

Phylogenetic Analysis of the Horned Lizards (Phrynosomatidae: *Phrynosoma*): Evidence from Mitochondrial DNA and Morphology

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The phylogenetic relationships among all but one horned lizard species were inferred from mitochondrial ribosomal rRNA gene sequences (251 bp of 12S and 457 bp of 16S) and morphology (32 informative characters). Phylogenies were reconstructed based on separate and combined analyses using parsimony and maximum-likelihood methods. The separate mtDNA and morphological hypotheses were largely incongruent. Bootstrap analyses suggested that most of the incongruence was the result of weak support (bootstraps <70%) for alternative relationships (e.g., placement of *Phrynosoma asio*). However, there were strongly supported alternative placements of *Phrynosoma ditmarsii*. Mitochondrial DNA strongly placed *P. ditmarsii* with the short-horned lizards (i.e., *Phrynosoma orbiculare* and *Phrynosoma douglasi sensu lato*), whereas morphology strongly supported its exclusion from the large clade containing the short-horned lizards and placed *P. ditmarsii* with the southern *Phrynosoma braconieri* + *Phrynosoma taurus*. Various consensus methods for assessing topological congruence (= taxonomic congruence) between the separate phylogenies indicate little congruence (i.e., strict consensus) or misleading congruence (i.e., Adam's consensus). Also, although bootstrap analyses suggested strong incongruence only involving the placement of *P. ditmarsii*, the Wilcoxon signed-ranks test suggests the incongruence between datasets is significant, even when *P. ditmarsii* is pruned from the trees. Combined analysis of the mtDNA and morphological data resulted in a single most-parsimonious tree. All resolved clades, except two, were also discovered in the separate analyses of mtDNA or morphology.

THE horned lizard genus *Phrynosoma* occurs from southern Canada to Guatemala but reach their highest diversity in the southwestern United States and Mexico. Because of their unique morphology and geographic proximity to North American biologists, horned lizards have generated much interest, and many aspects of their biology and natural history have been studied, such as behavior (Lynn, 1965), morphology/osteology (Presch, 1969; Montanucci, 1987, 1989a,b), physiology (e.g., Schmidt et al., 1989; Sherbrooke, 1997), diet and ecology (e.g., Pianka and Parker, 1975), reproduction (Howard, 1974; Zamudio and Parra-Olea, 2000), and evolution of sexual size dimorphism (Zamudio, 1998).

To date, three hypotheses have been proposed for the interrelationships among the species of *Phrynosoma* (Fig. 1) (Reeve, 1952; Presch, 1969; Montanucci, 1987). The evolutionary hypothesis proposed by Reeve (1952; Fig. 1A) was largely intuitive but represented the first com-

prehensive study. Like Reeve (1952), Presch (1969) used osteological data to hypothesize relationships (Fig. 1B) but based his decisions on presumed shared-derived characters. Although Reeve (1952) and Presch (1969) disagreed on several aspects of *Phrynosoma* phylogeny, they both hypothesized a short-horned lizard group (*Phrynosoma ditmarsii*, *Phrynosoma douglasi*, and *Phrynosoma orbiculare*), a group recently supported by Zamudio et al., (1997) and a southern Mexican Plateau group (*Phrynosoma braconieri* and *Phrynosoma taurus*).

Using scalation and osteology, Montanucci (1987) provided the most recent and rigorous comprehensive phylogenetic study of *Phrynosoma*, and his results largely refuted the hypotheses of the previous two studies. The preferred phylogenetic hypothesis presented by Montanucci depicted a fully resolved phylogeny (Fig. 1C). However, to fully resolve the relationships among the species, Montanucci performed two separate analyses. In the first analysis, the phy-

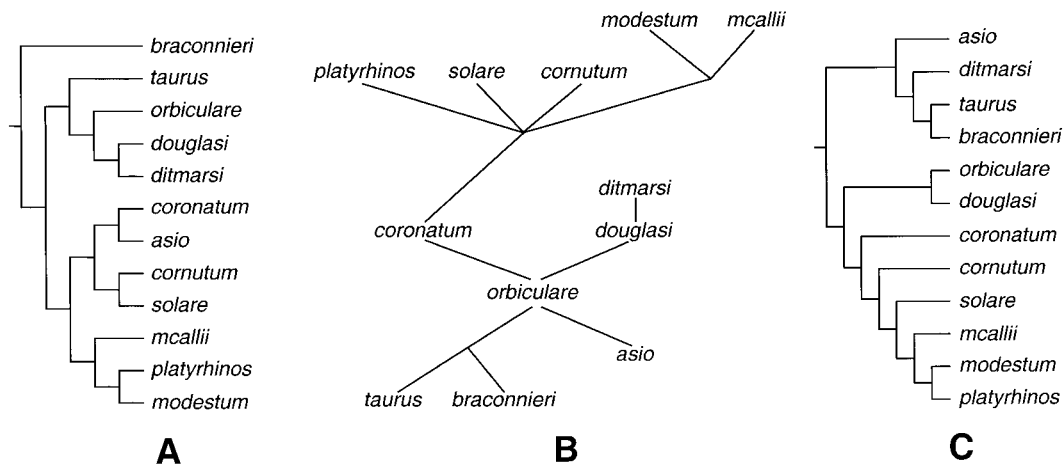


Fig. 1. Previous phylogenetic hypotheses for *Phrynosoma*. (A) Modified from Reeve (1952). (B) Modified from Presch (1969). (C). Preferred hypothesis of Montanucci (1987).

lognetic relationships were completely resolved, except for one polytomy involving four species (*Phrynosoma mcallii*, *Phrynosoma modestum*, *Phrynosoma platyrhinos*, and *Phrynosoma solare*). A second analysis was conducted using a subset of the original data that was relevant to the resolution of relationships among these four species, as well as six new characters. The polarity of the new characters was based on the relationships of the remaining *Phrynosoma* species and the more distant outgroups used in the original analysis. Even though the phylogenetic relationships could be completely resolved, some nodes were supported by only one to three unambiguous characters state changes. Some of the relationships that were weakly supported were those used to polarize the new characters in the second analysis; thus, some of the polarity decisions may be suspect.

Because some of the hypothesized clades of *Phrynosoma* are weakly supported, additional characters are needed to resolve the phylogenetic relationships within this clade of lizards. In this study, the existing morphological data will be augmented with mitochondrial DNA sequence data and a few new morphological characters, and the two datasets will be analyzed separately and in combination. We will demonstrate the consequences of using different methods of assessing incongruence between subsets or partitions of the total data. The hypothesis inferred from the combined analysis will be taken as the best estimate of phylogeny.

MATERIALS AND METHODS

Molecular data.—The molecular data consist of DNA sequences from the 12S and 16S ribosomal

RNA (rRNA) genes, collected from a total of 11 *Phrynosoma* species (see Material Examined). Sequence data were collected following the methods of DNA isolation, amplification, and sequencing described in Reeder (1995).

The mitochondrial rDNA sequences were initially aligned under varying gap costs using the program Clustal W (Thompson et al., 1994). The initial pairwise alignment parameters for guide tree estimation were held constant during all alignments. The main multiple alignment parameter that was varied between alignments was the Gap Opening Penalty (6, 8, 10, and 12). All other multiple alignment parameters were held constant. Additional details on the procedures, parameters, and use of rRNA secondary structure are described in Wiens and Reeder (1997). Regions of sequence were considered alignment-ambiguous if nucleotide positional homologies differed among the different gap cost alignments (Gatesy et al., 1993). These ambiguously aligned regions were excluded from phylogenetic analysis.

