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# Phylogenetic Relationships among Bufonoid Frogs (Anura: Neobatrachia) Inferred from Mitochondrial DNA Sequences

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**Nucleotide sequences of portions of the mitochondrial 12S and 16S ribosomal RNA genes were used to extend a recent study of anuran phylogeny (Hay *et al., Mol. Biol. Evol.* 12: 928–937, 1995) and to further evaluate phylogenetic relationships within the Neobatrachia. An analysis of almost 900 nucleotides from each of 8 new representatives of the Dendrobatidae, Hylidae, Leptodactylidae, and Myobatrachidae, plus 14 available members of the Neobatrachia provides support for 2 major lineages (Bufonoidea and Ranoidea) within this anuran suborder. The neotropical Bufonoidea and their derivatives are monophyletic. There is an interesting association of the 2 Australian myobatrachids with the South African Heleophrynidae, and the Sooglossidae is one of the basal bufonoid lineages. Within the New World bufonoid frogs, a monophyletic Dendrobatidae is strongly supported. An Australian hylid (Pelodryadinae) shows close affinity with the South American hylid Phyllomedusinae. A group composed of Hylinae (*Hyla* and *Smilisca*), Centrolenidae, Bufonidae, and the hylid Hemiphractinae, with the latter two clustered, was supported significantly. The addition of new taxa has more clearly defined some relationships within the suborder Neobatrachia and has indicated that the families Hylidae, Leptodactylidae, and Myobatrachidae may not be monophyletic.** © 1996 Academic Press, Inc.

## INTRODUCTION

Among living amphibians the vast majority (88%) of the more than 4500 species belong to the order Anura, most of which (96%) are classified as advanced frogs (suborder: Neobatrachia). Phylogenetic relationships within this diverse group remain obscure despite numerous studies of their evolutionary history using morphological, behavioral, ecological, and biochemical approaches (Duellman and Trueb, 1986; Cannatella and Hillis, 1993; Ford and Cannatella, 1993; Hedges and Maxson, 1993; Hillis *et al.*, 1993). Morphological traits are not always able to resolve branching patterns within the Anura largely because of the paucity of phylogenetically informative characters due to the strict conservation of the anuran body plan (Duellman and Trueb, 1986). Molecular methods that are useful for elucidation of specific and generic level relationships often are unable to resolve interfamilial relationships (Hillis, 1991; Maxson, 1992). Recently, DNA sequences of mitochondrial ribosomal RNA (rRNA) genes have been shown to be evolving at rates appropriate for resolving some aspects of amphibian phylogeny (e.g., Hedges and Maxson, 1993; Hay *et al.*, 1995). In addition, available fossil evidence indicates that major lineages of modern frogs diverged during a relatively short time span (Milner, 1988) which, if true, will complicate precise identification of relationships regardless of the method of phylogenetic inference employed.

The suborder Neobatrachia (Reig, 1958) was erected based on morphology and has recently received support from gene sequence data (Hedges and Maxson, 1993; Hillis *et al.*, 1993). The analysis of phylogenetic relationships among amphibian families based on more extensive mitochondrial rRNA gene sequences convincingly demonstrated the monophyly of this suborder (Hay *et al.*, 1995). The results of that study also corroborated the hypothesis of an early separation between the two major neobatrachian lineages: Bufonoidea and Ranoidea. However, only a few of the proposed relationships within either of these superfamilies were significantly supported.

One potential problem with most phylogenetic reconstructions is that of using only a single taxon to represent a large and diverse group. In such cases if the as-

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession Nos. U39968–U39991.

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sumption of the monophyletic origin of a group is not satisfied, the results of the analysis will be invalid. The impact of the species sampling scheme on the results of the phylogenetic analysis also can be significant (Lecointre *et al.*, 1993; Maxson *et al.*, unpublished data).

To improve the resolution of the neobatrachian portion of the anuran tree, we sequenced the same portions of the slowly evolving mitochondrial 12S and 16S rRNA genes that were used by Hay *et al.* (1995). The expanded data set from that study now includes representatives of all four currently recognized subfamilies of the Hylidae (Hemiphractinae, Hylinae, Pelodryadinae, and Phyllomedusinae), both subfamilies of the Myobatrachidae (Limnodynastinae and Myobatrachinae), two leptodactylid subfamilies (Leptodactylinae and Telmatobiinae), and three genera within the Dendrobatidae, a family traditionally associated with the ranoids (Duellman and Trueb, 1986; Ford, 1993), but according to the DNA sequence data, is nested within the Bufonoidea (Hedges and Maxson, 1993; Hay *et al.*, 1995).

In this study we address the following questions: (1) What are the relationships of the hylid subfamilies; in particular, what are the phylogenetic affinities of the Australian hylids (Pelodryadinae)? (2) Is the Myobatrachidae a monophyletic family and is it more closely related to the Heleophrynidae than to other bufonoid families? (3) Was the placement of the Dendrobatidae within the Bufonoidea (Hay *et al.*, 1995) an artifact of sampling or will its position remain the same with the inclusion in the analysis of additional members of the family? Finally, we anticipated that by including a wider representation of species, new patterns of relationships between different taxa might emerge.

## MATERIALS AND METHODS

### Taxa Examined

Eight new bufonoid species (see Appendix), representing the Dendrobatidae (*Colostethus*, *Phobobates*), Hylidae (*Gastrotheca*, *Litoria*, *Phyllomedusa*, *Smilisca*), Leptodactylidae (*Lithodytes*), and Myobatrachidae (*Pseudophryne*) were added to those species studied by Hay *et al.* (1995). All three mitochondrial ribosomal gene regions analyzed in this study were obtained from the same individual. Taxonomic assignment of examined species follows Duellman (1993).

### DNA Amplification and Sequencing

Total DNA was extracted from frozen (−20°C) tissue samples of whole blood, plasma, heart, or muscle using a standard phenol–chloroform protocol (Hedges *et al.*, 1991) or Puregene DNA isolation kit (Gentra). A region of the 12S and two adjacent regions of the 16S mitochondrial rRNA genes were sequenced using the following scheme. Double-stranded fragments were amplified in 30–35 cycles of the polymerase chain reaction (PCR; 95°C for 1 min, 50°C for 1 min, and 72°C for 2.5 min). Single-stranded sequencing template was produced in a second PCR amplification (25–35 cycles: 95°C for 1 min, 60°C for 1 min, 72°C for 2.5 min) with one primer as limiting, i.e., diluted to 1% of the original concentration (Gyllensten and Erlich, 1988). Slight variations in annealing temperatures and/or numbers of cycles were employed as needed to improve the quality of the DNA template. The primer pair 12L1/12H1 amplified a fragment of 12S rRNA gene (about 400 nucleotides long), while 16L1/16H1 (or 16L8/16H9) and 16L2a/16H10 yielded fragments of the 16S rRNA gene with approximate lengths of 500 and 570 nucleotides, respectively. Primers that were used for the double-stranded PCR were also used to generate a single-stranded template. In addition, a primer internal to the 16L2a/16H10 pair (16L2) was used. Location and sequences of all primers used were previously reported (Hedges, 1994), with the exception of 16H9 (5′-CCGGTCTGAACTCAGATCA CGT-3′), which is colocalized with the 16H1 primer. The template was purified using 30,000 molecular weight filters (Millipore) and sequenced (Sanger *et al.*, 1977) using *Taq* (*Thermus aquaticus*) DNA polymerase with second round PCR primers as sequencing primers. A more detailed description of the methods has been published (Hedges *et al.*, 1991).

### Sequence Analysis

Sequences were read from autoradiograms using the digitizing program GELIN (S. W. Schaeffer, Pennsylvania State University). We used only those portions of the new sequences which corresponded to the regions used in the analysis of amphibian phylogenetic relationships reported by Hay *et al.* (1995). The new data were combined with the sequences from representatives of 14 families of advanced frogs from that study. Since monophyly of both the Neobatrachia and the Archaeobatrachia is well established (Hay *et al.*, 1995) we used 4 representatives of the latter as outgroups in these analyses. The alignment was done by eye using the ESEE sequence editor (Cabot and Beckenbach, 1989). We omitted 359 sites from further analysis because of the moderate degree of length variation and presence of highly variable regions where homology could not be inferred with certainty.

Phylogenetic trees were constructed based on Jukes-Cantor corrected distances (Jukes and Cantor, 1969) using the neighbor-joining algorithm of Saitou and Nei (1987). We used the Jukes-Cantor distance in accordance with the recommendation of Kumar *et al.* (1993) since corrected distances were less than 0.3, average nucleotide frequencies did not deviate substantially from 25% (A, 32.7%; T, 22.6%; C, 23.7%; G, 21%), and transition/transversion ratios (range: 1–2.5, majority: 1.5–2) were not strongly biased. Nevertheless, for comparative purposes phylogenetic trees were constructed using more sophisticated distance estimation methods that (a) account for deviation of nucleotide frequencies from 25% (Tajima and Nei, 1984), (b) consider differences in transitional versus transversional substitution rates (Kimura, 1980), and (c) both of the above conditions (Tamura and Nei, 1993). The resulting tree topologies were identical to the neighbor-joining tree reported here.

