenidae lineages, or that limbs were lost once in the common amphisbaenian ancestor and then regained in the Bipedidae lineage. Limb loss is known to have occurred independently in many squamate lineages, and although Whiting et al.'s (2003) findings are consistent with potential reversal in skinks, no strong evidence for reversal beyond the possible reacquisition of a single phalanx is known for this trait (Greer, 1991). We therefore favor the former hypothesis. Kearney's (2003) recent morphological work on amphisbaenians found substantially different phylogenetic relationships among the families (e.g., a basal Bipedidae and a highly nested Rhineuridae), but she also found several problematic taxa that did not fit into any of the four families represented here. A well-sampled molecular phylogeny (Kearney and Stuart, 2004) provides an independent test of these findings. The results are consistent with ours regarding relationships within this group.

Major Systematic Implications

In addition to rejecting monophyly of Scleroglossa and of limbless squamates, several major taxonomic groupings are either suggested or confirmed by our new data and analyses.

The phylogenetic position of snakes aside, our favored phylogenetic hypothesis for Anguimorpha differs somewhat from morphology-based arrangements. The combined nuclear analysis statistically rejects monophyly of Varanoidea (families Helodermatidae and Varanidae), and the two nuclear data sets find an identical alternative arrangement grouping Varanidae with *Shinisaurus* and grouping Helodermatidae with Anguidae and *Xenosaurus*. Monophyly of Varanoidea has been recovered in virtually every morphological study since McDowell and Bogert (1954), and the list of morphological synapomorphies supporting it is long (see Estes et al., 1988). However, no previous molecular studies have included sufficient taxon sampling to test its monophyly. Although the mitochondrial analyses recover a monophyletic Varanoidea, support is very low. Ours is the second molecular study (see Macey et al., 1999) to find statistical support for nonmonophyly of Xenosauridae (*Xenosaurus* + *Shinisaurus*), which strongly suggests critical reevaluation of morphology in these taxa.

Our placement of Eublepharinae as the sister taxon of a clade containing Sphaerodactylinae, Gekkoninae, and *Teratoscincus* is unconventional, requiring, among other things, two separate cases of eyelid fusion in non-eublepharine geckos. Eublepharines are generally considered the sister group to a clade containing all

other gekkonids (Kluge, 1967, 1987). If the morphological hypothesis is correct, the *RAG-1* deletion pattern has two possible explanations, the first being that separate identical, four-codon deletions occurred in the two lineages leading to Eublepharinae and Sphaerodactylinae/Gekkoninae (including *Teratoscincus*), respectively. Deletions are more prone to homoplasy than insertions, and it is possible that the gekkonid lineage is for some reason prone to lose these codon positions. However, several studies (e.g., van Dijk et al., 1999, and references therein) have suggested that indels (especially those involving multiple codons, see Simmons et al., 2001) are generally reliable phylogenetic characters less prone to homoplasy than base substitutions (but see Cunningham et al., 1997). The second possibility is that polymorphism for presence/absence of the deletion existed in the common ancestor to all geckos, and that this polymorphism persisted through the divergence of eublepharines from all other geckos and also through the split between gekkonines/sphaerodactylines and diplodactylines/pygopodines; finally, the deletion became fixed in all but the latter clade. Polymorphism persisting through two separate higher-level divergences seems unlikely; we have found no documentation of lineage sorting occurring at a similar hierarchical level. The simplest explanation, especially in light of independent evidence from both nuclear data sets for eublepharine/gekkonine/sphaerodactyline monophyly, is that a single deletion occurred in the common ancestor to this clade.

Our findings regarding intrafamilial relationships of skinks are largely congruent with those of Whiting et al. (2003), which uses different molecular data and wider taxon sampling. Scincinae is paraphyletic with respect to the limbless Feyliniinae (which is nested within a clade of African scincines), but the southern African limbless Acontinae is the sister taxon to a clade containing all other skinks. Our Xantusiidae-Cordylidae clade agrees with Estes' (1983) conclusions, and is also strongly supported by Whiting et al. (2003).