Most nucleotide sites were alignment-invariant for the various gap costs. In all, 708 nucleotide positions were aligned (251 12S and 457 16S), with only 37 positions (four 12S and 33 16S) excluded from phylogenetic analysis. All DNA sequences are deposited in GenBank (accession numbers L40437–38, L40440, L40444–49, L40457, L41418, L41420, L41434, L41436–38, L41441–42, L41445, L41450–55, L41464, L41468, L41470, L41485, L41487–89, AF346839–52). The PAUP* matrix (including mtDNA and morphological data) is available upon request (from TWR) and/or can be downloaded from the following Web site:

http://www.bio.sdsu.edu/tod/data_sets/phrynosoma.matrix.

Morphological data.—Over 700 specimens (alcohol-preserved, cleared-and-stained skeletons, and dried skeletons) were examined, representing *Phrynosoma* and outgroup taxa. A wide range of phylogenetically informative morphological characters were derived from the squamation, osteology, and life history of *Phrynosoma*. A list of the specimens examined is reported in Montanucci (1987).

All multistate characters were treated as ordered (for justification, see Campbell and Frost, 1993), unless otherwise noted in the character description. For most of the multistate characters that were unordered, it was possible to determine the plesiomorphic condition, thus polarizing the unordered transformation series. Characters that could not be polarized are noted in the character descriptions. The 32 morphological characters are listed in the Appendix.

Phylogenetic analysis.—All extant species of *Phrynosoma*, except *P. douglasi* sensu stricto, were represented in our study. Zamudio et al. (1997) recently split *P. douglasi* sensu lato into two separate species, *P. douglasi* sensu stricto and *Phrynosoma hernandesi*. *Phrynosoma cerroense* was not included because recent studies (Brattstrom, 1997; Grismer and Mellink, 1994) support the placement of this taxon in the synonymy of *P. coronatum*. Because of the lack of tissue samples, *P. braconnieri* was coded only for the morphological data. However, this species was still included in the combined phylogenetic analysis (following Wiens and Reeder, 1995).

Character polarity was determined by using the outgroup method (Wiley, 1981). The phylogenetic placement of *Phrynosoma* within the Phrynosomatidae is well established, as are phrynosomatid intergeneric relationships (Reeder and Wiens, 1996). The first outgroup to *Phrynosoma* is the sand lizard clade (*Uma* (*Calisaurus* (*Cophosaurus* + *Holbrookia*))), and the second outgroup is the *Sceloporus* group (*Uta* (*Petrosaurus* (*Sceloporus* + *Urosaurus*))). The algorithm of Maddison et al. (1984) was used to reconstruct a hypothetical ancestor, which was used to root the ingroup (= *Phrynosoma*) tree(s).

Phylogenetic analyses were performed with PAUP* 4.0b2. The branch-and-bound algorithm was used in parsimony analyses and the heuristic algorithm was used in likelihood analyses. When multiple shortest trees were discovered, the trees were summarized with a strict consen-

sus tree (Sokal and Rohlf, 1981). A character state change was considered to support a clade unambiguously if it was placed along a branch by both ACCTRAN (Farris, 1970) and DELTRAN (Swofford and Maddison, 1987) optimizations.

Each of the separate datasets and the combined dataset were examined using the g_1 -statistic (Hillis, 1991; Hillis and Huelsenbeck, 1992; Huelsenbeck, 1991) to test for levels of character covariation significantly greater than expected for random data. Critical values for random data were extrapolated from tables 1 and 2 of Hillis and Huelsenbeck (1992). The g_1 -values for the datasets were estimated by examining the tree length frequency distribution of 10,000 randomly generated trees (as implemented in PAUP*). Support for individual clades was assessed by nonparametric bootstrapping (Felsenstein, 1985a). Parsimony bootstrap analyses were based on 500 branch-and-bound searches. Maximum-likelihood bootstrap analyses were based on 100 heuristic searches (TBR branch swapping with two random taxon addition replicates/pseudoreplicate). Clades with bootstrap values of $\geq 70\%$ were considered strongly supported, following Hillis and Bull (1993; but see their caveats).

The DNA and morphological datasets were analyzed separately and then combined for phylogenetic analysis. When comparing the hypotheses resulting from the separate analyses (= taxonomic congruence approach; Mickevich, 1978), three different consensus techniques were used: (1) strict (Sokal and Rohlf, 1981); (2) Adams (Adams, 1972); and (3) agreement subtrees (Swofford, 1991). Incongruence between the morphological and mtDNA phylogenies was also examined with the Wilcoxon signed-ranks test (Felsenstein, 1985b; Larson, 1998; Templeton, 1983), as implemented in PAUP*. For the Wilcoxon signed-ranks tests, the generally more conservative two-tailed test was used (Felsenstein, 1985b). The examination and testing of phylogenies inferred from the separate analyses of the mtDNA and morphological data allows an assessment of the degree of incongruence exhibited by these subsets of the total data. However, because it uses the maximum number of characters and phylogenetic accuracy generally increases with increasing number of characters (Wiens and Chippindale, 1994), the hypothesis resulting from the combined phylogenetic analysis was chosen as the best estimate of phylogeny for *Phrynosoma* (Kluge, 1989).

A slight transition bias exists in these DNA sequences (ti:tv = 3:1; estimated via maximum-

likelihood analysis); thus, corroborating a previous assessment of transition bias in phrynosomatid mitochondrial rDNA sequences (Reeder, 1995). Reeder (1995) also demonstrated that transitions were as informative as transversions in phrynosomatid mitochondrial rDNA. Therefore, differential weighting between these two classes of nucleotide substitutions was not performed in the parsimony analyses. Although more complex and/or realistic a priori weighting schemes could be devised for the DNA data (e.g., Arevalo et al., 1994; Cunningham, 1997), philosophical and methodological difficulties arise within the context of a combined phylogenetic analysis. Thus, the application of uniformly weighted parsimony facilitated the comparison and combination of the mtDNA and morphological datasets.

When uniformly weighted parsimony analyses are performed, any incongruence between the separate mtDNA and morphological hypotheses may be an artifact of inappropriately weighted data (e.g., assuming transitions = transversion). Therefore, to further assess congruence, maximum likelihood (ML; implemented via PAUP*) was used to analyze the mtDNA, and the resulting phylogeny was compared to the morphological hypothesis. The likelihood approach was chosen because it provides a more objective way of estimating and choosing character weights (Felsenstein, 1981) and incorporates important aspects of molecular evolution that are difficult to implement in parsimony analyses (e.g., among-site rate variation, unequal base frequencies). Also, it has been demonstrated that likelihood is a consistent and efficient estimator of phylogenies under a variety of simulated conditions and is robust to perturbations of model and model parameters (Huelsenbeck, 1995; Yang, 1996). The preferred maximum-likelihood phylogeny was estimated following a successive approach similar to that described by Wilgenbusch and de Queiroz (2000) and Wiens et al. (1999), except many more models were evaluated and tested using Modeltest 3.0 (Posada and Crandall, 1998). For the most general model of sequence evolution (GTR + I + Γ), model parameters and likelihood scores were estimated (using PAUP*) for each tree from a pool of parsimony trees (the most-parsimonious DNA tree and those one step longer). The tree with the best likelihood score was selected to evaluate the other less general models (using Modeltest). In all, 56 different models of sequence evolution were tested using the likelihood ratio test statistic, as described in Huelsenbeck and Crandall (1997). The model (and its estimated parameters) that best fit the data was

used in a Heuristic ML tree search (50 random taxon addition replicates with TBR branch swapping). The test of Kishino and Hasegawa (1989) was used to evaluate the statistical significance of the best ML DNA phylogeny relative to alternative phylogenies (i.e., parsimony morphology and DNA trees).