It has been shown that bootstrap confidence values tend to underestimate the extent of statistical support of species clusters, particularly when the true tree is starlike and the number of sequences involved in analysis is large (Sitnikova *et al.*, 1995). These circumstances are likely in our study since divergences within the Neobatrachia occurred during a short period of time (Milner, 1988), so we assessed statistical significance of groupings by the interior-branch test (Rzhetsky and Nei, 1992). Both the tree construction and statistical tests were done as implemented in the METREE program (Rzhetsky and Nei, 1994). Because the interior-branch test may overestimate statistical confidence when both the test and the tree reconstruction are done using the same data set (Sitnikova *et al.*, 1995), interior-branch confidence values ($P_C$) were corrected using a computer program provided by Tatyana Sitnikova (Pennsylvania State University). This correction gives a conservative estimate of statistical significance; therefore we treat values above 0.95 as significant and those above 0.90 as strongly supported. All calculations were done using the MEGA package (Kumar *et al.*, 1993).

Using MEGA, we also obtained maximum parsimony trees using the heuristic search method which does not guarantee that the tree(s) of minimal length will be found. The size of our data set prohibits the use of exhaustive or branch-and-bound searches, which provide such a guarantee. However, for this large data set, with long branches and short internodes, we believe the neighbor-joining algorithm, which has been shown to be more efficient in computer simulations (Nei, 1991; Nei *et al.*, 1995), is the most appropriate analytical method to use for recovering a phylogeny.

## RESULTS

For each of the eight new taxa approximately 345 and 820 nucleotides were sequenced from the 12S and 16S rRNA genes, respectively. When combined with previously available data (Hay *et al.*, 1995) these sequences produced an alignment (Fig. 1) with a total length of 1258 nucleotides (376 for the 12S and 882 for the 16S). After conservative omission of sites where alignment was uncertain due to length and/or extensive sequence variation, a data set of 899 nucleotides was obtained (317 from the 12S and 582 from the 16S). Almost half (465) of those sites were variable; interestingly, the variation was equally distributed among both genes (169 sites in the 12S and 296 sites in the 16S). This implies that within the Anura the requirements of sequence conservation due to structural constraints may be similar for these two genes.

The shaded areas in Fig. 1 were omitted from all analyses. Examination of the remaining alignment reveals that there still are some sites remaining which show a limited amount of length variation and missing data. There are two ways of analyzing our data set. All sites with gaps and missing data can be eliminated from the data set prior to the analysis (complete deletion) or they can be deleted from each pair of compared sequences separately (pairwise deletion). Since we had relatively few missing sites and they were distributed uniformly throughout the alignment we chose the latter option (Kumar *et al.*, 1993). When complete deletion was used the number of variable sites was reduced by about 10% (to 422 sites).

The phylogenetic tree reconstructed by the neighbor-joining method (Fig. 2) provides support for many groupings within the Neobatrachia. The monophyly of both the Bufonoidea ($P_C = 0.96$) and Ranoidea ($P_C = 0.78$) is supported. The three dendrobatid genera are monophyletic ($P_C = 0.98$) and cluster within the Bufonoidea. The two hylid subfamilies Pelodryadinae and Phyllomedusinae cluster together ($P_C = 0.90$), the Australian Myobatrachidae clusters with the Heleophrynidae ($P_C = 0.69$), the leptodactylid subfamily Telmatobiinae is a sister taxon of the New World bufonoids and their derivatives ($P_C = 0.92$), and there are several strong associations within the latter. Importantly, a neighbor-joining tree constructed using the complete deletion option (not shown) differed little from the tree presented here. Discrepancies between the two trees primarily involved a rearrangement of branches that received little statistical support, notably the relationships within the outgroup (Archaeobatrachia) and the position of the Sooglossidae (it was a sister group of the Telmatobiinae + neotropical Bufonoidea). In addition, a cluster containing the Centrolenidae, Hylinae, and Bufonidae with the Hemiphractinae was expanded in the "complete-deletion" tree to include the Leptodactylinae and Rhinodermatidae ($P_C = 0.95$).

The tree topologies derived from maximum parsimony analyses (with and without gaps and missing data) were fairly consistent with the neighbor-joining tree. All trees supported the division of the Neobatrachia into the Bufonoidea and Ranoidea and the placement of the Australian Myobatrachidae and the Heleophrynidae (although not as a monophyletic group) outside of the monophyletic Telmatobiinae + New World Bufonoidea. Also supported were the Hylinae (*Hyla* + *Smilisca*) and the Pelodryadinae + Phyllomedusinae joined consecutively by the Pseudidae and Rhinodermatidae. The most conspicuous differences between the neighbor-joining and the maximum parsimony trees involved the positioning of lineages with

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                  <12S                                                                              80
Discoglossidae    CCGCCAGGGAACTACGAGCCTCAGCTTAAAACCCAAAGGACTTGGCGGTGCCCNAAACCCACCTAGAGGAGCCTGTTCTA
Leiopelmatidae    CCGCCAGGGTACTACGAGCTACAGCTTAAAACCCAAAGGACTTGGCGGTGCCCCACACCCACCTAGAGGAGCCTGTTCTA
Pelobatidae       CCGCCTGGGAACTACGAGCGCCAGCTTAAAACCCAAAGGACTTGGCGGTGCCC-AAACCCACCTAGAGGAGCCTGTTCTA
Rhinophrynidae    CCGCCTGGGAACTACGAGCCTAAGCTTAAAACCCAAAGGACTTGGCGGTGCCCCAAACCCACCTAGAGGAGCCTGTTCTA
Bufonidae         TCGCCAGGGAACTACGAGCTAA-GCTTAAAACCCAAAGGACTTGACGGTACCCCATATCCCCCTAGAGGAGCCTGTCCTA
Centrolenidae     TCGCCAGGGAACTACGAGCAAA-GCTTAAAACCCAAAGGACTTGACGGTACCCCACATCCACCTAGAGGAGCCTGTCCTA
Colostethus       TCGCCTGGGAACTACGAGCTAA-GCTTAAAACCCAAAGGACTTGACGGTACCCCATATCCCCCTAGAGGAGCCTGTCCTA
Dendrobates       TCGCCTGGGAACTACGAGCTAA-GCTTAAAACCCAAAGGACTTGACGGTACCCCATATCCCCCTAGAGGAGCCTGTCCTA
Heleophrynidae    TCGCCAGGGTATTACGAGCCCAAGCTTAAAACCCAAAGGACTTGACGGTGCCCCA-ATCCCCCTAGAGGAGCCTGTTCTA
Hemiphractinae    TCGCCAGGGAACTACGAGCAAA-GCTTAAAACCCAAAGGACTTGACGGTACCCCATATCCCCCTAGAGGAGCCTGTCCTA
Hyla              TCGCCAGGGAACTACGAGCAAA-GCTTAAAACCCAAAGGACTTGACGGTACCCCATATCCACCTAGAGGAGCCTGTCCTA
Hyperoliidae      ACGCCAAAGAACTACGAGCGCAAGCTTAAAACTTAAAGGACTTGACGGTGTCCCA--TCTACCTAGAGGAGCCTGTTCTA
Leptodactylinae   TCGCCAGGGAACTACGAGCTAT-GCTTAAAACCCAAGGGACTTGACGGTACCCCAAATCCACCTAGAGGAGCCTGTCCTA
Limnodynastinae   TCGCCCGGGAACTACGAGCCCAGCCTTAAAACCCAAAGGACTTGACGGTGCCCCACATCCCCCTAGAGGAGCCTGTCCTA
Mantellidae       GCGCCAGGGAATTACGAGCGTANNNTTAAAATCCAAAGGATTTGACGGTGTCCCA--CCCACCTAGAGGAGCCTGTTCTA
Microhylidae      CCGCCCGGGAATTACAAGCCCAAGCTTAAAACCCAAAGGACTTGACGGTGTCCCA--CCCACCTAGAGGAGCCTGTTCTA
Myobatrachinae    CCGCCCGGGTACTACAAGCTAAAGCTTAAAACCCAAAGGACTTGACGGTGCCCCACATCCACCTAGAGGAGCCTGTCCTA
Pelodryadinae     TCGCCCGGGAACTACGAGCAAA-GCTTAAAACCCAAAGGACTTGACGGTACCCCATATCCACCTAGAGGAGCCTGTCCTA
Phobobates        TCGCCCGGGAACTACGAGCTAA-GCTTAAAACCCAAAGGACTTGACGGTACCCCACATCCCCCTAGAGGAGCCTGTCCTA
Phyllomedusinae   ANGCCAGAGAACTACAAGCAAA-GCTTAAAACTCAAAGGACTTGACGGTACCCCATATCCACCTAGAGGAGCCTGTCCTA
Pseudidae         TCGCCTGGGAACTACGAGCCAA-GCTTAAAACCCAAAGGACTTGACGGTACCCCATATCCCTCTAGAGGAGCCTGTCCTA
Ranidae           GCGCCAGGGAACTACGAGCAAT-GCTTAAAACCCAAAGGATTTGACGGTGTCCCA--CCCAGCTAGAGGAGCCTGTTCTT
Rhinodermatidae   TCGCCCGGGAACTACGAGCAAA-GCTTAAAACCCAAAGGACTTGACGGTGCCCCATATCCCCCTAGAGGAGCCTGTCCTA
Smilisca          TCGCCAGGGGACTACGAGCAAA-GCTTAAAACCCAAAGGACTTGACGGTACCCCATATCCACCTAGAGGAGCCTGTCCTA
Sooglossidae      GCACCTGGGAACTACAAGCAAAAGCTTGAAACCTAAAGGACTTGACGGTGCCCCAAACCCACCTAGAGGAGCCTGTTCTA
Telmatobiinae     TCGTCAGGGAAATACAAGCAAA-GCTTTAAACCCAAAGGACTTGACGGTATTCCACATCCCCCTAGAGGAGCCTGTCCAA
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                                                                                                    160
Discoglossidae    TAATCGATAACCCCCGCTTAACCTCACCACTTCTTGCCAAA-ACCGCCTATATACCACCGTCGCCAGCCCACCCCGTGAG
Leiopelmatidae    TAATCGATAACCCCCGCTAAACCTCACCACTTATTGCCAAT-ACCGCCTATATACCACCGTCGCCAGCCCACCCTGTGAA
Pelobatidae       TAATCGATAATCCACGATCAACCTCACCACTTCTTGCCAAT-TCCGCCTATATACCGCCGTCGCCAGCTTACCCTTTGAA
Rhinophrynidae    TAATCGATAATCCCCGCTTTACCTCACCACTTCTAGCCAAT-ACCGCCTATATACCACCGTCGGCAGCCCACCCTGTGAG
Bufonidae         TAATCGATAATCCACGTTTAACCTCACCATTTCTAGTTTA--TCAGCCTGTATACCTCCGTCGTCAGCTTACCACGTGAG
Centrolenidae     TAATCGATACCCCCCGCTAAACCTCACCACCTCTCGCCCAC-TCAGCCTGTATACCTCCGTCGTCAGCTTACCACATGAG
Colostethus       TAATCGATAACCCCCGCTTTACCTCACCATTTTTTGCTAA--TCAGCCTGTATACCTCCGTCGTCAGCTTACCACGTGAG
Dendrobates       TAACCGATAATCCCCGTTTAACCTCACCATTTCTAGCTAAG-ACAGCCTGTATACCTCCGTCGTCAGCTCACCGCGTGAG
Heleophrynidae    TAATCGATGATCCCCGTTAAACCTCACCACTTCTCGCCCA--TCCGCCTGTATACCTCCGTCGCCAGCCCACCGCATGAG
Hemiphractinae    TCATCGATACTCCACGTTTAACCTCACCATTTCTAGTCTA--TCAGCCTGTATACCTCCGTCGTCAGCTTACCGCATGAG
Hyla              TAATCGATAATCCCCGCTAAACCTCACCATTTCTAGCTAG--TCAGCCTGTATACCTCCGTCGTCAGCTTACCACGTGAG
Hyperoliidae      TAATCGATAATCCTCGTTATATCTCACCTTTTTTAGCTTA--TCAGTCTGTATACTTCCGTCGTAAGCTTACCATATGAA
Leptodactylinae   TAATCGATAACCCCCGCTTAACCTCACCATTTTTTGTAAA--TCAGCCTGTATACCTCCGTCGTCAGCTTACCATGTGAA
Limnodynastinae   TAATCGATGATCCACGTTTTACCTCACCTCTTCTTGCCAA--CCCGCCTGTATACCTCCGTCGTCAGCTCACCGCATGAT
Mantellidae       TAATCGATAATCCTCGATATACCCAACCATTTCTTGCTTT--TCAGCCTGTATACCTCCGTCGCAAGCTTACCATTTGAA
Microhylidae      TAATCGATTCCCCCCGATACACCCCACCCCTTCTAGCCAC--TCAGTCTGTATACCTCCGTCGCAAGCTTACCATGTGAA
Myobatrachinae    TAATCGATGATCCACGTTTAACCTCACCACTTCTAGCCTC--CCAGCCTGTATACCTCCGTCGCCAGCTCACCGCATGAG
Pelodryadinae     TAATCGATAACCCCCGCTTAACCTCACCATTTTTAGCCTC--TCAGCCTGTATACCTCCGTCGTCAGCTTACCACGTGAG
Phobobates        TAATCGATAACCCCCGCTTAACCTCACCATTTTTTGCTAA--TCAGCCTGTATACCTCCGTCGTCAGCTTACCACGTGAG
Phyllomedusinae   TAATCGATAACCCCCGCTTTACCTCACCATTTTTAGTTAA--TCAGTTTGTATACCTCCGTCGTCAGCTTACCACGTGAG
Pseudidae         TAATCGATAATCCCCGTTTAACCTCACCATTTTTAGCCCAC-TCCGCCTGTATACCTCCGTCGTCAGCTTACCACGTGAG
Ranidae           TAATCGATGATCCCCGCTACACCTGACCATTTCTTGCTCA--TCAGTCTGTATACCTCCGTCGAAAGCTTACCATGTGAA
Rhinodermatidae   CAACCGATAACCCCCGATTAACCTCACCATTTTTAGTTAT--TCAGCCTGTATACCTCCGTCGTCAGCTTACCCCGTGAG
Smilisca          TAATCGATAACCCCCGTTTAACCTCACCATTTTTAGCCTC--TCAGCCTGTATACCTCCGTCGTCAGCTTACCACGTGAG
Sooglossidae      TAACCGACACTACCCGATAAACCTCACCACCACTAGCCATACCCAGCCCGTATACCTCCGTCGTCAGCTTATCACTCAAG
Telmatobiinae     TAATCGATAACCCTCGCTTTACCTCACCACATCTTGCTTA--TCAGCCTGTATACTTCCGTCGTAAGTAAGCCACATGAA
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**FIG. 1.** Alignment of portions of mitochondrial 12S and 16S rRNA genes for all species in this study. "–" designates alignment gaps; "N" denotes undetermined nucleotides. Shaded areas were omitted from the phylogenetic analysis.

