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# Molecular Tests of Phylogenetic Taxonomies: A General Procedure and Example Using Four Subfamilies of the Lizard Family Iguanidae

James A. Schulte II,*,[1] J. Robert Macey,* Allan Larson,* and Theodore J. Papenfuss†

*Department of Biology, Box 1137, Washington University, St. Louis, Missouri 63130; and †Museum of Vertebrate Zoology, University of California, Berkeley, California 94720

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**A general procedure is described for examining when results of molecular phylogenetic analyses warrant formal revision of taxonomies constructed using morphological characters. We illustrate this procedure with tests of monophyly for four subfamilies in the lizard family Iguanidae using 1561 aligned base positions (838 phylogenetically informative) of mitochondrial DNA sequences, representing coding regions for eight tRNAs, ND2, and portions of ND1 and COI. Ten new sequences ranging in length from 1732 to 1751 bases are compared with 12 previously reported sequences and 67 morphological characters (54 phylogenetically informative) from the literature. New morphological character states are provided for *Sator*. Phylogenies derived from the molecular and combined data are in agreement but both conflict with phylogenetic inferences from the morphological data alone. Strong support is found for the monophyly of the subfamilies Crotaphytinae and Phrynosomatinae. Monophyly of the Iguaninae is weakly supported in each analysis. All analyses suggest that the Tropidurinae is not monophyletic but the hypothesis of monophyly cannot be rejected. A phylogenetic taxonomy is proposed in which the Tropidurinae* is maintained as a *metataxon* (denoted with an asterisk), for which monophyly has not been demonstrated. Within the Phrynosomatinae, the close relationship of *Sator* and *Sceloporus* is questioned and an alternative hypothesis in which *Sator* is the sister taxon to a clade comprising *Petrosaurus, Sceloporus,* and *Urosaurus* is presented. Statistical tests of monophyly provide a powerful way to evaluate support for taxonomic groupings. Use of the *metataxon* prevents premature taxonomic rearrangements where support is lacking.** © 1998 Academic Press

***Key Words:*** **Reptilia; Squamata; Iguania; Iguanidae; Crotaphytinae; Iguaninae; Phrynosomatinae; Tropidurinae; *Sator;* mitochondrial DNA; phylogenetics; taxonomy; monophyly tests; *metataxon*.**

Molecular phylogenetic analyses often are used to examine monophyly of taxonomic groupings constructed from morphological characters. There is no general procedure, however, for judging what constitutes sufficient evidence for monophyly of a taxonomic grouping and what taxonomic procedure should be followed when results are ambiguous. We suggest a general method for evaluating support for monophyly of taxonomic groupings and illustrate this procedure with phylogenetic analyses of the iguanid lizard subfamilies Crotaphytinae, Iguaninae, Phrynosomatinae, and Tropidurinae. This method uses statistical tests to ask whether the molecular and morphological data analyzed separately and in combination can reject alternative hypotheses of either nonmonophyly or monophyly for the taxa in question and whether these data partitions contain significant conflicts (Larson, 1994). Taxonomic groupings whose monophyly receives support from analyses of the relevant data deserve formal taxonomic recognition. Taxonomic groupings for which monophyly is not found but not statistically rejected can be recognized as *metataxa* (Estes *et al.*, 1988; Gauthier *et al.*, 1988) pending more definitive phylogenetic results.

Phylogenetic relationships of iguanid lizards have been highly controversial. Frost and Etheridge (1989) divided the Iguanidae into eight new families corresponding to the eight monophyletic groups of Etheridge and de Queiroz (1988) because their analysis of morphological data could neither confirm nor reject monophyly of the Iguanidae (*sensu lato*). This taxonomy has been a topic of extensive controversy (for a review, see Schwenk, 1994). Recently, Macey *et al.* (1997c) presented data supporting monophyly of the Iguanidae (*sensu lato*) and suggested a taxonomy recognizing the eight families of Frost and Etheridge (1989) as subfamilies. However, monophyly of the eight iguanid subfamilies has never been tested rigorously.

The eight iguanid subfamilies as proposed by Macey *et al.* (1997c) are the Corytophaninae, Crotaphytinae, Hoplocercinae, Iguaninae, Oplurinae, Phrynosomatinae, Polychrinae, and Tropidurinae. Recently, molecu-

[1] To whom correspondence should be addressed. Fax: (314) 935-4432. E-mail schulte@biodec.wustl.edu.



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lar studies have investigated relationships within several iguanid subfamilies but have not adequately tested monophyly of these subfamilies. The only subfamily that has been tested for monophyly using representatives of all other subfamilies is the Crotaphytinae (*Crotaphytus* and *Gambelia;* Macey *et al.,* 1997c). Two recent studies have addressed phylogenetic relationships of the Iguaninae but neither study included representatives of more than three of the other iguanid subfamilies (Rassman, 1997; Sites *et al.,* 1996). Molecular phylogenetic studies within the Phrynosomatinae (Reeder, 1995; Reeder and Wiens, 1996) included representatives of four additional iguanid subfamilies as outgroups, but these taxa were used to construct a hypothetical ancestor based on the topologies identified in an analysis of morphological data by Frost and Etheridge (1989). Macey *et al.* (1997c) showed that the morphological data of Frost and Etheridge (1989) have little phylogenetic information for resolving relationships between iguanid subfamilies. In an investigation of the phylogenetic relationships of the Oplurinae, Titus and Frost (1996) included members of three other iguanid subfamilies as outgroups. While the Oplurinae was found to be monophyletic, the Tropidurinae was not. Titus and Frost (1996) included members of two of the three groups of the Tropidurinae. The three groups of the Tropidurinae (*sensu* Macey *et al.,* 1997c) were recognized previously as subfamilies, the Liolaeminae, Leiocephalinae, and Tropidurinae, by Frost and Etheridge (1989).

Here, hypotheses of monophyly are tested for four of the eight iguanid subfamilies, the Crotaphytinae, Iguaninae, Phrynosomatinae, and Tropidurinae. Ten new mitochondrial DNA sequences are reported for the same region sequenced by Macey *et al.* (1997c). This sequence extends from the end of the gene encoding ND1 to the beginning of the gene encoding COI and includes all of the ND2 and eight tRNA genes.