RESULTS

Morphological data.—Analysis of the lengths of 10,000 randomly sampled trees resulted in a g_1 -index of -0.652 for the morphological data (outgroup removed). The critical g_1 -values for random data for 10 taxa for a two-state character for 10 characters is -0.44 ($P = 0.05$) or -0.59 ($P = 0.01$).

Phylogenetic analysis of the 31 phylogenetically informative morphological characters resulted in two shortest trees [length (L) = 163] with a CI of 0.60 and RI of 0.71. The strict consensus of these two trees is shown in Figure 2A. The only area of ambiguity is found in the clade containing *Phrynosoma mcallii*, *P. modestum*, *P. platyrhinos*, and *P. solare* (Fig. 2B). Although several of Montanucci's original characters were recoded, the strict consensus tree (of the three trees inferred in this study) is still consistent with the preferred phylogenetic hypothesis of Montanucci (1987; Fig. 1). However, the specific placement of *P. mcallii* and *P. solare* by Montanucci [1987; i.e., (*P. solare*, (*P. mcallii*, (*P. modestum* + *P. platyrhinos*)))] is not congruent with either of the alternative equally parsimonious arrangements supported in this new analysis. The numbers of unambiguous synapomorphies supporting the branches of the phylogeny are given in Figure 2.

Five of the 10 inferred clades (not counting the ingroup node) are strongly supported (bootstrap proportions $\geq 70\%$). Of the two basal *Phrynosoma* clades, only Clade E is strongly supported by the morphological data (four synapomorphies; bootstrap = 77%). The other basal clade (Clade B) is also supported by four synapomorphies, but this clade was supported only in 61% of the bootstrap replicates. However, within this clade, there is strong support (97%) for the *P. braconieri* + *P. taurus* clade.

Within Clade E, three synapomorphies support a *Phrynosoma hernandesi* + *P. orbiculare* clade and four synapomorphies support its placement as the sister taxon of the remaining species (Clade G). Both relationships are strongly supported in the bootstrap analysis (95% and 77%, respectively). The basal placement of *P. coronatum* within Clade G is strongly supported, as is the basal placement of *P. cornutum* within Clade

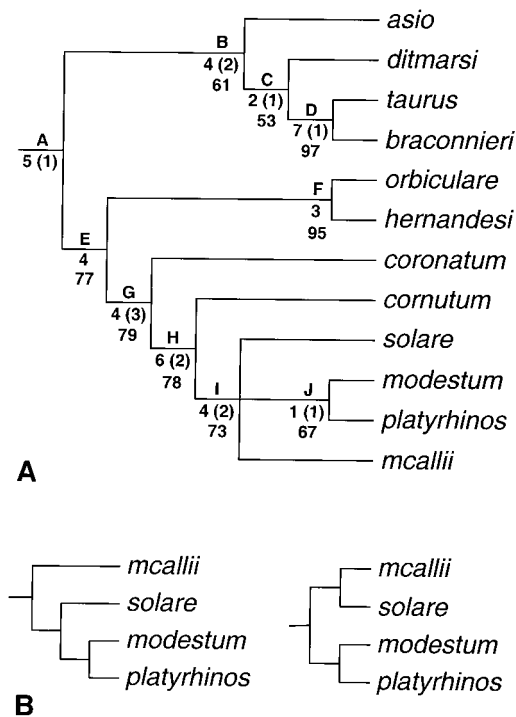


Fig. 2. (A) Strict consensus of two equally parsimonious shortest phylogenies (L = 164, CI = 0.60, RI = 0.71) inferred from the morphological data. Letters above the branches denote different clades of the strict consensus tree. The first set of numbers below the branches are the number of unambiguously placed morphological synapomorphies (those shared by clades in both equally parsimonious trees), with the number of unique (nonhomoplastic) synapomorphies given in parentheses. Numbers below the synapomorphies are bootstrap values. Branches without bootstrap values were supported in < 50% of the replicates. (B) The two equally parsimonious hypotheses of relationships within Clade I.

H. Clade I (containing *P. mcallii*, *P. modestum*, *P. platyrhinos*, and *P. solare*) is strongly supported (73%), but the interrelationships within Clade I are ambiguous and/or weakly supported (bootstrap < 70%).

DNA data.—The g_1 -value for the mtDNA data is -0.694. The critical g_1 -values for random data for 10 taxa for a four-state character for 50 characters is -0.28 ($P = 0.05$) or -0.36 ($P = 0.01$).

Phylogenetic analysis of the 66 parsimony informative DNA characters resulted in a single shortest tree (Fig. 3A; L = 486) with a CI of 0.52 and RI of 0.44. The numbers of unambiguous synapomorphies supporting the branches of the phylogeny are given in Figure 3A.

Only two of the nine clades (not counting the

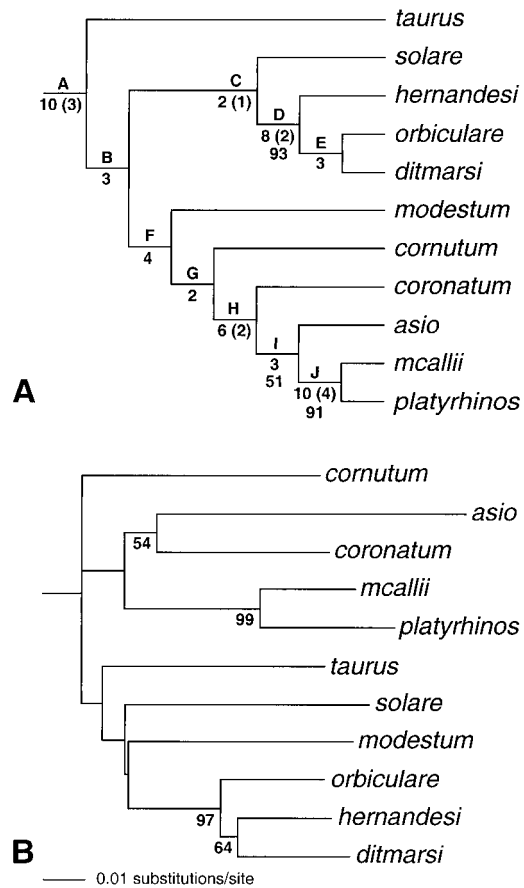


Fig. 3. Phylogenies based on mitochondrial 12S and 16S rDNA data. (A) Single shortest parsimony phylogeny (L = 486, CI = 0.52, RI = 0.44) inferred from the mtDNA data. Letters above the branches denote different clades. The first set of numbers below the branches are the number of unambiguously placed DNA synapomorphies, with the number of unique (nonhomoplastic) synapomorphies given in parentheses. Numbers below the synapomorphies are bootstrap values. Branches without bootstrap values were supported in < 50% of the replicates. (B) One of the two best likelihood phylogenies (-Ln = 2062.1017). Numbers below the branches are bootstrap values. Branches without bootstrap values were supported in < 50% of the replicates.

ingroup node) are strongly supported by the DNA data (bootstrap proportions $\geq 70\%$). Three synapomorphies support the placement of *P. taurus* as the sister taxon of all remaining *Phrynosoma*. However, this relationship is weakly supported (bootstrap < 50%). The monophyly of the short-horned lizards (Clade D) is strongly supported (bootstrap = 93%) by eight DNA synapomorphies. The only other strongly supported relationship is a clade containing *P. mcallii* and *P. platyrhinos* (Clade J; 91%). This clade

TABLE 1. MAXIMUM-LIKELIHOOD PARAMETER ESTIMATES AND SUBSTITUTION RATES FOR THE MITOCHONDRIAL 12S AND 16S rDNA.