Relationships within Iguania are largely concordant with previous molecular findings. Recent molecular studies (Macey et al., 1997b; Schulte et al., 1998) found strong evidence for monophyly of the traditional Iguanidae, and both our *RAG-1* and mitochondrial results (with taxon sampling from all major iguanid sublineages) confirm this finding. Agamidae is monophyletic in all but the *RAG-1* and*RAG-1/c-mos* likelihood analyses, and bootstrap- support is fairly high in the *RAG-1/c-mos*/mtDNA combined MP and Bayesian analyses. Joger (1991) found Agamidae monophyletic using albumin immunological distances, and Honda et al. (2000) reported strong heuristic support for its monophyly (MP bootstrap of 100%, but no statistical testing was performed) using 12S and 16S mitochondrial data. Macey et al. (2000) also recovered a monophyletic Agamidae, although support was not strong. Thus, although the morphological evidence is equivocal (Frost and Etheridge, 1989), the molecular consensus leans heavily in favor of a monophyletic Agamidae.

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APPENDIX
SPECIMEN INFORMATION

Museum numbers of voucher specimens and GenBank accession numbers are given below. Acronyms are AMNH, American Museum of Natural History, New York; AMS, Australian Museum, Sidney; CAS, California Academy of Sciences, San Francisco; CM, Craig Moritz Collection, University of Queensland, Australia; EBU, Evolutionary Biology Unit, South Australian Museum; FM, The Field Museum, Chicago;

LG, Laboratory of Cytogenetics of Vertebrates, São Paulo, Brazil; JW, John Wombey field number; LJV, Laurie J. Vitt field number; LSUMZ, Louisiana Museum of Natural History; MRT, Miguel Trefaut Rodrigues field number; MZUSP, Museo de Zoologia, University of São Paolo, Brazil; MVZ, Museum of Vertebrate Zoology, University of California at Berkeley; QCAZ, Museo de Zoología de la Pontificia Universidad Católica del Ecuador, Quito; SAMA, South Australian Museum, Adelaide, Australia; SBH, S. Blair Hedges field number; SD, Savel Daniels field number; SDSU, San Diego State University; TMT, senior author's private collection; TNHC, Texas Memorial Museum, Austin; UM, University of Madeira Collections; USNM, United States National Museum, Washington, DC; WHT, Wildlife Heritage Trust, Colombo, Sri Lanka; ZISP, Zoological Institute, St. Petersburg, Russia.

Mitochondrial and RAG-1 *Data*

Except where noted, specimens used for these two regions were identical. After each taxon name, mitochondrial accession numbers are given first, followed by those for *RAG-1*.

Outgroups: *Alligator mississippiensis* (mtDNA: no voucher, Y13113; *RAG-1:* AMNH OTC73, AF143724); *Gallus gallus* (mtDNA: no voucher, X52392; *RAG-1*: no voucher, M58530); Chelonia *mydas* (mtDNA: no voucher, NC_000886; *RAG-1*: no voucher, tissue from SBH collection, AY687907; unpublished sequence provided by Jim Krenz); *Sphenodon punctatus* (St. Louis Zoo, ISIS 373002; AY662533, AY662576)

Acrodonta: *Brookesia thieli* (FM 13949; AF448780, AY662577); *Chamaeleo rudis* (CAS 201711; AF448761, AY662578); *Calumma brevicornis* (FM 13715; AF448734, AY662579); *Ctenophorus salinarum* (SAMA R18178; AF375640, AY662580); *Physignathus lesueurii* (SAMA R33417; AF128463, AY662581); *Physignathus cocincinus* (MVZ 222159; U82690, AY662582); *Hydrosaurus* sp. (TNHC 54902; AF128476, AY662583); *Calotes calotes* (WHT 1679; AF128482, AY662584); *Japalura tricarinata* (CAS 177397; AF128478, AY662585); *Phrynocephalus raddei* (CAS 179770; U82691, AY662586); *Leiolepis belliana* (mtDNA: MVZ 215497, U82689; *RAG-1:* CAS 210725, AY662587); *Uromastyx acanthinurus* (MVZ 162567; U71325, AY662588)