Mitochondrial DNA sequences for both genera of the Crotaphytinae, *Crotaphytus* and *Gambelia,* were previously published by Macey *et al.* (1997c) and we further test the monophyly of this subfamily with sequences from 10 additional iguanid genera. Within the Iguaninae, a basal taxon, *Dipsosaurus,* is compared with a representative member of the Iguanini (de Queiroz, 1987), *Sauromalus,* from Macey *et al.* (1997c). Mitochondrial DNA sequences for all remaining major groups in the Phrynosomatinae, *Petrosaurus, Sator, Sceloporus, Uma* [a member of the "sand lizard" clade (Etheridge and de Queiroz, 1988)], *Urosaurus,* and *Uta* are reported and compared with the *Phrynosoma* sequence from Macey *et al.* (1997c). Within the Tropidurinae, members of the three groups recognized as subfamilies by Frost and Etheridge (1989) are included in the analysis. *Leiocephalus, Stenocercus,* and *Phymaturus* are included as a comparison with the previously published *Liolaemus* sequence from Macey *et al.* (1997c).

Outgroups were selected from two separate lineages, the Acrodonta, which is the sister taxon to the Iguanidae (Macey *et al.,* 1997c), and the Scleroglossa, which is the sister taxon to the Iguania (Estes *et al.,* 1988; Macey *et al.,* 1997a; Schwenk, 1988). *Chamaeleo* and *Leiolepis* were selected as representatives of the Acrodonta, and *Elgaria* as a representative member of the Scleroglossa using the previously published sequences of Macey *et al.* (1997c).

## MATERIALS AND METHODS

### *Specimen Information*

Museum numbers and localities for voucher specimens from which DNA was obtained and GenBank Accession Numbers are presented. Acronyms are MVZ for Museum of Vertebrate Zoology, University of California, Berkeley, and SDSU for San Diego State University, San Diego. The acronym followed by a dash RM represents the field number of the second author for an uncatalogued specimen being deposited in the Museum of Vertebrate Zoology. **Iguaninae:** *Dipsosaurus dorsalis,* beach flats, Bahia de Los Angeles, Baja California Norte, Mexico (MVZ 161172, AF049857). **Phrynosomatinae:** *Petrosaurus thalassinus,* 32.2 miles north of Parador Punto Prieta, Mexico Highway 1, Baja California Norte, Mexico (MVZ 161010, AF049858); *Sator angustus,* Baja California Sur, Mexico (MVZ 137666, AF049859); *Sceloporus graciosus,* Mescalero Sands, 37.5 miles east of Roswell on Highway 380, Chaves County, New Mexico (MVZ 180319, AF049860); *Uma scoparia,* 3.8 miles south of Parker on Highway 95, La Paz County, Arizona (MVZ 182602, AF049861); *Urosaurus graciosus,* Kelso Dunes, approximately 4 miles SSW of Kelso, San Bernardino County, California (MVZ-RM3491, AF049862); *Uta stansburiana,* Mescalero Sands, 37.5 miles east of Roswell on Highway 380, Chaves County, New Mexico (MVZ 180323, AF049863). **Tropidurinae:** *Leiocephalus carinatus,* Marsh Harbour, Abaco, Bahamas (no voucher, AF049864); *Phymaturus somuncurensis,* Meseta Somuncurá, Dept. Río Negro, Argentina (SDSU 1648, AF049865); *Stenocercus crasicaudatus,* Machu Pichu Ruins, Dept. Cuzco, Peru (MVZ 199531, AF049866).

The previously reported *Liolaemus* sequence (Macey *et al.,* 1997c) was misidentified as *L. tenuis.* The correct information for this specimen is as follows: *Liolaemus pictus,* Bariloche, 44 km west at Rio Castaño Overo, Dept. Rio Negro, Argentina (MVZ 162076, U82684).

### *Laboratory Protocols*

Laboratory protocols follow Macey *et al.* (1997a) except that cycle-sequencing reactions were run with a denaturation at 95°C for 35 s, annealing at 45–60°C for 35 s, and extension at 70°C for 1 min for 30 cycles. Two primer pairs were used to amplify genomic DNA from the ND1 gene to the COI gene: L3878 and H4980, and

L4437 and H5934. Both strands were sequenced using L3878, L4221, H4419a, L4437, L4645, L4882, H4980, L5002, L5556b, L5638b, H5692, L5706, and H5934. All primers are from Macey *et al.* (1997a) except L3878 which is from Macey *et al.* (1998). Two primers used were modified from Macey *et al.* (1997a): L4882 5′-TGACAAAAACTAGCACC-3′ and L5002 5′-AACCAGACACAAATACGAAAAAT-3′. Primer numbers refer to the 3′ end on the human mitochondrial genome (Anderson *et al.*, 1981), where L and H correspond to light and heavy strands, respectively.

*Sequence Alignment and Character Homology*

Alignments of DNA sequences were constructed based on amino acids using MacClade (Maddison and Maddison, 1992) for protein-coding genes and secondary structural models (Kumazawa and Nishida, 1993; Macey and Verma, 1997) for tRNA genes. Of the 1824 aligned positions, 263 positions were judged unsuitable for phylogenetic analysis. The alignment has been deposited in GenBank. Protein-coding genes were alignable for most regions but some regions encoding amino acids at the C-terminal ends of ND1 and ND2 (positions 76–94, 1274–1370) had to be excluded because of questionable alignment.