Substitution rates			Site rate parameters		Base frequencies			
A↔G	C↔T	ivs ^a	I	Γ	A	C	G	T
3.444	10.245	1.0	0.657	0.675	0.361	0.228	0.177	0.233

^a All transversions assumed to have the same rate.

is supported by 10 synapomorphies, four of which are unique.

When testing the different models of sequence evolution, the TrN + I + Γ model (= three nucleotide substitution parameters, some sites assumed to be invariable, variable sites following a gamma distribution, and nucleotide base frequencies were estimated; parameters given in Table 1) was determined to be the model that best explained the evolution of the mitochondrial rDNA data. The maximum-likelihood tree search using the TrN + I + Γ model yielded two best trees ($-\ln = 2062.1017$, Fig. 3B). The two tree topologies were identical, except for slight differences in branch length. Two of the nine ingroup clades are considered strongly supported by ML analysis of the DNA data. These well-supported groups (i.e., *P. mcal-*

lii + *P. platyrhinos* and the short-horned lizards) represent the same two strongly supported clades from the parsimony analysis. All other inferred relationships are supported by bootstrap values < 70%.

Incongruence between morphological and DNA data.—The two morphological trees (with *P. braconnieri* pruned) and the single parsimony DNA tree were assessed for taxonomic congruence using three different consensus techniques. The strict consensus approach resulted in a completely unresolved tree (not shown). Such an hypothesis indicates that no clades are shared between the separate parsimony hypotheses. Comparison of the morphological trees and the maximum-likelihood DNA tree also resulted in a completely unresolved tree (not shown). Even the parsimony and likelihood DNA hypotheses were largely incongruent, with only the three following clades being shared: (1) *P. mcallii* and *P. platyrhinos*; (2) *P. coronatum*, *P. asio*, *P. mcallii*, and *P. platyrhinos*; and (3) *P. orbiculare*, *P. hernandesi*, and *P. ditmarsii*.

Unlike the strict consensus approach, the Adams consensus technique (applied to the separate parsimony trees) generates a consensus tree with six resolved clades (Fig. 4A). The Adams consensus tree depicts a trichotomy at the base of the tree ((*P. taurus*) (*P. asio* + *P. ditmarsii*)) (remaining *Phrynosoma* species)). The relatively basal position of *P. taurus* is congruent with both the morphological and parsimony mtDNA hypotheses, whereas other relationships are more characteristic of either the morphological trees (e.g., *P. hernandesi* + *P. orbiculare*) or the mtDNA tree (e.g., *P. mcallii* + *P. platyrhinos*). Application of the agreement subtrees consensus approach (applied to the separate parsimony trees) resulted in two completely resolved subtrees for six *Phrynosoma* species (Fig. 4B), with *P. taurus* placed as the sister species to the remaining five species in each. The two subtrees are identical, except that either *P. cornutum* or *P. coronatum* may be placed as the sister species to the *P. mcallii* + *P. platyrhinos* clade.

Although the morphological and mtDNA hypotheses (parsimony and likelihood) are largely

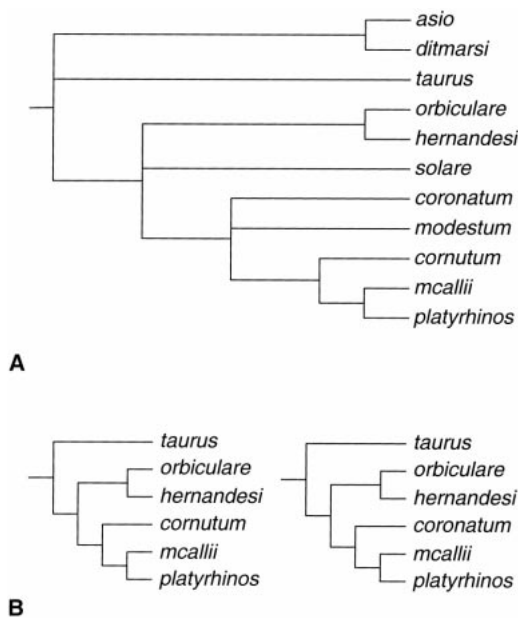


Fig. 4. (A) Adams consensus tree based on the fundamental phylogenies inferred in the separate parsimony analyses of the morphological and mtDNA data. (B) Two largest agreement subtrees based on the fundamental phylogenies inferred in the separate parsimony analyses of the morphological and mtDNA data.

TABLE 2. RESULTS OF THE WILCOXON SIGNED-RANKS TESTS USED TO EVALUATE INCONGRUENCE BETWEEN THE MORPHOLOGICAL AND MITOCHONDRIAL DNA DATASETS.

Alternative hypotheses tested ^a	N ^b	P ^c
1. Morphology on parsimony DNA tree	23	0.0012*
2. Morphology on ML DNA tree	24	0.0009*
3. Morphology on DNA tree (w/o <i>P. ditmarsis</i>)	21	0.0029*
4. Morphology on combined tree	14	0.1796 n.s.
5. DNA on morphology trees ^d	33 or 34	<0.0001*
6. DNA on morphology trees (w/o <i>P. ditmarsis</i>) ^d	23 or 24	0.0004*
7. DNA on ML DNA tree	18	0.1266 n.s.
8. DNA on combined tree	14	0.0129*

^a Because of the conflicting strong bootstrap support for the alternative placements of *Phrynosoma ditmarsis* in the separate analyses, alternative hypotheses with *P. ditmarsis* pruned from the trees were also tested.

^b Number of characters differing in minimum numbers of steps on paired topologies.

^c Significant differences between shortest tree and an alternative tree(s) are denoted by an asterisk. A significant result indicates that the alternative tree(s) is rejected by the test data.

^d Multiple values are given because there are two equally parsimonious morphological trees.

incongruent, there is little strongly supported conflict between the trees when one compares the bootstrap values for the competing clades. All but one of the competing relationships are either weakly supported by both datasets or only strongly supported in one of the separate analyses. The only strongly supported conflict between the separate phylogenetic hypotheses involves the placement of *P. ditmarsis*. In both mtDNA phylogenies, *P. ditmarsis* is strongly

placed with *P. hernandesi* and *P. orbiculare* (Fig. 3). However, the morphological data strongly support the exclusion of *P. ditmarsis* from the large monophyletic group (Clade E; Fig. 2A) that includes *P. hernandesi* and *P. orbiculare*, as well as the majority of *Phrynosoma* species.