long branches (notably, the Sooglossidae and, to a lesser extent, Telmatobiinae and Hyperoliidae), an effect described by Felsenstein (1978). It should be reemphasized that because of the size of our data set (26 sequences of 899 nucleotides) we were unable to conduct a maximum parsimony search that guaranteed finding the shortest tree (see Hedges *et al.* (1992) for discussion of a similar case). Furthermore, the nature of the tree (large number of taxa, long terminal branches, and short internal nodes) makes it difficult for a maximum parsimony algorithm to identify the correct tree (Nei *et al.*, 1995). Nonetheless, only nodes

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                                                                                            240
Discoglossidae     GGGAATGCAGTAGGCAAAAT-GGC----------------CCCCGCCAGAACGTCAGGTCAAGGTGTA-GCGTATGAAGT
Leiopelmatidae     GGACTTCAAGTAGGCACAAT-GGT----------------CACCACCAAAACGTCAGGTCAAGGTGTA-GCACATGAAGT
Pelobatidae        GGTTAAAAAGTAAGTAAAAT-GGT----------------ACTCACCAAAACGTCAGGTCAAGGTGTA-GCGAATGAAGT
Rhinophrynidae     -GCTGATTAGTAGGCTCAAT-GA-----------------CACCATCAACACGTCAGGTCAAGGTGTA-GCGTATGGAGT
Bufonidae          CGCCAATTAGTGAGCTTAAT-GTTT---------------TTTCACCAACACGTCAGGTCAAGGTGCA-GCAAATGAAAT
Centrolenidae      CGCAATTTAGTGAGCTTAAT-GCCC---------------ATACGCCAACACGTCAGGTCAAGGTGCA-GCAAATGAGAT
Colostethus        CGTC----AGTGAGCCCAAT-GTTT---------------GTTCAACCACACGTCAGGTCAAGGTGCA-ACATATAAAGT
Dendrobates        CGTCAGTGAGTGAGCCTAAT-GTTA---------------ATTCAACTTAACGTCAGGTCAAGGTGCA-ACATATGTGAT
Heleophrynidae     CGTGAGAAAGTGGGCCTAAA-GAAC---------------CTTTTCCAATACGTCAGGTCAAGGTGCA-GCACATGAAGT
Hemiphractinae     CGACCCCCAGTGAGCTTAAT-GGCG---------------TTACACCAATACGTCAGGTCAAGGTGCA-GCAAATGAAAT
Hyla               CGTCACCCAGTGAGCCTAAT-GTTC---------------TTACACCAACACGTCAGGTCAAGGTGCA-GTAAATGAAAT
Hyperoliidae       TGTATT--AGTAAGTTAAATAGTAATAAC-----------AATTACCAAAACGTCAGGTCAAAGTGCA-GCCAACAAAAA
Leptodactylinae    CGTTTTATAGTGAGCTTAAC-GCTT---------------ATTCACCAGTACGTCAGGTCAAGGTGCA-GCTAATGAAAT
Limnodynastinae    CGGTCAATAGTGAGCAAAAT-GGCC---------------AAGCGCCAAAACGTCAGGTCAAGGTGCA-GCACATGAATA
Mantellidae        TGTAAAAGAGTAGGTTTAAG-GATC---------------CCCCATCAATACGTCAGGTCAAGGTGCA-GCCAATGTAAT
Microhylidae       CGAAATA-AGTGAGCTTAAA-GGCC---------------CCCCGCCAACACGTCAGGTCAAGGTGCA-GCTCACGAAGC
Myobatrachinae     CGCCTAATAGTGAGCAAGAT-GGCCC--------------TTTCGCCAAAACGTCAGGTCAAGGTGCA-GCTAATGAAGC
Pelodryadinae      CGCAAATCAGTAAGCTTAAT-GTCC---------------TTGCGTCAATACGTCAGGTCAAGGTGCA-GTAAATAAAAT
Phobobates         CGTCAGTGAG----CTTAAT-GTTT---------------TTCCAACTATACGTCAGGTCAAGGTGCA-ACATATAAAAT
Phyllomedusinae    CGAACCACAGTGAGCTTAAT-GCCA---------------GAACGCCAATACGTCAGGTCAAGGTGCAACTAACAAAATG
Pseudidae          CGCTTTA-AGTGAGCCCAAT-GTCT---------------TCTCGTCAATACGTCAGGTCAAGGTGCA-GTAAATAAAAT
Ranidae            CGTCTTC-AGTAGGCTCAAT-GATCATAA-----------TTACATCAATACGTCAGGTCAAGGTGCA-GCTTAAGAAAT
Rhinodermatidae    CGAAATATAGTGAGCTTAAT-GCCT---------------TTACACCAATACGTCAGGTCAAGGTGCA-GCAAATAAAAT
Smilisca           CGTTATTTAGTGAGCTTAAT-GTCT---------------CTACATCAATACGTCAGGTCAAGGTGCA-GTAAATAAAAT
Sooglossidae       TGAATTTTAATAAGCCAAAT-GGC----------------CCCCACCAATACGTCAGGTCAAGGTGCA-GCATATGTCGT
Telmatobiinae      TGAACCTTAGTAAGCCCAAT-GTCTACACATAAATGTGTTGAACAACAATACGTCAGGTCAAGGTGCA-GCGGATGATGT