All iguanids sequenced had the typical vertebrate mitochondrial gene order (Macey *et al.*, 1997a,c). *Chamaeleo* and *Leiolepis* have the genes for $tRNA^{Ile}$ and $tRNA^{Gln}$ switched in order (Macey *et al.*, 1997c). These gene sequences in *Chamaeleo* and *Leiolepis* were changed to the typical vertebrate gene order to align with the ingroup taxa (for *Chamaeleo* GenBank U82688 positions 72–151 are placed after position 219, and for *Leiolepis* GenBank U82689 positions 81–165 are placed after position 235). Among tRNA genes, several loop regions were unalignable as were noncoding regions between genes. The dihydrouridine (D) and TψC (T) loops for the genes encoding $tRNA^{Ile}$ (positions 108–115, 148–154), $tRNA^{Trp}$ (positions 1384–1392, 1425–1431), and $tRNA^{Cys}$ (positions 1692–1696, 1653–1659) were excluded from the analyses. *Basiliscus* has an unusual $tRNA^{Asn}$ in which the variable loop is 7 bases (Macey *et al.*, 1997c) instead of the standard 3–5 bases, making this loop unalignable (positions 1558–1564). The variable loop of the $tRNA^{Cys}$ gene (positions 1665–1669) was not alignable. The D-loop was excluded from the genes for $tRNA^{Gln}$ (positions 220–227) and $tRNA^{Tyr}$ (positions 1774–1780). Noncoding sequences between the genes encoding $tRNA^{Ile}$ and $tRNA^{Gln}$ (positions 167–169), $tRNA^{Gln}$ and $tRNA^{Met}$ (positions 241–253), $tRNA^{Trp}$ and $tRNA^{Ala}$ (positions 1445–1454), $tRNA^{Ala}$ and $tRNA^{Asn}$ (positions 1525–1532), and $tRNA^{Cys}$ and $tRNA^{Tyr}$ (positions 1710–1721) were not used.

All taxa used for phylogenetic analysis appear to have a recognizable origin for light-strand replication ($O_L$) between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes by the criteria outlined in Macey *et al.* (1997a). However, the outgroups, *Chamaeleo* and *Leiolepis*, have strange stem-and-loop structures that contain a shortened stem of 7–8 bp in length (Macey *et al.*, 1997c). In addition, the $O_L$ stem is nearly invariant in the ingroup and the $O_L$ loop is not alignable; therefore, this region (positions 1609–1639) was excluded. Coding regions other than the anticodon stem and loop in the *Chamaeleo* and *Leiolepis* $tRNA^{Cys}$ gene (positions 1640–1669, 1687–1709) were coded as missing data because this gene contains a D-arm replacement loop instead of a D-stem, and the AA- and T-stems may shift as a result (Macey *et al.*, 1997b).

Sixty-seven morphological characters from Frost and Etheridge (1989) were analyzed in combination with DNA sequences. Characters were available for all taxa included in this study except *Sator*. Newly coded character states for *Sator* are given in Appendix 1.

*Phylogenetic Analysis*

Phylogenetic analyses were conducted as in Macey *et al.* (1997c). The Wilcoxon signed-ranks test (Templeton, 1983) was applied to examine statistical significance of the shortest tree in each analysis relative to alternative hypotheses. The Wilcoxon signed-ranks test was initially conducted as a two-tailed test. Tests that were close to significance using this conservative criterion were reexamined using a correction for tied ranks (Zar, 1984) and one-tailed probabilities, both of which make the test less conservative. Felsenstein (1985a) showed that one-tailed probabilities are close to the exact probabilities for this test, but not always conservative.

## RESULTS

The 10 new mitochondrial DNA sequences range in size from 1732 to 1751 bases and are aligned with the 3 outgroup and 9 additional iguanid sequences of Macey *et al.* (1997c) as 1824 aligned positions. All newly reported iguanid sequences have a mitochondrial gene order of ND1, $tRNA^{Ile}$, $tRNA^{Gln}$, $tRNA^{Met}$, ND2, $tRNA^{Trp}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $O_L$ (origin for light-strand replication), $tRNA^{Cys}$, $tRNA^{Tyr}$, and COI, which is typical for vertebrates. Sequences reported here are inferred to be authentic mitochondrial DNA based on the criteria of Macey *et al.* (1997a,c). These sequences show strong strand bias against guanine on the light strand (G = 11.5–13.2%, T = 22.4–25.0%, A = 32.6–36.6%, and C = 27.6–33.2%), which is characteristic of the mitochondrial genome but not the nuclear genome. The aligned sequences contain 838 phylogenetically informative characters (parsimony criterion).

Approximately three-fourths of the variation and phylogenetically informative sites were from protein-coding regions (Table 1). First and second positions of codons are similar in numbers of phylogenetically informative sites and provided half of the informative characters from protein-coding genes. Transfer-RNA

**TABLE 1**

**Distribution of Phylogenetically Informative and Variable Positions**

| | Protein coding: Codon positions | | | tRNA | | Noncoding region[a] | All aligned sequence | All data[b] |
|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | Stem | Nonstem | | | |
| Informative sites | 194 | 114 | 316 | 163 | 50 | 1 | 838 | 892 |
| Variable sites | 244 | 157 | 333 | 224 | 69 | 1 | 1028 | 1090 |

[a] The noncoding region is between $tRNA^{Tyr}$ and COI genes.
[b] Combined molecular and morphological data.

stem regions provided three-fourths of the phylogenetically informative characters from tRNA genes. The morphological data contribute 54 informative characters. Therefore, no single class of characters is dominating the phylogenetic analysis.

*Phylogenetic Hypotheses*

Analysis of 67 morphological characters produced 147 trees, each with a length of 145 steps. The Crotaphytinae, Phrynosomatinae, and Iguaninae were present as monophyletic groups in all trees with fairly good heuristic support (Crotaphytinae, 96% bootstrap, decay index 4; Phrynosomatinae, 98% bootstrap, decay index 5; Iguaninae, 90% bootstrap, decay index 3). Only 3 of the 147 equally most-parsimonious trees contained a monophyletic Tropidurinae. Within the Tropidurinae, the sister-group relationship of *Liolaemus* and *Phymaturus* is present but with little support (81% bootstrap, decay index 1). These taxa were recognized previously as the subfamily Liolaeminae.

Analysis of the DNA sequence data produced a single most-parsimonious tree of 4503 steps. The Crotaphytinae, Phrynosomatinae, and Iguaninae were monophyletic in the shortest tree with good support for the former two and less support for the latter (Crotaphytinae, 100% bootstrap, decay index 41; Phrynosomatinae, 99% bootstrap, decay index 22; Iguaninae, 56% bootstrap, decay index 7). Nonmonophyly of the Tropidurinae was found with *Stenocercus* and *Leiocephalus* forming the sister taxon to the Crotaphytinae, and *Liolaemus* and *Phymaturus* forming the sister taxon to the Oplurinae. However, these nodes received little support. The sister-group relationship of *Liolaemus* and *Phymaturus*, previously recognized as the tropidurid subfamily Liolaeminae, was strongly supported (96% bootstrap, decay index 19).