Iguanidae: *Anolis paternus* (USNM 498070; U82679, AY662589); *Phrynosoma mcallii*, San Diego Co., California (MVZ 230681; AY297486, AY662590); *Sauromalus obesus* (MVZ 144194; U82687, AY662591); *Hoplocercus spinosus* (MZUSP 907931; U82683, AY662592); *Enyalioides laticeps* (LSUMZ H13573; AY528719, AY662593); *Phymaturus somuncurensis* (SDSU 1648; AF049865, AY662594); *Liolaemus pictus* (MVZ 162076; U82684, AY662595); *Uracentron flaviceps*, (QCAZ 2536; AF528747, AY662596); *Stenocercus crasicaudatus* (MVZ 199531; AF049866, AY662597); *Leiocephalus carinatus*, Marsh Harbor, Abaco, Bahamas (no voucher; AF049864, AY662598); *Basiliscus plumifrons* (MVZ 204068; U82680, AY662599); *Gambelia wislizenii* (MVZ 227883; U82682, AY662600); *Oplurus cuvieri* (MVZ-RM10468B; U82685, AY662601)

Anguimorpha: *Ophisaurus attenuatus* (MVZ-RM10468A; AF085625, AY662602); *Elgaria panamintina*, (MVZ 227761; U82692, AY662603); *Celestus enneagrammus* (MVZ 191045; AF085607, AY662604); *Anniella pulchra* (MVZ 228815; AF085606, AY662605); *Heloderma suspectum* (mtDNA: no voucher, AF085603; *RAG-1*: St. Louis Zoo, ISIS 100503, AY662606); *Xenosaurus grandis* (MVZ 137789; U71333, AY662607); *Varanus griseus* (ZISP 19576; U71334, AY662608); *Lanthanotus borneensis* (Cincinnati Zoo, probably ISIS 393113; AY662537, AY662609); *Shinisaurus crocodilurus* (MVZ 204291; AF085604, AY662610)

Serpentes: *Dinodon semicarinatus* (mtDNA; no voucher, AB008539); *Dinodon rufozonatum* (*RAG-1*; CAS 178042, AY662611); *Rhamphotyphlops braminus* (CAS 210151; AY662539, AY662612); *Cylindrophis ruffus* (CAS 210518; AY662538, AY662613); *Agkistrodon strauchii* (MVZ 216826; AY662540, AY662614)

Lacertidae: *Eremias grammica* (mtDNA; CAS 179206, U71331); *Eremias scripta* (*RAG-1*; CAS 179229, AY662615)

Ampisbaenia: *Bipes biporus* (MVZ 137543; U71335, AY662616); *Trogonophis wiegmanni* (MVZ 162541; AY662542, AY662617); *Amphisbaena xera* (CAS 200734; AY662541, AY662619); *Rhineura floridana* (*RAG-1*; CAS195955, AY662618)

Teioidea: *Cnemidophorus tigris* (MVZ 179799; U71332, AY662620); *Leposoma parietale*, Reserva Faunistica Cuyabeno, Sucumbios Province, Ecuador (LSUMZ 12574; AY662543, AY662621)

Gekkonidae: *Eublepharis turkmenicus* (CAS 184771; AF114248, AY662622); *Sphaerodactylus shrevei*, Haiti (SBH 194572; AY662547, AY662623); *Teratoscincus przewalskii* (CAS 171010; U71326, AY662624); *Gekko gecko* (MVZ 215314; AF114249, AY662625); *Pseudothecadactylus lindneri* (AMS R90195; AY369024, AY662626); *Crenadactylus ocellatus* (SAMA R22245; AY369016, AY662627); *Lialis jicari* (TNHC 59426; AY662546, AY662628)