                                                                                                 320
Discoglossidae     GGAAAGAAATGGGCTACATTTTCTGGCT--CAGAACAA-CACGAAAGGTCACT-ATGAAA-CCTGACC--CAAAGGAGGA
Leiopelmatidae     GGGAAGAAATGGGCTACATTTTCTACCC--TAGAACAA--ACGAACGATCATT-ATGAAA-CATGCTC--AGAAGGTGGA
Pelobatidae        GGAAAGAAATGGGCTACATTTTCTAATAA-CAGAATAT--ACGAACGATTACT-ATGAAA-AAGAATC--TGAAGGAGGA
Rhinophrynidae     GGGAAGAAATGGGCTACATTTTCTAAAT--TAGAAAAC-AACGGAAGACCATT-ATGAAA-CCTGGTC--TGAAGGCGGA
Bufonidae          GAAAAGAGATGGGCTACACTTTCTAATT--TAGAAAAT--ACGAAAAACTACCTATGAAA-CCTAGTT--AGAAGGCGGA
Centrolenidae      GGGAAGAGATGGGCTACACTCTCTAATA--TAGAAAAC--ACGGAAGACTACTTATGAAA-CCTAGTC--TAAAGGCGGA
Colostethus        GGAAAGAGATGGGCTACACTCTCTAACT--TAGAACAA--ACGAATAACTTA--ATGAAA-CCTAACT--AGAAGGCGGA
Dendrobates        GGGAAGAGATGGGCTACACTCTCTAACT--TAGAATAT--ACGAAAGACTACTTATGAAA-TCTAGTC--AAAAGGTGGA
Heleophrynidae     GGAAAGAAATGGGCTACACTCTCTAACC--TAGAAAAC--ACGAAAGACTGC--CTGAAACACCAGTC--TGAAGGCGGA
Hemiphractinae     GGGAAGAGATGGGCTACACTTTCTAAAT--TAGAAAAA--ACGAAAGACT--TTATGAAA-CCTAGTC--GGAAGGCGGA
Hyla               GGGAAGAGATGGGCTACACTTTCTAATC--TAGAACAC--ACGAAAGACTATTTATGAAA-CCTAGTC--GGAAGGCGGA
Hyperoliidae       GGGAAGAAATGGGCTACAATTTCTACTT--AAGAACAT--ACGAATTTAAGTAACTGAAA-ACTACTA--TGAAGGCGGA
Leptodactylinae    GGATAGAGATGGGCTACACTTTCTAACA--TAGAATAT--ACGAAAGATTACCTATGAAA-TCTAATC--TGAAGGCGGA
Limnodynastinae    GGGTAGAGATGGGCTACATTTTCTAAAT--TAGAAAATTAACGGAAGACTCA--ATGAAA-CCCAGTC--AGAAGGCGGA
Mantellidae        GGAAAGTAATGGGCTACAATTTCTATAA--TAGAACAA--ACGAAACACTGC--ATGAAA-AACAGTCA-TGAAGGCGGA
Microhylidae       GGAAAGAAATGGGCTACAATCTCTATCT--TAGAACAA--ACGAAAGACCAC--ATGAAA-AACAGTCA-TGAAGGCGGA
Myobatrachinae     GGGCAGAGATGGGCTACATTTTCTACTC--TAGAAAACC-ACGAAAGACTGC--GTGAAA-CCCCGTC--AGAAGGAGGA
Pelodryadinae      GGGAAGAGATGGGCTACACTTTCTACCT--TAGAATAA--ACGGAAAACTATTTATGAAA-TCTAGTT--AGAAGGCGGA
Phobobates         GGAAAGAGATGGGCTACACTTTCTACCA--TAGAATAA--ACGAAAGACTAA--ATGAAA-CCTAGTC--AGAAGGCGGA
Phyllomedusinae    TGGAAGAGATGGGCTACACTCTCTAATT--TAGAACAT--ACGAAAAACTTT--ATGAAA-CCTAGTT--AGAAGGCGGA
Pseudidae          GGGAAGAGATGGGCTACACTCTCTACTA-TTAGAGTAA--ACGAAAGACCACCTATGAAA-CCTGGTC--AG-AGGCGGA
Ranidae            GGGAAGCAATTGGCTACAATTTCTAATA--TAGAACA---ACGAAAGGCTAT--GTGAAA-TCATAGCAGCGAAGGTGGA
Rhinodermatidae    GGGAAGAGATGGGCTACACTTTCTAACA--TAGAAAAT--ACGAAAGACTACATATGAAA-CCTAGTC--AGAAGGCGGA
Smilisca           GGGAAGAGATGGGCTACACTCTCTAACT--TAGAGTAC--ACGAATGACTATTTATGAAA-TCTAGTC--AGGGGGCGGA
Sooglossidae       GGGCAGAAATGGGCTACACTCCCTCACAACCAGGAAAA--ACAAAAGACC-T-AGAGAAA-CAAAGTC--AAAAGGCGGA
Telmatobiinae      GGAATGTGATTGGCTACACTTTTTAACT--TAAAAGAA--ACGGAAGACAAAAAATGAAA-CTCTGTC--AGAAGGCGGA
```

**FIG. 1**—*Continued*

poorly supported on the neighbor-joining tree varied in their position in the maximum parsimony trees.

## DISCUSSION

The addition of eight new sequences representing members of different bufonoid lineages and using a phylogenetically closer outgroup, the Archaeobatrachia, has allowed us to clarify some relationships with the Neobatrachia. There is support for a split at the base of the radiation between the two major groups (Bufonoidea and Ranoidea) within the suborder. We now can securely place the Sooglossidae in the Bufonoidea ($P_C = 0.96$). Previous morphological studies had