The combined morphological and molecular data produced 2 equally most-parsimonious trees with lengths of 4668 steps. One of these trees was identical to the tree produced from analysis of the molecular data. Support for individual nodes was similar to that obtained from the molecular data with a slight improvement at several nodes. The Iguaninae had an increased bootstrap value of 84% and a decay index of 10, and the sister-group relationship of *Stenocercus* and *Leiocephalus* was supported by a 63% bootstrap and a decay index of 10.

Independent analysis of the morphological and molecular data sets produced different phylogenetic hypotheses. When the Wilcoxon signed-ranks test was applied to the morphological data set, the hypotheses produced from the molecular and combined data (B and C in Appendix 2) were rejected as an alternative to a representative shortest tree obtained from the morphological data (A in Appendix 2), (B and C-1, $n = 15$, $T_S = 5.5$, $P < 0.002$**; C-2, $n = 14$, $T_S = 5$, $P < 0.003$**). When this test was applied to the molecular data, the topology produced from analysis of the DNA-sequence data (B in Appendix 2) was significantly more parsimonious than a representative tree from the 147 morphologically based topologies (A, $n = 255$, $T_S = 10417.5$, $P < 0.001$**). These results imply that the two data sets are in conflict.

When the Wilcoxon signed-ranks test was applied to the combined data set, the representative hypothesis produced from the morphological data (A in Appendix 2) was rejected as an alternative to the shortest estimates of phylogeny obtained from the combined data (C-1, $n = 270$, $T_S = 13228.5$, $P < 0.001$**; C-2, $n = 269$, $T_S = 13207.5$, $P < 0.001$**). Hence, the morphological and combined data sets are in conflict. Because the molecular tree is one of the two equally most-parsimonious trees obtained from analysis of the combined data, the molecular and combined data sets do not conflict.

*Monophyly of Taxonomic Groupings*

All analyses showed monophyly of the Crotaphytinae. The Wilcoxon signed-ranks tests using the DNA-sequence and combined data each showed that the shortest phylogenetic tree constrained to have a nonmonophyletic Crotaphytinae (G and K, respectively, in Appendix 2) was significantly longer than the overall shortest trees (B and C, respectively, in Appendix 2) (see Table 2 for results of the Wilcoxon signed-ranks tests). Using the morphological data, a representative of the 310 equally most-parsimonious alternative trees constrained to show a nonmonophyletic Crotaphytinae

**TABLE 2**

**Results from Wilcoxon Signed-Ranks Tests for Monophyly of Iguanid Subfamilies[a]**

| | Monophyletic crotaphytinae | Monophyletic phrynosomatinae | Monophyletic iguaninae | Nonmonophyletic tropidurinae |
|---|---|---|---|---|
| Morphological data | A vs D<br>$n = 13$, $T_s = 32.5$,<br>$P < 0.364$, ns | A vs E<br>$n = 13$, $T_s = 30$,<br>$P < 0.279$, ns | A vs F<br>$n = 15$, $T_s = 48$,<br>$P < 0.496$, ns | N/A |
| Molecular data | B vs G<br>$n = 111$, $T_s = 1960$,<br>$P < 0.001$**,[b] | B vs H<br>$n = 154$, $T_s = 5148.5$,<br>$P < 0.100$*,[b] | B vs I<br>$n = 198$, $T_s = 9577$,<br>$P < 0.735$, ns | B vs J<br>$n = 59$, $T_s = 720$,<br>$P < 0.213$, ns |
| Combined data | C-1 vs K<br>$n = 177$, $T_s = 5963$,<br>$P < 0.005$**,[b]<br>C-2 vs K<br>$n = 186$, $T_s = 6759$,<br>$P < 0.008$**,[b] | C-1 vs L-1<br>$n = 153$, $T_s = 4928$,<br>$P < 0.080$, ns<br>C-1 vs L-2<br>$n = 153$, $T_s = 4928$,<br>$P < 0.080$, ns<br>C-1 vs L-3<br>$n = 225$, $T_s = 11381$,<br>$P < 0.173$, ns<br>C-2 vs L-1<br>$n = 176$, $T_s = 6732$,<br>$P < 0.119$, ns<br>C-2 vs L-2<br>$n = 176$, $T_s = 6732$,<br>$P < 0.119$, ns<br>C-2 vs L-3<br>$n = 243$, $T_s = 13404$,<br>$P < 0.196$, ns | C-1 vs M-1<br>$n = 135$, $T_s = 4262.5$,<br>$P < 0.472$, ns<br>C-1 vs M-2<br>$n = 216$, $T_s = 11199$,<br>$P < 0.573$, ns<br>C-2 vs M-1<br>$n = 122$, $T_s = 3456.5$,<br>$P < 0.451$, ns<br>C-2 vs M-2<br>$n = 220$, $T_s = 11630$,<br>$P < 0.579$, ns | C-1 vs N-1<br>$n = 167$, $T_s = 6690$,<br>$P < 0.605$, ns<br>C-1 vs N-2<br>$n = 131$, $T_s = 4077.5$,<br>$P < 0.573$, ns<br>C-2 vs N-1<br>$n = 169$, $T_s = 6857$,<br>$P < 0.609$, ns<br>C-2 vs N-2<br>$n = 131$, $T_s = 4067$,<br>$P < 0.557$, ns |

[a] The null hypothesis being tested is that the shortest trees showing monophyly versus nonmonophyly of the group in question are equally parsimonious. Column headings denote the hypothesis that would be favored by a statistically significant result. Trees used in Wilcoxon signed-ranks tests are numbered as in Appendix 2. Two-tailed probabilities are shown; probability values should be halved for a one-tailed test.
[b] Tests that are significant with a two-tailed probability are denoted with two asterisks. Tests that are significant with a one-tailed probability and corrected for tied ranks are denoted with one asterisk.

(D in Appendix 2) was not significantly longer than the representative overall shortest tree (A in Appendix 2).

Monophyly of the Phrynosomatinae also was suggested by all analyses with high bootstrap values and decay indices. When the Wilcoxon signed-ranks test was applied to the molecular data set, the shortest alternative hypothesis showing a nonmonophyletic Phrynosomatinae (H in Appendix 2) could be rejected using the one-tailed probability in favor of the overall shortest tree (B in Appendix 2). When this test was applied to both the morphological and the combined data sets, trees showing a nonmonophyletic Phrynosomatinae (E and L in Appendix 2) could not be rejected in favor of the overall shortest trees (A and C in Appendix 2).