When the separate mtDNA and morphological phylogenies were tested against their respective alternative hypotheses (mtDNA data on the morphological hypothesis and vice versa), in all cases the alternative hypotheses were rejected in favor of the preferred tree(s) inferred from their own respective dataset (Table 2). These results of the Wilcoxon signed-ranks tests imply that significant incongruence exists between the mtDNA and morphological data. Finally, when the two DNA hypotheses (parsimony vs likelihood) were tested against each other, neither the Wilcoxon signed-ranks test (i.e., DNA on ML tree) nor the Kishino-Hasegawa test (i.e., DNA on parsimony tree) could reject the alternative hypothesis.

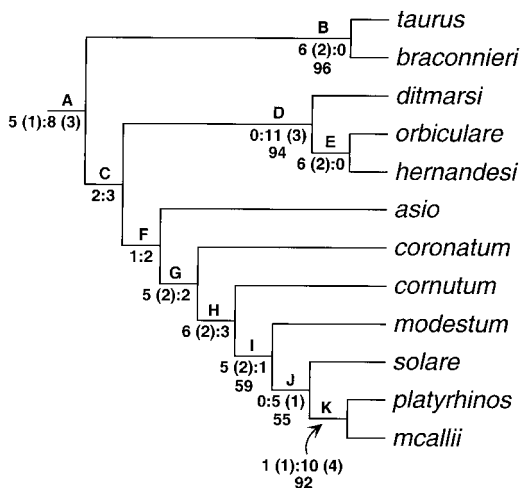


Fig. 5. Single shortest phylogeny (L = 708, CI = 0.50, RI = 0.47) inferred from the combined morphological and mtDNA data. Letters above the branches denote different clades. The first set of numbers below the branches are the number of unambiguously placed morphological and DNA synapomorphies (morphology:DNA), with the number of unique (nonhomoplastic) synapomorphies given in parentheses. Numbers below the synapomorphies are bootstrap values. Branches without bootstrap values were supported in < 50% of the replicates.

Combined analysis.—The combined data have a g_1 -value of -0.628 . The critical g_1 -values for random data for 10 taxa for a two-state character for 50 characters is -0.35 ($P = 0.05$) or -0.39 ($P = 0.01$). The combined morphological and DNA data included 98 phylogenetically informative characters. Parsimony analysis of these data resulted in a single shortest tree (L = 692; Fig. 5) with a CI of 0.50 and RI of 0.47.

The numbers of unambiguous synapomorphies supporting the branches of the phylogeny are given in Figure 5. Seven of the 11 clades are supported by unambiguously placed morphological and DNA synapomorphies. Two clades (Clades B and E) are supported by only morphological synapomorphies. However, the lack

of DNA data for *P. braconnieri* prevents the unambiguous placement of DNA synapomorphies for Clade B. Two clades (Clades D and J) are supported only by DNA synapomorphies.

Clade B (*P. braconnieri* + *P. taurus*) is supported by six synapomorphies (6 morphological:0 DNA; this convention followed throughout, only morphological characters listed): 2.2 (9–12 caudal vertebrae), 8.3 (jugal posterior border is flat), 13.1 (maxilla nasal process separated from nasal), 14.1 (enlarged nuchal scale row), 23.2 (surangular surface expanded with an angular edge) and 28.1 (coronoid overlap of dentary reduced). Five synapomorphies (2:3) support Clade C: postrectal present (29.1) external naris on canthal line (17.1 or 2). Clade C is divided into two clades, one containing the short-horned lizards (Clade D) and the other containing *P. asio* and the remaining northern species (Clade F). Clade D is supported by 11 DNA synapomorphies but no unambiguously placed morphological characters. The placement of *P. ditmarsii* as the sister species of the *P. hernandesi* + *P. orbicularis* clade (Clade E) is supported by six morphological synapomorphies: enlarged dorsals tapered and spinose (5.2), enlarged gulars absent (7.0), posterior border of jugal slopes posteriorly (8.1), enlarged nuchal scale row (14.1), enlarged triangular postlabials (16.2), and slanted postrectal (18.1). Clade F is supported by three synapomorphies (1:3): lower abdominal row of fringe scales extensive (12.2).

Within Clade F, *P. asio* is supported as the sister species of Clade G by seven synapomorphies (5:2): 3.4 (chinshield row margin strongly peaked), 5.2 (enlarged dorsals tapered and spinose), 7g.1 (horizontally projecting enlarged gulars), 8.2 (posterior border of jugal slopes anteriorly), and 22.1 (dentate supralabial margin). Clade H is supported by nine synapomorphies (6:3): 4.1 (protuberances or horns on angular), 13.1 (maxilla nasal process separated from nasal), 17.0 (external naris above canthal line), 20.1 (rostrifrontal angle acute), 23.1 (laterally directed horns on surangular), and 29.0 (postrectal absent). Six synapomorphies (5:1) support Clade I: 5.1 (enlarged dorsals flat), 12.0 (lower abdominal row of fringe scales absent), 15.1 (enlarged projecting parietal scale present), 19.1 (rostral lip present), and 28.1 (dentary/surangular overlap reduced). The exclusion of *P. modestum* from Clade J is supported by five DNA synapomorphies. *Phrynosoma mcallii* and *P. platyrhinos* are supported as sister species (= Clade K) by 11 synapomorphies: 16.1 (postlabials not enlarged).

Bootstrap values are shown in Figure 5. In the

combined phylogenetic analysis, three of the 10 inferred clades (not counting the ingroup node) are strongly supported (bootstraps $\geq 70\%$). These are as follows: Clade B (*P. braconnieri* + *P. taurus*; 96%), Clade D (*P. ditmarsii* + *P. hernandesi* + *P. orbicularis*; 94%), and Clade K (*P. mcallii* + *P. platyrhinos*; 92%). The clade containing *P. modestum*, *P. solare*, *P. mcallii*, and *P. platyrhinos* (= Clade I) is moderately supported (59%). All remaining clades in this analysis are weakly supported.

DISCUSSION

Phrynosoma phylogeny.—Phylogenetic analysis of the combined morphological and mtDNA data resulted in a completely resolved hypothesis of relationships for *Phrynosoma* (Fig. 5). The combined phylogeny is incongruent with each of the phylogenetic hypotheses inferred from the morphological and mtDNA data alone. The discovery of unique phylogenies is often the result when separate datasets are combined for phylogenetic inference (Chippindale and Wiens, 1994). Thus, our combined analysis has discovered relationships within *Phrynosoma* that would not have been found had the morphological and DNA data only been analyzed separately (Barrett et al., 1991).

Although the combined phylogeny is incongruent with the separate morphological and mtDNA hypotheses, most of the individual clades of this phylogeny are supported in either the morphological or mtDNA phylogeny. Overall, the combined phylogeny is more similar to the morphological phylogeny than to the mtDNA phylogeny (4 vs 3 clades; ingroup node and *P. braconnieri* + *P. taurus* clade not included). This is also reflected in Wilcoxon signed-ranks tests where the morphological data did not reject the combined phylogeny as a significantly worse explanation of the data (Table 1), whereas the DNA data did reject the combined phylogeny (Table 1). Two clades (Clades F and J) in the combined phylogeny are absent from both of the separate analyses. However, both clades are weakly supported (bootstrap values < 70%).