```
                                                                   12s><16s2          400
Discoglossidae     TTTAGTAGTAAAGAAAAACAA-GAGAGTTCTCTTTAAC-TCGGCCCTGGG-CGCGCCACAGCTCATGCAAAGCTTAACCA
Leiopelmatidae     TTTAGCAGTAAAAAGGAACAA-AAGAGTCCTTTTTAAT-CCGGCCCTGGG-CGCGTCACAGCTTNNGCACCCCTAACCCC
Pelobatidae        TTTAGCAGTAAAAGAGGATCA-TGATGCCCCTTTTAAA-CTGGCCCTGGG-CGCGCCACAGCTCCCCCTCAATTAACCTT
Rhinophrynidae     TTTAGCAGTAAAAAGAAACAG-GAGAGTTCTTTTTAAAGGCGGCCCTTGGGCGCGCGCAAGCT--ATCTACCATAACCAA
Bufonidae          TTTAGCAGTAAAAAGGAATCA-GCATGTCCATTTTAACCCGCACACTGGG-TGTGTCANAGCTTAATTGTTCTAACGCCT
Centrolenidae      TTTAGAAGTAAAAAAAAAAAT-GAATGTCCTTTTTAACTGGGGCACTGGGGTGTGTCAAANCTTAATCNANCCACTAATT
Colostethus        TTTAGCAGTAAAATGAAACTA-GAGCGTTCATTTAAAT-TAGGCACTGGGGTGTGTCAAAGCTTCCTCATCTGTTCTGCC
Dendrobates        TTTAGAAGTAAAACGTAACAA-GAGAGTCCTTTTTAAC-ATGGCACTGGG-TGTGTCAAAGCTTTCTTACACCCAAAGAT
Heleophrynidae     TTTAGTAGTAAAAAGAAACAATGAGAGTTCTTTTTAAC-TCGGCCCTGGGGTGTGTCAAAGCTTCACCATCTACACCCAA
Hemiphractinae     TTTAGAAGTAAGGGGAAATCA-GAATGTCCCCNTTAAC-TTG-CACTGGGGTNTGTCAAAGCTTATTCGTACTCTTATTT
Hyla               TTTAGAAGTAAAAAAAGATCA-TAAAACTCTTTTTAAC-CCGGCACTGGGGTGTGTTAAAGCTCCATTATCCCCCGCTCC
Hyperoliidae       TTTAGAAGTAAAATGAAAATA-AAGTGTTCATTTTAAC-AATGCTCTGGGACGTGTCAAAGCTTAAATACATAATAAAAA
Leptodactylinae    TTTAGAAGTAAAAAGAAACCA-GAGTGTTCTTTTTAAC-TCGGCACTGGGGTGTGTCAAAGCTCACTCGCAACTTCTGAT
Limnodynastinae    TTTAGCAGTAAAAGGAAACCA-GAAAGTCCTTTTTAAC-ACGGCCCAGGG-GATGTCATAGCTTAAACACACCCTCAACA
Mantellidae        TTTAGTAGTAAAAGGGGAATA-GAGAGCCCCTTTTAAC--AGGCCCTGGGACGTGTNAAAGCTTAACATTTTCTTTTTCT
Microhylidae       TTTAGTAGTAAAAAGAAAATA-AAGTGTTCTTTTTAAT-TAGGCCCTGGGACGCGTTACAGCTTCCTCACCAGTCACTTT
Myobatrachinae     TTTAGTAGTAAAGCAGAGATCAATGAGCTCTCTTTAAC-AAGGCACAGGGGCATGTCAAAGCTTTTACATCTCATCAACT
Pelodryadinae      TTTAGTAGTAAAAAGAAATCA-GAGAGTTCTTTTTAAC-CCGGCACTGGGGTGTGTCAAAGCTTAATTATTTTTTTAAAA
Phobobates         TTTAGCAGTAAGGTGAAACTA-GAGCGTTCACTTAAAT-ACGGCACTGGGGTGTGTCAAAGCTCCCTCATTTNTTCCTCC
Phyllomedusinae    TTTAGCAGTAAAAAGAAACTA-GAGAGTTCTTTTTAAT-AAGGCACTGGGGTGTGTCAAAGCTTATTTGTAAATTAACTC
Pseudidae          TTTAGCAGTAAAAAAGGACCA-GAGAGCCTTTTTTAAC-CTGGCACTGGGGTGTGTCATAGCTTACTTGTTATAACATTT
Ranidae            TTTAGTAGTAAAAAGAAAATA-GAGTGTTCTTTTTAAC-CCGGCTCTGGGATGCGTNNAGGCTTAGTTCTACACACTTAC
Rhinodermatidae    TTTAGCAGTAAAAAGATAACA-GAGAGTTCTTTTTAAT-TTGGCACTGGGGTGTGTCAAAGCTTAATTGTCTTATTATTA
Smilisca           TTTAGGAGTAAAAAGAGACAA-GAGAGCTCTTTTTAAC-TTGGCACTGGG-TGTGTCAAAGCTTCATTATTTTTTTCTTT
Sooglossidae       TTTAGTAGTAAAAAGAAAACA-GAGTGTTCTTTTTAAC-TCGGCCCTGGGACACGTCACAGCTCACCTTCAAAACCAAAA
Telmatobiinae      TTTAGAAGTAAGAAAAAGATA-TCATATTTTTCTTAATAAGGGCACTGGAGTATGTNNNNNNTTAGCTATTATACTTAAC
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                                                                                       480
Discoglossidae     A--CAATACCGTTAAACCC-TCTAAACCCATAAATACTAATGAGCCTCTCTATATTTT--TATAGAACAGTTTATGCTAG
Leiopelmatidae     CAATAAT-ACGAATAACCAATCAAAACCCATCAACACTACTGGGCCTATCTATAAACT--CATGGATACGCTTATGCTGG
Pelobatidae        TT--AAT-TAGGACNCAAATTCAAAACCCATTAAAACTATTGAGTATTCCTATACGTT--TATAGGAGAATTAATGCTAG
Rhinophrynidae     C---AAT----TTAAACCTAATCAAACCCTAACACACTACTGAGCTGTTCTATACACC--TATAGAACCACTTATGCTAG
Bufonidae          ----AATTACTAAACTTCCATCTTAATCCTTCACTAATACTGAGTAATTCTATATTCT--TATAGAAAATTTTATGTTAA
Centrolenidae      ----AAT-CCCAACATTACTTAAGAACCCTTACATAGTACTGAGTAATTCTATATCAC--TATAGAAGACTTTATGTTAA
Colostethus        T---AATTCCTCTACCTAT-CATTAACCCCTTCCCCCCAAAGAGCAATTCTATATCTT--TATAGAAACTATTATGTTAA
Dendrobates        ----AATTCCAANACCTTTCTCTACCCCTTTACCCCCTATAGAGCAACTCTATACTTC--TATAGAAACTTTTATGTTAA
Heleophrynidae     T---AAT-CCCACAACAATCTCAAAACCCTCACCCACTATCAAGCAATTCTATATCCC--TATAGAACATCTTATGCTAG
Hemiphractinae     TA--AATTTCCTCACCATT-AATTAACCCTTCAACAATACTGAGTAATTCTATACTGC--TATAGAAATTTTTATGTTAA
Hyla               C---AAT-ACCTCAAATGATCCAGAACCCTTCCTCACTAATAAGTACTTCTATAATCC--TATAGAACATTTTATGTTAA
Hyperoliidae       A---AATTTTTTAATTGACTATAAAACCCACCATCAATACCAAATAACTTTATATTAA--TATAAAAAATATTATGTTAG
Leptodactylinae    T---AATACCACTAACTAA-AGAGAACCCCACTTCAATATTAAGCAATCCCATAACCC--TATGGNAGGTTTTATGTTAA
Limnodynastinae    TC--AAT-ACCACCATCCCAACAAAACCCTTCTCCAGTATCAAGCAATTCTATAGAGA--TATAGAAAAACTTATGCTAG
Mantellidae        ----AAT-TCCTCAAACCACCTAAAACCCCTCCCCAATATTGAGTAATTCCATAAAAA--TATGGAAGATATTATGCTAG
Microhylidae       ----AAT-ACCAAAAACACTACATAACCCTCACCCAGTACTAAATAACTCTATACCCC--TATAGAAATTATTATGCTAA
Myobatrachinae     A---AATACCTCCCACCGA-TCAAAACCCACACACACTATTGAGTGATTCCATATTT---TATGGAAGACCCTATGCTAG
Pelodryadinae      ----AATTCCAAAATTTAT-AAAAAACCCTTAACTACTATTAAGTACTTCCATATCTT--TATGGAATTTACAATGTTAA
Phobobates         ----AATTCCTCCTCTTTT-TAACAACCCATTTTCCCCAGAGAGCAATTCTATATTTA--TATAGAAAATTTTATGTTAA
Phyllomedusinae    ----AATCCCCCTAGTTAT-TAAAAACCCTTAACTAATATTAAGTAATTTTATAAAAT--TATAAAACATATAATGTTAA
Pseudidae          TTC-AAT-ACCCCAATTTTCCCAGACCCCTTTATTTTTATTGAGTAGTTCTATAATAA--TATAGAAATTATACTGTTAA
Ranidae            ----AAT-TTCTAAAATTTTGATCAACCCTTTATCCCTACTGAATTATTTTATATCCT--TATAAAAGTAATAATGCTAG
Rhinodermatidae    ----AAT-TTCTTCATCAAATATTAACCCTTTAATAGTACTGAGCACTTCTATATTTT--TATAGAATTTTTTATGTTAA
Smilisca           ----AAT-ACCACAAATTATTTAGAACCCTTCATCATTATTGAGAACTTCTATATCCC--TATAGAATATTTTATGTTAA
Sooglossidae       ----AAT-CCCCTCAACACTACAGAACCCTGATACACCATTAAGCAATCCTATTTTCCCATAT-GAAC-TATTATGCTAA
Telmatobiinae      ----AAATTTCTAAAATAAATTAAAACCCTACCTTAATACTGAATAATTCCATATATA--TATGGAAGCTATTATGTTAA
```

**FIG. 1**—*Continued*

alternately located this family in the Bufonoidea (Laurent, 1979), or the Ranoidea (Duellman, 1975). Placement of Sooglossidae close to the Myobatrachinae (Ford and Cannatella, 1993; Lynch, 1973) within the Bufonoidea remains a possibility, since our phylogenetic tree does not definitively identify a sister-group of the Sooglossidae.

The Heleophrynidae is still most closely associated with the Australian Myobatrachidae (Limnodynastinae) as found by Hay *et al.* (1995). However, inclusion of representatives from both myobatrachid subfamilies suggests that the Myobatrachidae may not be monophyletic. In their review of phylogenetic relationships among major frog lineages, Ford and Cannatella (1993) found evidence for the monophyly of the Limnodynastinae and of the Myobatrachinae, but not of the family