All data sets produced trees with a monophyletic Iguaninae but no data set showed strong support for this node. When the Wilcoxon signed-ranks test was applied to each of the three data sets, trees showing a nonmonophyletic Iguaninae (F, I, and M in Appendix 2) could not be rejected in favor of the overall shortest trees (A, B, and C in Appendix 2).

Only the morphological data set produced trees compatible with a monophyletic Tropidurinae. For the morphological data, numerous trees that show a nonmonophyletic Tropidurinae are the same length as the three shortest trees showing monophyly. When the Wilcoxon signed-ranks test was applied to the molecular and combined data sets, the shortest trees showing a monophyletic Tropidurinae (J and N in Appendix 2) could not be rejected in favor of the overall shortest trees, which depict nonmonophyly (B and C in Appendix 2).

### *Phylogenetic Relationships within the Phrynosomatinae*

The topology of the Phrynosomatinae acquired from analysis of both the molecular and the combined data is identical to the combined analysis of Reeder and Wiens (1996) except for the position of *Sator*. The morphological data suggest that *Sator* is closest to *Sceloporus* as reported by Wiens (1993) and Reeder and Wiens (1996), whereas the molecular and combined data place *Sator* in a more basal position. For the molecular data set, the topology acquired by Reeder and Wiens (1996) placing *Sator* as the sister taxon to *Sceloporus* requires 14 extra steps, and for the combined data it requires 11 extra steps; however, neither the molecular nor the combined data can reject this topology as being significantly less parsimonious than the favored tree. When the Wilcoxon signed-ranks test is applied to the morpho-

logical data to compare these two hypotheses, the topology acquired from analysis of the molecular and combined data could not be rejected in favor of the overall shortest morphological tree in which *Sator* and *Sceloporus* form a monophyletic group ($n = 3$, $T_S = 0$, $P < 0.109$, ns, using the representative topology of A in Appendix 2). When this same test was conducted on the molecular and combined data, these hypotheses also could not be distinguished (molecular data, B, $n = 82$, $T_S = 1411$, $P < 0.179$, ns; combined data, C-1, $n = 85$, $T_S = 1591$, $P < 0.300$, ns, C-2, $n = 84$, $T_S = 1572.5$, $P < 0.343$, ns).

In the morphological data set, only three characters [as scored by Frost and Etheridge (1989) and Etheridge and de Queiroz (1988) for *Sator*] differ in number of steps between the phrynosomatine topology of Reeder and Wiens (1996), which places *Sator* and *Sceloporus* as sister taxa, and the topology that we acquired with the molecular and combined data sets (Fig. 1). The presence of ventrolateral belly patches in males is variable within each of the genera *Petrosaurus, Sator, Sceloporus, Urosaurus,* and *Uta* (as coded by Reeder and Wiens, 1996); Frost and Etheridge (1989), however, scored *Petrosaurus* and *Uta* as lacking the patches and *Sceloporus* and *Urosaurus* as having them. Etheridge and de Queiroz (1988) scored *Sator* as having the ventrolateral patches. If all five of these genera are acknowledged as being variable for this character, it no longer discriminates the alternative topologies shown in Fig. 1. The gular fold is lost to varying degrees within both *Sator* and *Sceloporus* (Etheridge and de Queiroz, 1988; Reeder and Wiens, 1996) and in other iguanian lizards (Etheridge and de Queiroz, 1988; Frost and Etheridge, 1989). This character therefore seems not to be a particularly strong synapomorphy for a group comprising *Sator* and *Sceloporus*. The position of the innervation of the dorsal shank muscle also requires considerable homoplastic evolution on all of the topologies resulting from all analyses. These three morphological characters therefore do not constitute strong evidence for grouping *Sator* and *Sceloporus* to the exclusion of *Petrosaurus* and *Urosaurus*.

Wiens and Reeder (1997) recently placed *Sator* in synonymy with *Sceloporus*. Their phylogenetic analysis of morphological and molecular characters placed *Sator* within *Sceloporus;* however, their analysis constrained *Sator* and *Sceloporus* to form a monophyletic group. Their analysis therefore did not permit examination of the topology favored by our analyses (Figs. 1B and 1C). Our data suggest that the sinking of the genus *Sator* into *Sceloporus* by Wiens and Reeder (1997) is unwarranted, and we therefore recommend retention of the genus *Sator*. The phylogenetic position of *Sator* has been controversial for a long time, and further work is needed to test the possibility that *Sator* may be the sister taxon to a clade comprising *Petrosaurus, Sceloporus,* and *Urosaurus*.