Scincidae: *Mabuya aurata* (CAS 179697; U71330, AY662629); *Ctenotus robustus*, (JW R6061; AY662548, AY662630); *Scincella potanini* (CAS 194923; AY662549, AY662631); *Eumeces inexpectatus*, (MVZ 137529; AY662550, AY662632); *Eumeces skiltonianus* (CAS 220815; AY662551, AY662633); *Eumeces anthracinus* (MVZ-RM 10668; AY662552, AY662634); *Scelotes anguineus*, Grahamstown, Eastern Cape, South Africa (SD 294; AY662559, AY662635); *Proscelotes eggeli* (CAS 168961; AY662558, AY662636); *Feylinia polylepis* (CAS 219338; AY662556, AY662637); *Chalcides ocellatus*, pet trade (TMT47; AY662557, AY662638); *Acontias meleagris* (CAS 206704; AY662553, AY662639); *Typhlosaurus gariepensis* (CAS 214519; AY662555, AY662640); *Typhlosaurus lomii* (CAS 206872; AY662554, AY662641)

Xantusiidae: *Xantusia vigilis* (MVZ 228254; U71328, AY662642)

Cordylidae: *Cordylus polyzonus* (CAS 193440; AY662561, AY662643); *Zonosaurus sp.*(TNHC 55947; AY662560, AY662644)

Dibamidae: *Dibamus sp.* (MVZ 224112; AY662562, AY662645)

C-mos Data

Outgroups: *Crocodylus porosus* (SAMA R34528, AF039484); *Gallus gallus* (no voucher, M19412); *Pelomedusa subrufa* (captive animal, AF109208) *Sphenodon punctatus* (CM43, AF039483)

Acrodonta: *Chamaeleo jacksonii* (CAS 199070, AY662563); *Uromastyx aegyptia* (AF137531); *Leiolepis guentherpetersi* (AF137529); *Physignathus lesueurii* (AF137524); *Ctenophorus decresii* (SAMA R42978, AF039475); *Physignathus cocincinus* (EBU 0188218, AF039476); *Phrynocephalus mystaceus* (AF137527); *Calotes versicolor* (AF137525)

Iguanidae: *Sauromalus obesus* (AF315400); *Oplurus sebae* (AF315391); *Leiocephalus sp.* (AF315388); *Corytophanes cristatus* (AF315390); *Sceloporus grammicus* (CM331, AF039478)

Anguimorpha: *Elgaria multicarinata* (CM199, AF039479); *Varanus salvator* (AF435017); *Lanthanotus borneensis* (Cincinnati Zoo, probably ISIS 393113; AY662564); *Shinisaurus crocodiluris* (MVZ 204291, AY662565); *Heloderma suspectum* (St. Louis Zoo, ISIS 100503, AY662566); *Xenosaurus grandis* (MVZ 137789, AY662567)

Serpentes: *Agkistrodon piscivorus* (AF471096); *Dinodon rufozonatum* (AF471163); *Cylindrophis ruffus* (AF471133); *Ramphotyphlops australis* (SAMA R36502, AF039474)

Lacertidae: *Gallotia galloti* (AF315394)

Amphisbaenia: *Diplometopon zarudnyi* (AF148709); *Bipes biporus* (CM22, AF039482); *Amphisbaena xera* (CAS 200734, AY662568)

Teioidea: *Tupinambus quadrilineatus* (LG 1132, AF420863); *Bachia dorbignyi* (MRT 977273, AF420861)

Gekkonidae: *Coleonyx variegatus* (AF315386); *Pseudothecadactylus lindneri* (AMS R90194, AF090846); *Lialis burtonis* (SAMA R29312, AF090850); *Teratoscincus przewalskii* (CAS 171010, AY662569); *Tarentola boettgeri* (AF315387); *Sphaerodactylus shrevei* (SBH 194572, AY662570)

Scincidae: *Mabuya delalandii* (UM R43, AF335080); *Eumeces skiltonianus* (AF315396); *Feylinia polylepis* (CAS 219338, AY662571); *Acontias meleagris* (CAS 206704, AY662572); *Proscelotes eggeli* (CAS 168961, AY662573)

Xantusiidae: *Xantusia vigilis* (AF148703); **Cordylidae:** *Cordylus cordylus* (AF148711)

Dibamidae: *Dibamus sp.* (MVZ 224112, AY662574)