```
                                                                                                        560
Discoglossidae     AACTAGTAAC-ACGAAATA----ATTCTCT--AAATGCAAGTGTAAATCAGATCGAAAACCTCACTGATAATNAACGTAC
Leiopelmatidae     AATGAGTAAC-AAGAATTAAAC-CATCTCC--CAATGTAAGTGCAACCCAGACCGAATCAATCACTGGCAATTAACGGCC
Pelobatidae        AACTAGTAACTCAGAATTAAAC-ATTCTCT--TAATGTAAGTGTAAATCAGATTGAATAAATCACTGATAATTAACAGCC
Rhinophrynidae     AACTAGTAAC-ATGAATTT----ATTCTCCC-AAATGCAAGTGTAAATCAGATCGAAACAATCACTGATAATTAACGACC
Bufonidae          AACTAGTAAT-AAGAAGAAGATTCTTCTCTT-TAATGTAAGTGTAAATCAGAAAGGACAAACCACTGATATTTAACGTCA
Centrolenidae      AACTAGTAAT-AAGAAGAAGAA-CTTCTCTT-ACATGCAAGTGTAAATCAGATAGGACACTCCACTGATAATTAACACCA
Colostethus        AACTAGTAAT-AAGAAGTAAGACCTTCTCTT-TAATGCAAACCTACATCAGCAAGGACACCCCACTGATTCTTATCGCCC
Dendrobates        AACTAGTAAT-AAGAAGCAGACCCTTCTCTT-AAACGCAAGTTTGCGTCAGTTCGGACACCCCCCTGACAATTAACGTTT
Heleophrynidae     AACTAGTAAC-AAGAAGATGAA-CTTCTCCA-CAATACACGTGTAAATCAGATTGGACCACCCCCTGATAATTAACGCCA
Hemiphractinae     AACTAGTAAC-AAGAAGAAGCC-CTTCTCTT-AAATGCGCCCGTAAATCAGAAAGGACCCCCCACTGATAATTAACGCCT
Hyla               AACTAGTAAC-AAGAAGAAGAA-CTTCACTT-AAATGCAAGTGTAAGTCAGAAAGGACCTACCACTGACTTTTATCATAC
Hyperoliidae       AATGAGTAAT-AAGAAAAGAA--TTTCTCCA-AAATATAAGCATAATGCAAAATAAACTAAA-ACTGCCAATTACTGTAT
Leptodactylinae    AACTAGTAAC-AAGAAGAAGAA-CTTCTCAT-TAGTGCATGTGTAAATCAGACAGTACATTCCACTGATCCCTAACACTA
Limnodynastinae    AACTAGTAAC-AAGAAATAGAC-CTTCTCTC-CAATATAAGTGTACATCAAAATGGACACACCACTGATAACTAACGCCA
Mantellidae        AACTAGTAAC-AAGAAATAACT-TTTCTCCA-AAATGTAAGTCTAAGCCACATTAGATAAACTAATGGCAATTAACGAAA
Microhylidae       AACTAGTAAC-ATGAAGAAGCC-CTTCTCCT-AAATGTACATGTACGCCAAAAAGAACAATTCATTGGCATTTAACGCTA
Myobatrachinae     AATTAGTAAT-CCGAAGAACCCCCTTCTCTA-CAACACAAGTGTAAATCAGAACGGACACCCCCCTGATAATTAACGCCC
Pelodryadinae      AACTAGTAAC-AAGAAGAAGCC-CTTCTCTT-AAATGCAAGTGTAAATCAGAAAGGACACACCACTGATAATTAACACCT
Phobobates         AACTAGTAAT-AAGAAGCAAGCCCTTCTCTT-TAATGCAAACTTAAATCAGTCAGGACCATCCGCTGATCTTTAACGCTT
Phyllomedusinae    AACTAGTAAC-AAGAACTAGAA-CTTCTCTA-TAATGCAAGTGTATGTCAGATTGGACAAACCGCTGACTCTTAACATCT
Pseudidae          AACTAGTAAC-AAGAAGAAGCC-CTTCTCTT-AAATGCGAGTGTATATCAGAAAGGACAAACCACTGATAATTAATGCCC
Ranidae            AACTAGTAAC-AAGAAACTGCCCATTCTCCT-AAATGCAAGCATAAACCAGAATAGACACCCTACTGGTAATCAACGTAA
Rhinodermatidae    AACTAGTAAC-AAGAACAAGAC-CTTCTCTAACAATGCATATGTAAATCAGAAAGGACACACCACTGCTAATTAACATGC
Smilisca           AACTAGTAAC-AAGAAGTAGCA-CTTCACTT-AAATGTAAGTGTAAATCAGAAAGGACTCACCACTGACTATTAACATTT
Sooglossidae       AACTAGTAAT-AAGGGCATCAA-CCCCTCTT-TAATGCAAACATACATCAGCACGGATAAACCACCGATACTTAACACCC
Telmatobiinae      AACAAGTAAC-ATGAAGATAC--CTTCTCTT-AAATGTAAACTTAAGTCAGACCGGATAAAACACTGACAATTAACAGC-
```

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                                                                                                   640
Discoglossidae     CACA-TGAAGGAACTGTAGTAACTCAT----CAAGAAAA---TCCTAC-AACCCCCACCGTTAATCTAACACAAGAGCAT
Leiopelmatidae     TCAA-TGAGACAAATGTAGCAACTCCA----CAAGAAAA---CCCTAC-AACCACCACCGTTAACCTAACACAAGAACAT
Pelobatidae        TTAC-AGAAAGCAATGTAACAACTCAC----CAAGAAAA---CCTTAC-ATCAAAAACTGTTAATCTTACACAAGAACAC
Rhinophrynidae     TTAAATGAAAAAAATGTAGCAACCCAC----CAAGAAAA---CCCTAT-ACCACACACCGTTAAACTTACACAAGAGCAT
Bufonidae          C----TGAGTTAAAAGTAAAAACTTAT----CAAGAAAA---CTCTAC-TTCCATCAACGTTAACCTAACACAAGAACAT
Centrolenidae      A----TGAACAAAAAGTAGTAACTCAA----CAAGAAAA---CTCTAC-TACATTTTATGTTAATCTAACACCAGAGCAT
Colostethus        T----TAAAAACATGGCAGCAACTTAT----CAAGAAAA---CACTCC-CTCCATCAGCGTTAACCTAACACCAGAGCAT
Dendrobates        T----TAATTTTAAAGTAGTAACTTAA----CAAGAAAA---GCCTAC-TAAAACTTACGTTTACCTTACACTAGCGCGT
Heleophrynidae     -----TGAACTTAGGGTAATAACGACA----CAAGAGAA---ACCTACCCACCCCCCCGTTAACCTAACACAAGAGCAT
Hemiphractinae     A----TGACCCTAAAGTAAAAACTTAT----CTAGAAAA---ACTTAC-TAAACGCGCCGTAAACCTAACACAAGAGCAT
Hyla               A----TGCAAACAAATTAATAAATTTTT---CAAGAAAA---ACTTAA-TTTTAACTATGTTAATCTAACACAAGAGCAT
Hyperoliidae       A----TTAAACTAAAGTAGTAACTTAA----CTAGAAAA---CCCTAC-TTTTCTAAACATTAATCTAACACAAGAATAT
Leptodactylinae    T----TGAATCTTAA-TAGCAACTTTA----CAAGAAAA---CCCTAT-TATTTTATATGTTAATCTAACACAAGAGCAC
Limnodynastinae    C----TGAACCCATAGTAGCAACCCAC----CAAGAAAA---CCCTAC-TAACCCCAACGTTAACCTAACACTAGAGTAT
Mantellidae        A----TGAACCCCCTTTAGTAACTAAT----CAAGAAAA---TTCTAC-AAACCAAAACGTTAACCTTACACTAGAACAT
Microhylidae       C----TGACCCTAAAGCAACAACTATA----CAAGAGAA---TCTTGC-TAACACCAACGTTAACCTAACACAAGAACAT
Myobatrachinae     A----TGAAAAAAGAACAGTAACTTAA----CTAGAAAA---TCCTGC-TCTATAATCCGTTAACCTAACACAAGAGTGT
Pelodryadinae      A----TGAATACAAAGTAGTAACTTCA----CAAGAAAA---TTCTAC-TAAATCTAATGTTAACCTAACACAAGAGCAT
Phobobates         T----TAAACCACCTGTAGTAACCCAT----CAAGAAAA---CACTAC-CTCACTTAGCGTTAACCTGACACAAGAGCAT
Phyllomedusinae    -----TGAAATCAAAGTAACAACTTAT----CAAGAAAA---TTTTAC-CAAACACAGTGTTAATCTAACACAAGAGCAT
Pseudidae          A----TAAAATAATAGTAATAACATAA----CAAGAAAA---CCTTAC-TCTCTTCAGCATTAACCTAACACAAGAGCAT
Ranidae            A----TGTCACTTTTATAGTAACATAG-----TAGAAAA---TCCTATAATCCCCTTACGTTAACCTTACACTAGAGCAT
Rhinodermatidae    C----TGAATATAAAGTAGTAACCCAA----CAAGAAAA---TTCAAC-TAAATCTCATGTTAACCTAACACAAGAGCAT
Smilisca           A----TGAAAATACATTAGCAACTCCA----CAAGAAAA---ACCTAA-TTGTAACCATGTTAACCTAACACCAGAGCAT
Sooglossidae       AC---TAATCCCGCAGCTACAACAAAACACACAAGAAAA---TTTACC-TAAGCCAAACGTTAACCTAACACAAGCGCAT
Telmatobiinae      -----TTAAACTAAAGTGATAACCCAA----CAAGAAAATCTCACTAT-TAACTCATCTGTTAACCTAACACAAGAACAT
```

**FIG. 1**—*Continued*

Myobatrachidae, which consists of both lineages. Expanded sampling of members within both of these old lineages (Maxson, 1992) will be necessary to identify the sister group of the South African Heleophrynidae.

The position of the Dendrobatidae within the Neobatrachia has elicited much controversy. It was previously placed both within the Ranoidea (Duellman and Trueb, 1986; Ford, 1993) and the Bufonoidea (Lynch, 1973). With the addition to the analysis of two more dendrobatid genera we substantiated the placement of the Dendrobatidae within the bufonoids. Relationships within this family will be discussed in more detail elsewhere (I. Ruvinsky, B. J. Smith, and L. R. Maxson, unpublished data).