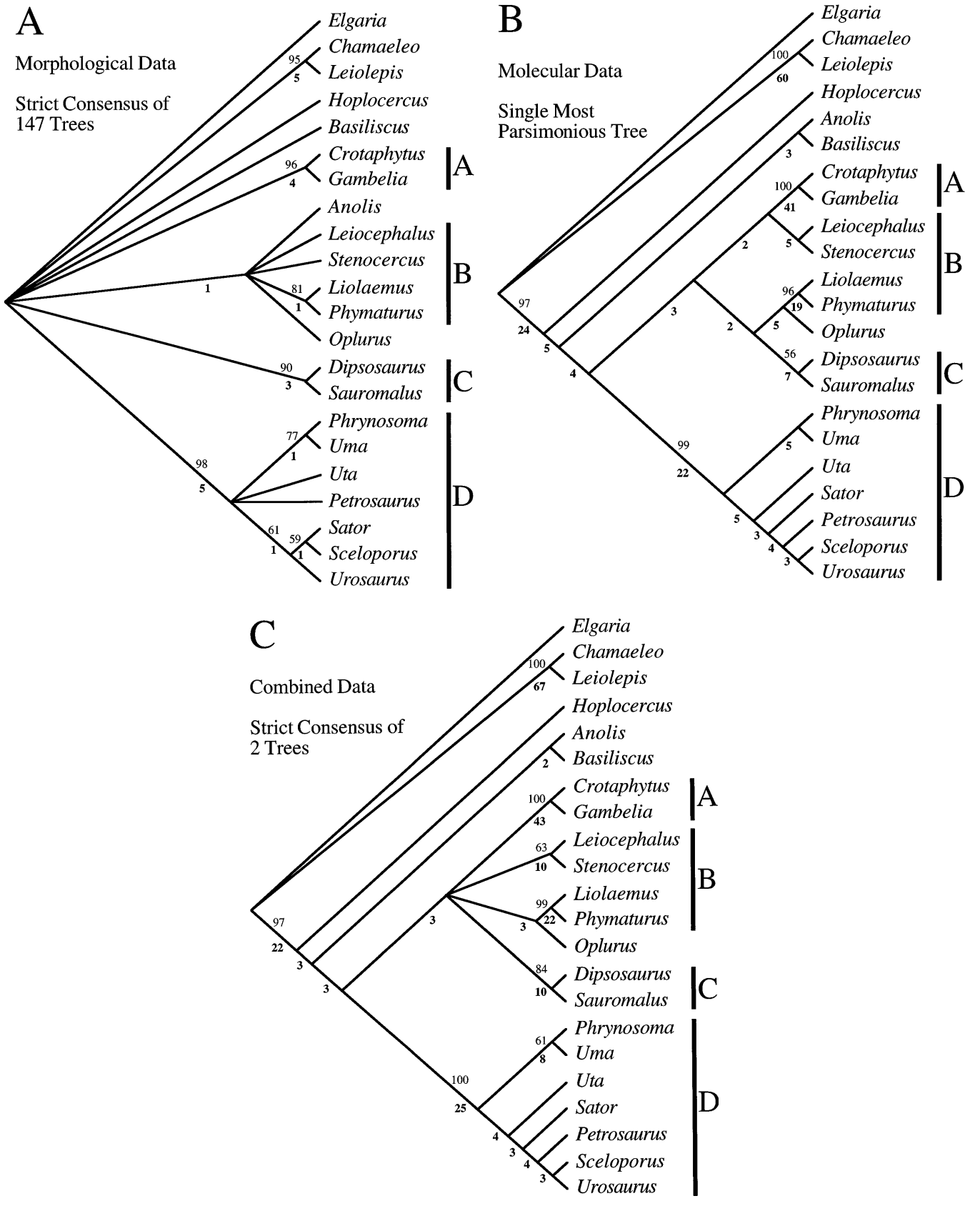
## DISCUSSION

Phylogenetic taxonomies have great potential for practical utility by providing information on monophyletic groups. These taxonomies are subject to change as new phylogenetic information is gathered. Any taxonomy loses utility if it has no stability. Phylogenetic taxonomies could benefit from rigorous testing of alternative phylogenetic hypotheses prior to taxonomic rearrangement.

Our results illustrate a general statistical procedure using molecular and morphological data to examine monophyly, and therefore validity, of taxonomic groups. The first step is to ask whether recognized taxa form monophyletic groups on the most-parsimonious topologies constructed from the molecular and morphological data sets analyzed separately and in combination. Our molecular and morphological data analyzed separately and in combination consistently specified monophyly of the iguanid lizard subfamilies Crotaphytinae, Iguaninae, and Phrynosomatinae.

For taxa that appear monophyletic on the most-parsimonious trees derived from the molecular, morphological, and combined data, the second step is to examine strength of support for monophyly using statistical analyses. A heuristic assessment of support for monophyly can be obtained by examining bootstrap values (Felsenstein, 1985b) and decay indices ["branch support" of Bremer (1994)] for the branch immediately basal to the group being examined. A combination of bootstrap values above 95% (Felsenstein and Kishino, 1993) and decay indices of at least 4 (Felsenstein, 1985a) indicates potentially strong support for a branch by the data being analyzed. A more definitive statistical criterion is to apply the Wilcoxon signed-ranks test (Felsenstein, 1985a; Templeton, 1983) to ask whether the shortest tree violating monophyly of the taxon in question is significantly longer than the most-parsimonious tree. According to this criterion, our molecular data analyzed alone or in combination with the morphological data strongly support monophyly of the Crotaphytinae (Table 2). The morphological data alone do

**FIG. 1.** Phylogenetic hypotheses obtained from analyses of morphological, molecular, and combined data sets. Bootstrap values are presented above branches and decay indices are shown in bold below branches. Subfamilies are labeled with letters as follows: A, Crotaphytinae; B, Tropidurinae; C, Iguaninae; D, Phrynosomatinae. (A) Strict consensus tree of 147 equally most-parsimonious trees generated from analysis of morphological data (length = 145, CI 0.503). (B) Phylogenetic tree generated from analysis of molecular data (length = 4503, CI 0.387). (C) Strict consensus of two equally most-parsimonious trees generated from analysis of the combined morphological and molecular data (length = 4668, CI 0.389) [CI, consistency index; Swofford (1993)].


A
Morphological Data
Strict Consensus of
147 Trees
Elgaria
Chamaeleo
Leiolepis
Hoplocercus
Basiliscus
Crotaphytus
Gambelia
Anolis
Leiocephalus
Stenocercus
Liolaemus
Phymaturus
Oplurus
Dipsosaurus
Sauromalus
Phrynosoma
Uma
Uta
Petrosaurus
Sator
Sceloporus
Urosaurus
A
B
C
D
B
Molecular Data
Single Most
Parsimonious Tree
C
Combined Data
Strict Consensus of
2 Trees

not provide statistical support for monophyly of the Crotaphytinae using the Wilcoxon signed-ranks test despite heuristic support (96% bootstrap; decay index 4). Support for monophyly of the Phrynosomatinae is marginally significant using the Wilcoxon signed-ranks test with the molecular data alone but not significant by this criterion when morphology is analyzed alone or in combination with the molecular data. Statistical support for monophyly of the Iguaninae is lacking for the molecular, morphological, and combined data using the Wilcoxon signed-ranks test.

Although strength of support for monophyly varies among the Crotaphytinae, Iguaninae, and Phrynosomatinae, we recommend continued taxonomic recognition of all three subfamilies. All relevant data indicate monophyly of these taxa, and the Wilcoxon signed-ranks test as applied here is a more rigorous criterion for monophyly than those used in standard taxonomic practice. Further testing of the monophyly of these groups is necessary, especially for the Iguaninae (Fig. 2).