In the combined phylogeny, two clades are supported only by unambiguous morphological synapomorphies (Clades B and E), whereas two additional clades are supported only by unambiguous DNA synapomorphies (Clades D and J). The lack of DNA data for *P. braconnieri* prevents the placement of unambiguously optimized DNA characters for Clade B. However, this clade is still strongly supported by six unambiguous morphological synapomorphies

(bootstrap = 96%), two of which are unique (2.2 and 8.3).

Phylogenetic placement of Phrynosoma ditmarsii.—Based on bootstrap analysis, the only strongly supported incongruence in this study involves *P. ditmarsii* and its relationship to *P. hernandesi* and *P. orbiculare*. The current separate morphological analysis and the original morphological analysis by Montanucci (1987) place *P. ditmarsii* as the sister species of the *P. braconnieri* + *P. taurus* clade. Although this specific placement of *P. ditmarsii* is weakly supported, there is strong morphological support for the exclusion of *P. ditmarsii* from the large *Phrynosoma* clade (Clade E; Fig. 2A) containing *P. hernandesi* and *P. orbiculare*. Alternatively, the separate mtDNA analyses (parsimony and likelihood) strongly support the placement of *P. ditmarsii* in a clade with *P. hernandesi* and *P. orbiculare* (Fig. 3).

Zamudio et al. (1997) used mitochondrial cytochrome *b* and ND4 gene sequences to infer the phylogenetic relationships within the short-horned lizard clade (including *P. orbiculare*, *P. ditmarsii*, and *P. douglasi* sensu lato). Their data strongly support a close relationship between *P. ditmarsii* and *P. hernandesi*. However, the precise placement of *P. ditmarsii* was ambiguous; it was either the sister species of *P. hernandesi* or nested within *P. hernandesi*. These results are largely congruent with our study. Parsimony analysis of our mitochondrial 12S and 16S rDNA data weakly support a sister group relationship between *P. ditmarsii* and *P. orbiculare* (< 50%), whereas likelihood analysis more strongly supports a *P. ditmarsii* + *P. hernandesi* clade (64%).

The short-horned lizard clade is also strongly supported in the combined analysis by 11 DNA synapomorphies (3 unique), but there are no morphological synapomorphies. Although support for this clade in the combined analysis is dictated primarily by the DNA data, there are four ambiguously optimized morphological synapomorphies that may support this monophyletic group [tuberosities on jugal surface (10.2), external naris above canthal line (17.2), viviparity (27.1), and ≤ 10 interfemoral scales (32.1)]. Finally, the present study and Zamudio et al. (1997) are not the first to suggest a close relationship between these taxa. Without comment, Reeve (1952) placed *P. ditmarsii*, *P. douglasi* sensu lato, and *P. orbiculare* into a single species group (= *orbiculare* group). Also, based on geographic distribution and presence of relatively short horns, Presch (1969) hypothesized that *P. ditmarsii* was derived from *P. douglasi* sensu lato.

Phylogenetic placement of Phrynosoma asio.—Within *Phrynosoma*, *P. asio* is the largest species and ranges the furthest south, reaching southwestern Guatemala (Reeve, 1952). The placement of *P. asio*, therefore, has important implications for understanding the evolution of *Phrynosoma*. Unfortunately, the placement of this species varies greatly among the different phylogenetic analyses. *Phrynosoma asio* is relatively basal in the morphology tree and Montanucci (1987) has previously hypothesized that *P. asio* most closely resembles the most recent common ancestor of *Phrynosoma*. However, parsimony analysis of the mtDNA data places *P. asio* as deeply nested in the tree, as the sister species to the *P. mcallii* + *P. platyrhinus* clade. The maximum-likelihood and combined analyses also place *P. asio* with the northern desert species, instead of with the southern radiation (e.g., *P. braconnieri*, *P. taurus*) as suggested by the morphological data. Although the phylogenetic placement of *P. asio* differs according to dataset and analysis, all of the alternatives are weakly supported. Thus, at this point we must conclude that the phylogenetic placement of *P. asio* is still uncertain.

Implications of different consensus approaches.—When attempting to assess levels of taxonomic congruence among differing phylogenetic hypotheses, one is left with the option of choosing among several consensus techniques (Kluge, 1989). When there is little incongruence between phylogenies, the various consensus approaches will generally yield similar conclusions. However, in cases of greater degrees of incongruence, different consensus techniques may result in quite different conclusions. In such situations, some techniques may underestimate or obscure the levels of congruence, whereas others may be misleading. Both of these undesirable outcomes are evident in this phylogenetic study of *Phrynosoma*.

Strict consensus is the most commonly used consensus technique for evaluating taxonomic congruence among different phylogenetic hypotheses. When the strict consensus approach is applied to the separate *Phrynosoma* phylogenies, the result is a completely unresolved consensus tree. The absence of shared clades implies there is no congruence between the morphological and DNA phylogenetic hypotheses. However, because of the conservative nature of the strict consensus approach, a few unstable taxa (or just one) can result in little or no resolution. In this study, the conflicting (but weak) placements of a few species (e.g., *P. asio*, *P. solare*) obviously contribute to the lack of resolution in the strict consensus tree. Such a result

may obscure other relatively congruent relationships.

A potentially desirable property of the Adams consensus approach is that it identifies unstable taxa and places them at relatively lower positions in the consensus tree, while maintaining the relative relationships among the other taxa higher in the tree (Adams, 1972; Hillis, 1987). In this study, the Adams consensus technique yielded a more resolved tree, indicating there may be more congruence between the morphological and DNA phylogenies than suggested by the strict consensus approach. For example, the Adams consensus tree depicts a clade containing *P. cornutum*, *P. coronatum*, *P. mcallii*, *P. modestum*, and *P. platyrhinos*. In the morphological phylogeny, these species form a clade with one additional species (*P. solare*; Clade G of Fig. 2A), although in the DNA phylogeny, *P. asio* is nested among these five species (Clade F of Fig. 3). Thus, both separate phylogenies suggest these five species are relatively closely related. The two species causing the incongruence and collapse of this clade in the strict consensus tree (i.e., *P. asio* and *P. solare*) are removed and placed at relatively lower levels in the Adams consensus tree. Another instance of congruence not evident in the strict consensus tree involves the relatively close relationship between *P. hernandesi* and *P. orbiculare*.

Although the Adams consensus approach demonstrates some congruence between the morphological and DNA hypotheses, one must still interpret these results with caution. The resolved clades generally indicate relatively close relationships, but the resolved relationships may not be strictly congruent with any of the fundamental phylogenies. In fact, resolved relationships may actually be misleading. For example, *P. asio* and *P. ditmarsii* are depicted as sister species in the Adams consensus tree. Although these two species are members of a small clade (with *P. braconnieri* and *P. taurus*) in the morphological phylogeny, they are only distantly related in the molecular phylogeny.

Like the Adams consensus technique, the agreement subtrees consensus approach also uncovers congruence between the morphological and DNA phylogenies that was obscured in the unresolved strict consensus tree. The agreement subtrees consensus technique demonstrates that there are characters in each dataset that support six species in a clade (i.e., *P. cornutum*, *P. coronatum*, *P. hernandesi*, *P. mcallii*, *P. orbiculare*, and *P. platyrhinos*) to the exclusion of *P. taurus* (Fig. 5B). There are also morphological and DNA characters supporting *P. cornutum*, *P. coronatum*, *P. mcallii*, and *P. platyrhinos* as being

more closely related to each other than any of them are to *P. hernandesi* and *P. orbiculare*. Although a disadvantage of the agreement subtrees technique is that it generally produces consensus trees with fewer taxa than the Adams consensus approach (which generates a consensus tree for all taxa), the relationships depicted in the subtrees are "strict" (= relationships are completely congruent with the fundamental trees). Thus, the resolved relationships are not misleading, as can be the case with Adams consensus trees.