Monophyly of a bufonoid subgroup (containing all taxa except the Heleophrynidae, Myobatrachidae,

```
                                                                                          720
Discoglossidae    AA-AAGAAAGATTAAAAGATATAAAAGGAACTCGGCAAACATGAACTTCGCCTGTTTACCAAAAACATCGCCTCCTGCTC
Leiopelmatidae    ACTAGGAAAGATTAAAAAACAAGGAAGGAACTCGGCAAATATGAACCTCGCCTGTTTNCCAAAAACACCGCCTCTTGCAA
Pelobatidae       ACCAAGAAAGATTAAAAGATATAGAAGGAACTCGGCAAATCTAGGTCCCGCCTGTTTACCAAAAACATCGCCTCTTGCCC
Rhinophrynidae    TCCAGGAAAGATTTAAAGACTTGGAAGGAACTCGGCAAACAAGAACCCCGCCTGTTTACCAAAAACATCGCCTCTTGCCC
Bufonidae         TTCAAGAAAGATTAAAAGAAAAAGAAGGAACTCGGCAAATATTAACCTCGCCTGTTTACCAAAAACATCGCCTCTTGA--
Centrolenidae     TACAAGAAAGATTAAAAGAAAGAGAAGGAACTCGGCAAATATTAACCTCGCCTGTTTACCAAAAACATCGCCTCTTGAA-
Colostethus       TTTAAGAAAGATTTAAAGAAAAAGAAGGAACTCGGCAAATAATAGCCCCGCCTGTTTACCAAAAACATCGCCTCTTGC--
Dendrobates       TACAAGAAAGATTAAAAGAAAAAGAAGGAACTCGGCAAATATTAGTCTCGCCTGTTTACCAAAAACATCGCCTCTTGCTT
Heleophrynidae    TTCTGGAAAGATCTAAAGGAGAGGAAGGAACTCGGCAAATTTTGGCCCCGCCTGTTTACCAAAAACATCGCCTCTTGAAT
Hemiphractinae    TTCAAGAAAGATTAAAAGAAAAAGAAGGAACTCGGCAAATATTAGCCCCGCCTGTTTACCAAAAACATCGCCTCTTGA--
Hyla              TTCAAGTACGATTTAACGAAAAAGAAGGAACTCGGCAAATATTAACCTCGCCTGTTTACCAAAAACATCGCCTCTTGAC-
Hyperoliidae      TACTGGAAAGATTAAAAGAATGGGAAGGAACTCGGCAAATATAAACCCCGCCTGTTTACCAAAAACATCGCCNCTNNCTT
Leptodactylinae   TTCAAGAAAGATTAAAAGAAAAAGAAGGAACTCGGCAAATCTTAACCCCGCCTGTTTACCAAAAACATCGCCTCTTGA--
Limnodynastinae   TTTGAGAAAGATTAAAAAAAAGGGAAGGAACTCGGCAAATATTAACCTCGCCTGTTTACCAAAAACATCGCCTCTTGAG-
Mantellidae       TAATGGAAAGATTAAAAGAAAAAGAAGGAACTCGGCAAATCTTAACCCCGCCTGTTTACCAAAAACATCGCCTCTTGAAA
Microhylidae      TTCAGGAAAGATTAAAAGAGGTGGAAGGAACTCGGCAAATTTTAACCCCGCCTGTTTACCAAAAACATCGCCTCCTGCA-
Myobatrachinae    TAATAGAAAGATTAAAAAGGACGGAAGGAACTCGGCAAATACCAACCCCGCCTGTTTACCAAAAACATCGCCTCTTGA--
Pelodryadinae     TAAAAGAAAGATAAAAAGGAAAAGAAGGAACTCGGCAAATATTAACCTCGCCTGTTTACCAAAAACATCGCCTCTTGAT-
Phobobates        TCCAAGAAAGATTAAAAGAAAAAGAAGGAACTCGGCAAACACCAGCCCCGCCTGTTTACCAAAAACATCGCCTCTTGTT-
Phyllomedusinae   TTTCAGACAGATTAAAAGGAAAAGAAGGAACTCGGCAAATATTAACCTCGCCNNTTTACCAAAAACATCGCCTCTTGACA
Pseudidae         TACAAGAAAGATTAAAAAAAGAGGAAGGAACTCGGCAAATATTAACCCCGCCTGTTTACCAAAAACATCGCCTCTTGAA-
Ranidae           TTCAGGAAAGATTAAAAGAGAAAGAAGGAACTCGGCAAACTTCAGCCTCGCCTGTTTACCAAAAACATCGCCTCTTGAAA
Rhinodermatidae   TACAAGAAAGATTAAAAGAAAAAGAAGGAACTCGGCAAATATTAACCCCGCCTGTTTACCAAAAACATCGCCTCTTGACT
Smilisca          TTCAAGTAAGATTAAAAGAAAAAGAAGGAACTCGGCAAATATTAACCTCGCCTGTTTACCAAAAACATCGCCTCTTGATT
Sooglossidae      TACAAGAAAGATTACAAGGAAAAGAAGGAACTCGGCAAATTTCAGCCTCGCCTGTTTACCAAAAACATCGCCTTCTGCCT
Telmatobiinae     TTTAAGAAAGACTTAAAGAGAAAGAAGGAACTCGGCAAAAATTGATTCCGCCTGTTTACCAAAAACATCGCCTCCTGAAT

                                                                   16s2><16s1           800
Discoglossidae    T---ACATGTATAGGAGGTCCAGCCTGCCCAGTGACGTAAATGTTTAACGGCCGCGGTATTNNNNNNNNNNNNNNNNNCCT
Leiopelmatidae    ACAAACATATATAAGAGGTATAGCCTGCCCAGTGACAATT--GTTCAACGGCCGCGGTATTNNNNNNNNNNNNNNNNNACC-
Pelobatidae       AA---TCTGTATAAGAGGTCCCGCCTGCCCAGTGACATCA-TGTTTAACGGCCGCGGTATTNNNNNNNTTAAAGAA-GACT
Rhinophrynidae    AA---TTCAAATAAGAGGTCCAGCCTGCCCAGTGACCATG-TGTTCAACGGCCGCGGTATTNNNNNNNNNNAATAGAGACT
Bufonidae         ----ATACTCATAAGAGGTCCAGCCTGCCCAGTGACCAT---GTTCAACGGCCGCGGTATCNNNNNNNNNNAATGAGGACT
Centrolenidae     ----ATAAATATAAGAGGTCCAGCCTGCCCAGTGACTCT---GTTCAACGGCCGCGGTNNNNNNNNNNNNNNNNNNNNNACT
Colostethus       -----CACCTATAAGAGGTCCAGCCTGCCCAGTGACTCT---GTTCAACGGCCGCGGTATCNNNNNNNNNNAATGAGGACT
Dendrobates       -----AAACAATAAGAGGTCCAGCCTGCC-AGTGACTCATT-GTTCAACGGCCGCGGTATCGTTCT-TTAATTGAGGACT
Heleophrynidae    --------ACATAAGAGGTCCAGCCTGCCCAGTGACTTT---GTTCAACGGCCGCGGNNTTNNNNNNNNNNNNNNNNNGACT
Hemiphractinae    -----AAAACATAAGAGGTCCAGCCTGCCCAGTGACACT---GTTCAACGGCCGCGGTATCNNNNNNNNNNNNNNNNNNNNN
Hyla              -----AAAATATAAGAGGTCCAGCCTGCCCAGTGACTTT---GTTCAACGGCCGCGGTATCTGTCT---TAATGAGACC-
Hyperoliidae      ------AAATATAAGAGGTCCCGCCTGCCCCGTGATTTA---GTTTAACGGCCGCGGTATCNNNNNNNNNNNNNNNNNNNNN
Leptodactylinae   -----CAACTATAAGAGGTCCAGCCTGCCCAGTGACTCT---GTTCAACGGCCGCGGTATCNNNNNNNNNNNNNNNNNNNNN
Limnodynastinae   ------TTATATAAGAGGTCCAGCCTGCCCAGTGACCAT---GTTGANNNNNNNNNNNNNNNNNGTCT---TAAT-AGGACT
Mantellidae       T----CAAATATAAGAGGTCTAGCCTGCCCAGTGACAA----ATTCAACGGCCGCGGTACCNNNNNNNNNNNNNNNNNNNNN
Microhylidae      ------ACATATAGGAGGTCCAGCCTGCCCAGTGACCAA---GTTAAACGGCCGCGGTACCTGTCT---TAATGAGGACT
Myobatrachinae    ------CAAGATAAGAGGTCCAGCCTGCCCAGTGACTTA---GTTCAACGGCCGCGGTATCNNNNNNNNNNAATGAGGACT
Pelodryadinae     ------ACCCATAAGAGGTACAGCCTGCCCAGTGACTCT---GTTTAACGGCCGCGGTATCTGTTCTCTAAATAGGGACT
Phobobates        -----CAATTATAGGAGGTCCAGCCTGCCCAGTGACTTT---GTTCAACGGCCGCGGTATCNNNNNNNNNNNNNNNNNNNNC
Phyllomedusinae   -----AACATATAAGAGGTCTAGCCTGCCCAGTGACTCT---GTTCAACGGCCGCGGNNNNNNNNNNNNNNNNAATAAGGACT
Pseudidae         ------TATTATAAGAGGTCCCGCCTGCCCAGTGACTCT---GTTCAACGGCCGCGGNNNNGTTCT-TTAAATN-GGACC
Ranidae           ------AAATATAAGAGGTCCAGCCTGCCCAGTGACTAA---GTTTAACGGCCGCGGTACCTGTCT---TAATTGGGACT
Rhinodermatidae   -------AACATAAGAGGTCCAGCCTGCCCAGTGATATA---ATTCAACGGCCGCGGTATCNNNNNNNNNNNNNNNNNNNNN
Smilisca          ------AAATATAAGAGGTCCAGCCTGCCCAGTGACTCT---GTTCAACGGCCGCGGTATCTGTCT---TAATGAGGACT
Sooglossidae      ------AAACATAGGAGGTCCTGCCTGCCCAGTGATCCA---ATTCAACGGCCGCGGTATTTGTCT---TAAA-AGGACT
Telmatobiinae     -----AAATCATAAGAGGTCCAGCCTGCCCAGTGACTCT---GTTNAACGGCCGCGGTATCTGTTT---TAAT-TAAGCC
```

**FIG. 1**—*Continued*

Sooglossidae, and Telmatobiinae) is strongly supported, with the Telmatobiinae strongly supported as the sister taxon to this subgroup. These findings provide additional support for the bufonoid phylogeny reconstructed with less extensive species sampling (Hay *et al.*, 1995). Identification of a well-supported pair consisting of the Pelodryadinae and Phyllomedusinae provides a basis for some of the following discussion where we address evolutionary and biogeographic implications of the expanded phylogenetic scheme for the Bufonoidea.