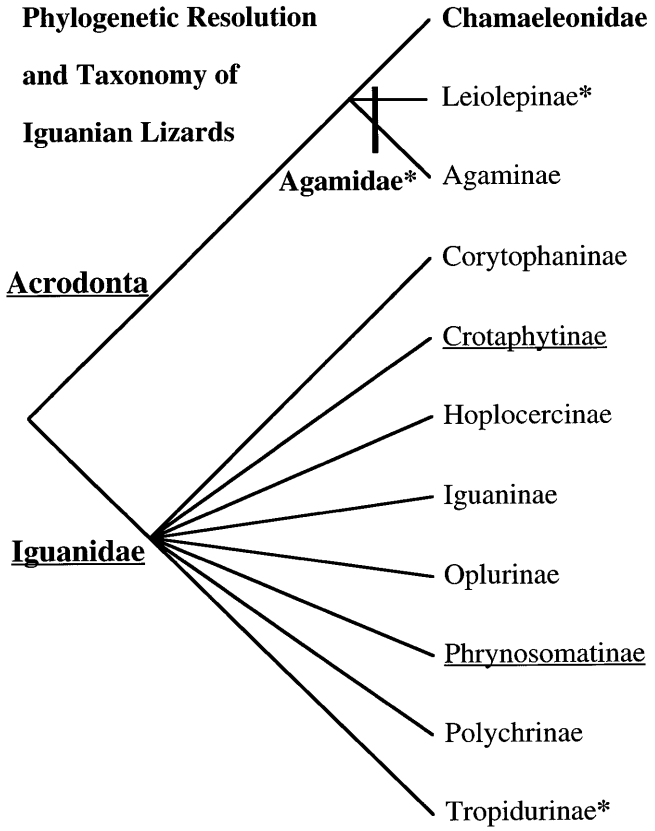



**FIG. 2.** Phylogenetic resolution and taxonomy in Iguanian lizards based on results presented in this study and Macey *et al.* (1997c). Higher taxa are bolded and subfamilies of the Agamidae* and Iguanidae are in plain type. Taxonomic groupings whose monophyly is well supported are underlined. Asterisks denote *metataxa*, whose monophyly is uncertain; the most-parsimonious trees from combined morphological and molecular data suggest nonmonophyly for these groups, but monophyly cannot be rejected. The vertical bar indicates phylogenetic branches included in the *metataxon* Agamidae*. The subfamilies Agaminae and Iguaninae appear monophyletic in our analyses (here and in Macey *et al.*, 1997c) but the hypothesis of monophyly is not strongly supported and needs further testing. We have not examined in detail the subfamilies Corytophaninae, Hoplocercinae, Oplurinae, or Polychrinae, whose monophyly needs to be examined. Monophyly of the Chamaeleonidae has strong support from morphological characters but no molecular phylogenetic analysis has been conducted.

For taxa that appear nonmonophyletic on the most-parsimonious trees derived from the molecular, morphological, and combined data, the second step in the analysis is to determine whether monophyly is statistically rejected by the data. The iguanid subfamily Tropidurinae appears as both a monophyletic and a nonmonophyletic group among the numerous equally most-parsimonious trees derived from the morphological data (Fig. 1A). The molecular and combined data specify topologies incompatible with monophyly of the Tropidurinae; however, these data are unable to reject monophyly of the Tropidurinae using the Wilcoxon signed-ranks test.

We suggest that the subfamily Tropidurinae*, whose monophyly is neither supported nor strongly rejected, be retained for convenience as a *metataxon* (Estes *et al.*, 1988; Gauthier *et al.*, 1988), denoted by an asterisk. This taxonomy avoids premature rearrangement where phylogenetic information is not definitive. Our use of statistical criteria for recognizing *metataxa* differs from the original use of the concept, which referred to topology alone. The *metataxon* was defined originally to include groups whose phylogenetic relationships are unresolved in the overall shortest topology or topologies. In our usage, a *metataxon* may appear nonmonophyletic on the shortest topology or topologies, but monophyly cannot be rejected statistically. If additional data permit statistical rejection of tropidurine monophyly, recognition of this *metataxon* should be discontinued. Alternatively, if further studies establish statistical support for a monophyletic Tropidurinae, it then should be recognized as a taxon rather than a *metataxon*.

Future phylogenetic studies evaluating characters from diverse data sets should apply the statistical criteria illustrated here to provide stability in phylogenetic taxonomies.

## APPENDIX 1

Newly coded morphological character states for *Sator* from the literature and personal communication with Richard Etheridge. Numbering follows Frost and Etheridge (1989) with character states in parentheses. Characters 8, 60–65, and 67 were coded as missing data. Characters 1, 5–14, 17, 18, 20, 21, 23, 25, 27–30, 34–42, 44–52, 58, 59, and 66 are from Etheridge and de Queiroz (1988). Characters 3, 4, 15, 16, 19, 22, 24, 31–33, and 53–57 are from Etheridge (personal communication). Characters 2 and 26 are from Estes *et al.*

(1988). Character 43 is from Reeder and Wiens (1996).

1-(0), 2-(0), 3-(0), 4-(0), 5-(0), 6-(0), 7-(0), 9-(0), 10-(0), 11-(0), 12-(0), 13-(0), 14-(1), 15-(0), 16-(0), 17-(0,1), 18-(1), 19-(0), 20-(0), 21-(1), 22-(0), 23-(0), 24-(1), 25-(1), 26-(0), 27-(1), 28-(1), 29-(0), 30-(1), 31-(0), 32-(0), 33-(1), 34-(0), 35-(1), 36-(1), 37-(2), 38-(1), 39-(1), 40-(0), 41-(0), 42-(1), 43-(0), 44-(0), 45-(0), 46-(1), 47-(1), 48-(0), 49-(0), 50-(0), 51-(0), 52-(0), 53-(1), 54-(0), 55-(0), 56-(0), 57-(0), 58-(0), 59-(1), 66-(1).