Statistically significant incongruence.—Recently, Wiens (1998) proposed a methodology for evaluating and combining datasets that may have different phylogenetic histories (= exhibit strongly supported incongruence). Wiens advocated that the total data should be divided into logical partitions, which are analyzed separately. Support for individual clades in the separate analyses is assessed by nonparametric bootstrapping, and strongly conflicting clades are identified. The partitions are then combined for phylogenetic analysis, but those areas of the phylogeny that involve strongly supported conflict in the separate analyses are considered weakly supported or unresolved. This approach was informally applied in a previous phylogenetic study of the large clade *Sceloporus* (Wiens and Reeder, 1997), another group of phrynosomatid lizards.

In the present study, the methodology outlined by Wiens (1998) was used to evaluate and analyze the morphological and mtDNA data available for *Phrynosoma*. As previously mentioned, separate analyses of these partitions of the total data reveal a great deal of incongruence when the separate phylogenies are compared to one another using different consensus techniques. Although there is obvious incongruence, bootstrap analysis suggests that the only strongly supported incongruence involves the phylogenetic placement of *P. ditmarsii*.

Wiens (1998) suggested that tests of overall incongruence (e.g., Wilcoxon signed-ranks test, Kishino-Hasegawa test) may not be as useful as methods that attempt to assess individual clade support (e.g., nonparametric bootstrapping). In the present study, the application of the Wilcoxon signed-ranks test implies that the incongruence between datasets is statistically significant (= strong incongruence). However, this test gives no indication of where the incongruence is localized within the competing hypotheses (if in fact it is localized), whereas the bootstrap analyses indicate the strong incongruence involves *P. ditmarsii*. To further investigate the sen-

sivities of these two approaches of assessing incongruence, *P. ditmarsii* was excluded from a new series of separate analyses. With *P. ditmarsii* removed, the bootstrap analyses no longer detect strong incongruence (i.e., no conflicting clades with bootstrap values $\geq 70\%$; results not shown). However, the Wilcoxon signed-ranks test still detects significant incongruence between the datasets (Table 2). It may be possible that the alternate (but weakly supported) placements of other species (i.e., *P. asio* and/or *P. solare*) may be contributing to the overall significant incongruence. Thus, it would appear that the simultaneous application of global incongruence tests and bootstrapping may be useful in evaluating partitions of the total data.

Finally, strong incongruence between the morphological and mtDNA data has been detected. In the case of the mtDNA-based placement of *P. ditmarsii*, the presumably close relationship with *P. hernandesi* (strongly supported by Zamudio et al., 1997) or *P. orbiculare* could be the result of past cytoplasmic capture resulting from past introgressive hybridization (Gyllenstein and Wilson, 1987; for additional examples, see Avise, 1994). The restricted distribution of *P. ditmarsii* and its close geographic proximity to *P. hernandesi* (~19 km apart; Zamudio et al., 1997) may lend support to such an hypothesis. However, the source of the remaining incongruence (as suggested by the analyses excluding *P. ditmarsii*) is unclear. Given the many specialized adaptations within this peculiar group Montanucci, 1987), it is possible that correlated changes in a suite of morphological characters may be leading the morphological hypothesis astray, thus significantly contributing to the incongruence between the morphology and mtDNA. The results of our study suggest that not only should the placement of *P. ditmarsii* be considered weakly supported or tenuous (as would be recommended by Wiens, 1998) but the overall relationships within *Phrynosoma* should be considered uncertain until new data (preferably from unlinked loci) can be applied to this group. However, until such data are collected, we offer a new working hypothesis that comparative biologists may use to explore various evolutionary transformations of interest (e.g., Zamudio and Parra-Olea, 2000).

MATERIAL EXAMINED

Voucher specimens for molecular analyses.—Except for the following, institutional abbreviations follow Leviton et al. (1985): ASU = Appalachian State University, RRM = Richard R. Montanuc-

ci, Clemson University; RWM = Robert W. Murphy, Royal Ontario Museum.

Phrynosoma.—*Phrynosoma asio*: Mexico, no specific locality data, RRM 2499. *Phrynosoma cornutum*: USA, Arizona, Cochise Co., 4.7 mi east of Portal, LSUMZ 48807. *Phrynosoma coronatum*: Mexico, Baja California Sur, 30 miles south La Paz, RRM 2479. *Phrynosoma ditmarsii*: Mexico, Sonora, Rancho La Palma, 26 km east-northeast of Baviacora, RRM 2459. *Phrynosoma hernandesi*: USA, Colorado, Weld Co., near Stoneham (southwestern Weld Co., near Pawnee Natl. Grasslands), Stoneham hogbacks, RRM 2470. *Phrynosoma mcallii*: Mexico, Sonora, San Pedro microondas, ROM 13876. *Phrynosoma modestum*: USA, New Mexico, Hidalgo Co., Peloncillo Mts., 5.6 mi east US 80 on NM 9, LSUMZ 48831. *Phrynosoma orbiculare*: Mexico, no specific locality data, RRM 2480. *Phrynosoma platyrhinus*: USA, CA, San Diego Co., Hwy S-2, 19.6 rd mi northwest of Jct with I-8, ASU 15250. *Phrynosoma solare*: Mexico, Sonora, Bahia Kino, ROM 15044. *Phrynosoma taurus*: Mexico, Puebla, near Zapotitlan Salinas, UTA-R4841, 9092, 11389–90, 11394–96, 19386–89, 19390–400, or 22590–92 (tissue came from one of these individuals, but exact voucher unknown).

Outgroups.—*Callisaurus draconoides*: USA, New Mexico, Hidalgo Co., 12.4 mi north and 0.9 mi west Jct US 80 and Portal Road (NM 533), LSU 48811. *Cophosaurus texanus*: USA, New Mexico, Hidalgo Co., 9.5 mi east Jct US 80 and I-10, LSUMZ 48758. *Holbrookia maculata*: USA, Arizona, Cochise Co., 6.3 mi east Portal, LSUMZ 48805. *Petrosaurus mearnsi*: USA, California, Imperial Co., Mountain Springs, ROM 13743. *Petrosaurus thalassina*: Mexico, Baja California Sur, Arroya Santa Agueda, 4.9 mi west Hwy 1 on road to Ramon a Santa Agueda, RWM 2223. *Sceloporus grammicus*: Mexico, Oaxaca, Sierra Juarez, 12.6 mi south Vista Hermosa (7000 ft), UTA R-23970. *Sceloporus merriami*: USA, Texas, Val Verde Co., Amistad Reservoir, 2.2 mi north Jct US 90 and Spur 349, LSUMZ 48844. *Sceloporus occidentalis*: USA, California, Alpine Co., 0.1 mi south Tamarack Lodge, MVZ 137487. *Urosaurus microscutatus*: Mexico, Baja California Sur, Arroya Santa Agueda, 4.9 mi west Hwy 1 on road to Ramon a Santa Agueda, RWM 2234. *Urosaurus ornatus*: USA, Arizona, Cochise Co., 43.3 mi south NM 533, just off west side of US 80, LSUMZ 48828. *Uta palmeri*: Mexico, no voucher. *Uta stansburiana*: USA, New Mexico, Dona Ana Co., 11.3 mi south Jct NM Co Hwy A14 and Co Hwy A17, LSUMZ 48840.