## APPENDIX 2

*Trees Used in Wilcoxon Signed-Ranks Tests*

Lengths of trees and consistency indices (Swofford, 1993) are given in parentheses. A–C are overall shortest trees from analyses of the different data sets and D–N are alternative suboptimal trees. When large numbers of equally most-parsimonious trees were found in phylogenetic analyses, a single representative tree was selected for comparisons using the Wilcoxon signed-ranks test. Numbers correspond to the following taxon names as presented in Fig. 1: 1, *Elgaria;* 2, *Chamaeleo;* 3, *Leiolepis;* 4, *Hoplocercus;* 5, *Anolis;* 6, *Basiliscus;* 7, *Crotaphytus;* 8, *Gambelia;* 9, *Leiocephalus;* 10, *Stenocercus;* 11, *Liolaemus;* 12, *Phymaturus;* 13, *Oplurus;* 14, *Dipsosaurus;* 15, *Sauromalus;* 16, *Phrynosoma;* 17, *Uma;* 18, *Uta;* 19, *Sator;* 20, *Petrosaurus;* 21, *Sceloporus;* 22, *Urosaurus.*

A. Representative shortest tree (145 steps, CI 0.503) of the 147 equally most-parsimonious trees from analysis of the morphological data. (1, ((((((2, 3), 4), 6), ((7, 8), ((16, 17), ((((19, 21), 22), 20), 18)))), (((5, (10, 9)), (11, 12)), 13)), (15, 14))).

B. Shortest tree (4503 steps, CI 0.387) from analysis of the DNA-sequence data (Fig. 1B).

C. Two equally most-parsimonious trees (4668 steps, CI 0.389) from analysis of the combined data sets. C-1, see Fig. 1B. C-2, (1, ((2, 3), (((5, 6), ((((7, 8), ((11, 12), 13)), ((15, 14), (10, 9))), ((16, 17), ((19, (20, (22, 21))), 18)))), 4))).

D. Representative tree (149 steps, CI 0.490) of the 310 equally most-parsimonious trees constrained to show a nonmonophyletic Crotaphytinae from analysis of the morphological data. (1, (((((2, 3), 4), (((((5, 13), (10, 9)), (11, 12)), ((((16, 17), ((19, 21), 22)), 18), 20)), 6)), 7, 8), (15, 14))).

E. Representative tree (150 steps, CI 0.487) of the 42 equally most-parsimonious trees constrained to show a nonmonophyletic Phrynosomatinae from analysis of the morphological data. (1, ((((2, 3), (((((5, 13), (10, 9)), (11, 12)), 6), ((7, 8), (4, (15, 14))))), (16, 17)), ((((19, 21), 22), 18), 20))).

F. Representative tree (148 steps, CI 0.493) of the 18 equally most-parsimonious trees constrained to show a nonmonophyletic Iguaninae from analysis of the morphological data. (1, (((((((2, 3), 6), (7, 8)), 4), 15), 14), ((((5, 13), (10, 9)), (11, 12)), ((16, 17), ((((19, 21), 22), 18), 20))))).

G. Shortest tree (4544 steps, CI 0.384) constrained to show a nonmonophyletic Crotaphytinae from analysis of the DNA-sequence data. (1, ((2, 3), (((((((5, 6), ((16, 17), ((19, (20, (22, 21))), 18))), (((11, 12), 13), (15, 14))), (10, 9)), 7), 8), 4))).

H. Shortest tree (4525 steps, CI 0.386) constrained to show a nonmonophyletic Phrynosomatinae from analysis of the DNA-sequence data. (1, ((2, 3), ((((((5, 6), (((7, 8), (10, 9)), (((11, 12), 13), (15, 14)))), ((20, 22), (18, 21))), (16, 17)), 19), 4))).

I. Shortest tree (4510 steps, CI 0.387) constrained to show a nonmonophyletic Iguaninae from analysis of the DNA-sequence data. (1, ((2, 3), (((((5, 6), ((((11, 12), 13), 14), 15)), (10, 9)), ((7, 8), 4)), ((((16, 17), (18, 21)), (20, 22)), 19)))).

J. Shortest tree (4514 steps, CI 0.386) constrained to show a monophyletic Tropidurinae from analysis of the DNA-sequence data. (1, ((2, 3), (((5, 6), ((((7, 8), ((11, 12), (10, 9))), (13, (15, 14))), ((16, 17), ((19, (20, (22, 21))), 18)))), 4))).

K. Shortest tree (4711 steps, CI 0.386) constrained to show a nonmonophyletic Crotaphytinae from analysis of the combined data sets. (1, ((2, 3), (((((((5, 4), 6), ((16, 17), ((19, (20, (22, 21))), 18))), (((11, 12), 13), (15, 14))), (10, 9)), 7), 8))).

L. Three shortest trees (4693 steps, CI 0.387) constrained to show a nonmonophyletic Phrynosomatinae from analysis of the combined data sets. L-1, (1, ((2, 3), (((((5, 4), 6), (((7, 8), (10, 9)), (((11, 12), 13), (15, 14)))), (16, 17)), ((19, 18), (20, (22, 21)))))). L-2, (1, ((2, 3), (((((5, 4), 6), (((7, 8), (10, 9)), (((11, 12), 13), (15, 14)))), ((19, 18), (20, (22, 21)))), (16, 17)))). L-3, (1, ((2, 3), ((((((5, 4), 6), (((7, 8), (10, 9)), (13, (15, 14)))), (11, 12)), (((16, 17), (19, 18)), (22, 21))), 20))).

M. Two shortest trees (4678 steps, CI 0.388) constrained to show a nonmonophyletic Iguaninae from analysis of the combined data sets. M-1, (1, ((2, 3), ((5, 6), (((7, 8), ((((((11, 12), 13), 14), 15), (10, 9)), ((16, 17), ((19, 18), (20, (22, 21)))))), 4)))). M-2, (1, ((2, 3), (((((((5, 4), 6), 14), 15), ((7, 8), ((11, 12), ((16, 17), ((19, 18), (20, (22, 21)))))))), (10, 9)), 13))).

N. Two shortest trees (4676 steps, CI 0.389) constrained to show a monophyletic Tropidurinae from analysis of the combined data sets. N-1, (1, ((2, 3), ((5, 6), (((7, 8), ((((11, 12), (10, 9)), (13, (15, 14))), ((16, 17), ((19, (20, 22)), (18, 21))))), 4)))). N-2, (1, ((2, 3), (((((5, 6), ((((11, 12), (10, 9)), (15, 14)), 13)), ((16, 17), ((19, 18), (20, (22, 21))))), (7, 8)), 4))).

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