ACKNOWLEDGMENTS

A Gaige Fund Award (American Society of Ichthyologists and Herpetologists) and a Theodore Roosevelt Award (American Museum of Natural History) supported fieldwork by TWR. Molecular laboratory work by TWR was funded primarily by a grant from Sigma Xi and a National Science Foundation Doctoral Dissertation Research Improvement Grant (BSR-9122823). Additional research support to TWR was provided during his tenure as a Kalbfleisch Research Fellow (American Museum of Natural History) and a Smithsonian Institution Postdoctoral Fellow. We are grateful to D. Hillis, A. Price, and W. Sherbrooke for providing some of the tissues for the molecular portion of this study. We thank K. Burns, D. Kizirian, K. Kozak, A. Leache, P. Chippindale, J. Richmond, J. Wiens, D. Wood, B. Yang, K. Zamudio, and two anonymous reviewers for valuable comments on various versions of this manuscript.

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APPENDIX MORPHOLOGICAL CHARACTERS

Characters 1–26 and 28–31 (and their character states) are described in detail in Montanucci (1987). Only character names are listed here, with details provided only if characters were coded differently than in Montanucci

(1987). Recoding of several of the original Montanucci (1987) characters was an attempt to reduce the number of assumptions in the present study. The additional characters listed (i.e., 27 and 32) are described herein.

(1) *Angular*.—The angular bone is variably present or absent in *Phrynosoma modestum* and absent in *Phrynosoma mcallii*. In Montanucci (1987), this transformation series was ordered and unscaled. In the present study, we scaled this polymorphic transformation series (following Campbell and Frost, 1993). (2) *Caudal vertebrae*. (3) *Chinshields*.—This transformation series was originally analyzed as ordered (Montanucci, 1987). In the present study, we analyzed this complex transformation series as unordered. (4) *Dentary surface*. (5) *Enlarged dorsals in median area*.—This transformation series was originally analyzed as ordered (Montanucci, 1987). In the present study, this complex transformation series is unordered. Montanucci (1987) hypothesized that the “0” condition was plesiomorphic. However, because the observed states in *Phrynosoma* are noncomparable in the outgroups, this transformation series is unpolarized in the present study. (6) *Ectopterygoid*. (7) *Enlarged gular scales*.—This transformation series was originally analyzed as ordered in Montanucci (1987). In the present study, this transformation series is unordered. (8) *Posterior border of jugal*. (9) *Jugal*. (10) *Jugal surface*.—(0) smooth; (1) rugose; (2) with tuberosities and/or processes. Ordered. Character state 2 was further subdivided in Montanucci (1987). (11) *Gular scales*. (12) *Lower abdominal fringe scales*. (13) *Nasal process of maxilla*. (14) *Paravertebral nuchal scale rows enlarged*.—(0) absent; (1) present. Montanucci (1987) coded only *Phrynosoma braconnieri* and *Phrynosoma taurus* as having the apomorphic condition (enlarged paravertebral row of scales) but noted that *Phrynosoma douglassi* sensu lato and *Phrynosoma orbiculare* also possessed enlarged paravertebral rows. However, Montanucci (1987) did not consider the states homologous, with the enlarged scales of the paravertebral rows being separated in *P. douglassi* sensu lato and *P. orbiculare* (juxtaposed in *P. braconnieri* and *P. taurus*) and more spinose. In the present study, the enlarged paravertebral scales in these four species are a priori presumed to be homologues. The resulting phylogeny will provide a final test of homology (congruence test of Patterson, 1982). (15) *Parietal horn*.—(0) enlarged, projecting scale at the base of horn is absent; (1) present; (2) second parietal horn is present. Montanucci (1987) analyzed this transformation series as ordered, assuming the second parietal horn of *Phrynosoma*

solare was a further transformation of the bony projection observed in *Phrynosoma mcallii*, *Phrynosoma modestum*, and *Phrynosoma platyrhinos* (evident externally as an enlarged, projecting scale at the base of the parietal horn). In the present study, we do not make such an assumption and have treated this transformation series as unordered. This character is also unpolarized. (16) *Postlabials*. (17) *External naris placement*.—(0) situated on the canthal line; (1) polymorphic, variable in relation to the canthal line; (2) well above the canthal line. This character was treated as unordered in the phylogenetic analysis. In Montanucci (1987), this transformation series was ordered and unscaled. In the present study, we scaled this polymorphic transformation series. (18) *Postorbital*. (19) *Rostral lip*. (20) *Rostrofrontal angle*.—Montanucci (1987) originally coded *P. solare* as possessing the plesiomorphic condition “0.” In the present study, *P. solare* is coded as possessing the derived condition “1.” (21) *Supraorbital bar*.—(0) incomplete; (1) polymorphic; (2) complete. *P. asio* is polymorphic for this character (Montanucci, 1987). In Montanucci (1987), this transformation series was ordered and unscaled. In the present study, we scaled this polymorphic transformation series. (22) *Supralabial margin*. (23) *Surangular*. (24) *Tympanum*.—*Phrynosoma modestum* and *P. platyrhinos* are polymorphic for this character (Montanucci, 1987). In Montanucci (1987), this transformation series was ordered and unscaled. In the present study, we scaled this polymorphic transformation series. (25) *Upper row of abdominal scales*. (26) *Ventral scales*. (27) *Mode of reproduction* (Montanucci, 1989b; Zamudio and Parra-Olea, 2000).—(0) oviparous; (1) viviparous. (28) *Coronoid*.—(0) lateral process of coronoid overlaps the dentary and surangular; (1) overlap reduced; (2) no overlap at all. Ordered. (29) *Postriotal*.—(0) postriotal absent; (1) postriotal present. (30) *Squamosal horns*.—(0) no squamosal horns; (1) one horn; (2) two horns; (3) three horns; (4) four horns. This character was treated as unordered and unpolarized in the phylogenetic analysis. (31) *Supracoracoid foramen*. (32) *Interfemoral scales* (median scales between femoral pore rows).—(0) 15 or more interfemoral scales; (1) 10 or fewer.

Three additional characters used by Montanucci (1987) were excluded from the present analysis. External naris entry (ENAR of Montanucci, 1987) was considered redundant with external naris placement (number 17 of this study; PNAR of Montanucci, 1987). Of these two characters, external naris placement was chosen for this study because it was more easily and objectively defined. Ventral scale micro-

nation (MVEN of Montanucci, 1987) was not deemed completely independent of ventral scale condition (number 26 of this study; VENS of Montanucci, 1987). Montanucci (1987) coded *Phrynosoma cornutum* as lacking mucronation of ventral scales. However, some *P. cornutum* individuals possess strongly keeled ventral scales with weak mucronation. Also, Montanucci (1987) coded *P. asio* as possessing mucronate ventral scales. However, some individuals of *P.*

asio possess weakly keeled ventral scales that lack mucronation. Thus, mucronation is dependent on the degree of keeling of ventral scales. The final excluded character was angle of ascent of the premaxilla (PMAX of Montanucci, 1987). This character is not independent of rostrofrontal angle (number 20 this study; RSFA of Montanucci, 1987), with angle of ascent of the premaxilla determining the abruptness of the rostrofrontal angle.