Members of the Sooglossidae are restricted to two islands of the Seychelles Archipelago which has been separated from the Indian continent for over 60 million years (Dickin *et al.*, 1986/1987). It can be proposed that the ancestor of the Sooglossidae was separated from the

| | 880 |
|---|---|
| Discoglossidae | AGTATGAATNGCACCACGAAAGTTCAACTGTCTCCTATATCTAATCAGTGAAACTGATCTCCCCGTGCAGAAGCGGGGAT |
| Leiopelmatidae | AGTATGAACGGCACCACGAGGGCTCAACTGTCTCCCCTGTTTAATCAGTGAAATTGATTTCCCAGTGCAGAAGCTGGGAT |
| Pelobatidae | GGTATGAACGGCATCACGAAGACCTTACTGTCTCCTATATCCAATCAGTGAAACTGATCTCCCCGTGAAGAAGCGGGGAT |
| Rhinophrynidae | GGTATGAATGGCACCACGAAGGTTCAACTGTCTCCCAAATCTAATCAGTGAAACTGATCTCCCCGTGCAGAAGCGGGGAT |
| Bufonidae | AGTATGAATGGCACCACGAAGGTTATACTGTCTCCTTTTTCTAATCAATGAAACTAATCTCCCCGTGAAGAAGCGGGGAT |
| Centrolenidae | TGTATGAATGGCTCCACGAGGGTTATACTGTCTCCTCTTTCCAATCAGTGAAACTAATCTCTCCGTGAAGAAGCGGGGAT |
| *Colostethus* | AGTATGAATGGNCCCACGAGCGGTACGCTGTCTCCTTTTTCTAATCAATGAAACTAATCTTCTCGTGAAGAAGCGAGAAT |
| *Dendrobates* | AGTATGAACGGCTTCACGAAGACTACACTGTCTCCTTTTTCTAATCAATGAAACTAATCTCCCCGTGAAGAAGCGGGGAT |
| Heleophrynidae | TGTATGAATGGCTCCACGAGGGTTGAACTGTCTCCCTCCCCCAATCAGTGAAACTGATCTCCCCGTGAAGAAGCGGGGAT |
| Hemiphractinae | NNNNNNNNNNNNACCACGAGGGTTATACTGTCTCCTTTTTCTAATCAGTGAATCTAATCTCCCCGTGAAGAAGCGGGGTT |
| *Hyla* | AGTATGAATGGCATCACGAAGGTTACACTGTCTCCTTTTTCCAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGGAT |
| Hyperoliidae | NNNATGAACGGCATNACGAGGGTTTAACTGTCTCCCCATTTTAATCAATGAAATTNATCTTCCCGTGAAGAAGCGGGGAT |
| Leptodactylinae | NNNNNNNNNNNNNNNNCGAGGGTTGCACTGTCTCCTTTCTCTAATCAGTGAAACTAATCTTCCCGTGAAGAAGCGGGAAT |
| Limnodynastinae | AGTATGAATGGCATCACGAGGGTTATACTGTCTCCCCTTTTTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGGAT |
| Mantellidae | NNNATCAACGGCATCANGAGGGTTACACTGTCTCCTTTTTCCAATCAGTGAAACTGATCTCTCCGTGAAGAAGCGGAGAT |
| Microhylidae | AGTATGAACGGCATCACGAGGGTTATACTGTCTCCCACCTCCAATCAGTGAAACTGATCCCCCCGTGAAGAAGCGGGGAT |
| Myobatrachinae | AGTATGAATGGCATCACGAGGGTTATACTGTCTCCTTTCCCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGGAT |
| Pelodryadinae | AGTATGAATGGCATCACGAAGGTTGCACTGTCTCCCATCCTTAATCAGTGAAATTAATCCCCCCGTGAAGAAGCGGGGAT |
| *Phobobates* | CGTATGAATGGCCCCACGAGGGCTGCACTGTCTCCTTTTTCTAATCAATGAAACTAATCTCCCCGTGAAGAAGCGGGAAT |
| Phyllomedusinae | AGTATGAATGGNACCACGAAGGTTATACTGTCTCCTTTCCCTAATNAGTGAAACTAATCTCCCCGTGAAGAAGCGAGGAT |
| Pseudidae | AGTATCAACGGCATCACGAGGACTCAACTGTCTCCTCTCCCAAATCAGTGAAACTAATCTCCCTGTGAAAAAGCAGGAAT |
| Ranidae | CGTATCAACGGCATCACGAAGGCTGCACTGTCTCCTTTCTCTAATCAGTGAAACTGATCTCCCCGTGAAGAAGCGGGGAT |
| Rhinodermatidae | NGTATGAACGGCTTCACGAAGGTTATNCTGTCTCCTTTCTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGGAT |
| *Smilisca* | AGTATGAACGGCACCACGAAGGTTATACTGTCTCCTTTTTCTAATCAGTGAAACTAATCTCCCCGTGAAGAAGCGGGGAT |
| Sooglossidae | AGTATGAACGGCCCAACGAAGGCTGCACTGTCTCCTTTCCCCCATCAATGAACCTGATTTCCCCGTGAAGAAGCGAGGAT |
| Telmatobiinae | TGTATGAAAGGCATCACGAGAATCACACTGTCTCCTTTCTTTAGTCAGTGAAACTAATCTCCCCGTGAAAAAGCGAGGAT |

| | 960 |
|---|---|
| Discoglossidae | ATCAACATAAGACGAGAAGACCCCATGGAGCTTTAAACTCTAAG--CACCCACTTTACCTATAAACCCCACGGATTAAAT |
| Leiopelmatidae | AATAACATAAGACGAGAAGACCCCATGGAGCTTAAGACGCATCTCCCCCCCCCCCCCATAGTTTAGGCAGCCA----- |
| Pelobatidae | TTCACCATAAGACGAGAAGACCCCATGGAGCTTTAAACTTAAAT--CACTTGCTTCAATCACTAATCCACGGATAAACA |
| Rhinophrynidae | TTCCACATAAGACGAGAAGACCCTATGGAGCTTTAAACT---AGGACCACCT-GTTTTTCTTTTCAAACCAATCAGGCAT |
| Bufonidae | AAACCTATAAGACGAGAAGACCCTATGGAGCTTTAAACAACATAG-CACCTACCTTATAACAT---AAAATTTCCCGAAC |
| Centrolenidae | AAACACATAAGACGAGAAGACCCTACGGAGCTTTAAACTACAGCAAGCATACTGCTCTGACCCACTTAAATTTCAGAAAA |
| *Colostethus* | AATTTTATAAGACGAGAAGACCCTATGGAGCTTTAAATAACTGAAAAATTTGCACATCCAATTTTTATCTTCCGAGCTCC |
| *Dendrobates* | AAACCTATAAGACGAGAAGACCCTATGGAGCTTTAAACAAACGAAATACCTGCTATTTAACTTTATACCTTCTGAAAACT |
| Heleophrynidae | AAAAATATAAGACGAGAAGACCCCTTGGAGCTTCAAACAACCTGGCACCATGCTTAATAACTTTTTAACTTCGGAATTNC |
| Hemiphractinae | ACAACTATAAGACGAGAAGNNCCTATGGAGCTTTAAACAAAA-CAACATTTGCTAAGTCACACACGAATCTCAGGAAATT |
| *Hyla* | AAATCTATAAGACGAGAAGACCCTATGGAGCTTTAAACTAAATAA-CACTTGCTTTCACACAATCTGCCTCCAGAGCAAC |
| Hyperoliidae | AAGACTATAAGACGAAAAGACCCCATGGAGCTTTAAACCTATCAC-CACTTACCAAACCCTAAAACATACCCCTGGTAAA |
| Leptodactylinae | AAAAATATAAGACGAGAAGACCCTATGGAGCTTTAAACTAAACAATAATAGCCAACCCACTTACACAACTCCAGACGAAT |
| Limnodynastinae | ATTCCTATAAGACGAGAAGACCCCATGGAGCTTTAAACAAACAG--CATATGCTCACCTCACCTACTCTCCGTAAAGTCA |
| Mantellidae | TTTTTTATAAGACGAGAAGACCCCATGGAGCTTTAAACCCACCT------GCACTCCTATTTTCTATTACCTCTAAAAC |
| Microhylidae | AAAATTATAAGACGAGAAGACCCCATGGAGCTTTAAACTCAG---CACCAACCACCTACAACCACATCATCTTAAACAA |
| Myobatrachinae | GAACATATAAGACGAGAAGACCCTATGGAGCTTTAAACTAAATTACATTTGCCTAAAATATAAAAATTTCAGAAAAATAA |
| Pelodryadinae | AAAATTATAAGACGAGAAGACCCCGTGGAGCTTAAAACTAAAGGCACCTGCTTACTCCATATAAAAGTCTAGCACCC--- |
| *Phobobates* | TATCCTATAAGACGAGAAGACCCTATGGAGCTTTAAACAACTGAAACATTTGCTTTTCTCCTAACTCCTCCCGAGCCCTT |
| Phyllomedusinae | AAATATATAAGACGAGAAGACCCTATGGAGCTTAAAACTTTCGGCAATTATCTTATTTATACCCACCCAAGGGGCAATCA |
| Pseudidae | AACAATATAAGACGAGAAGACCCCATGGAGCTTTAAACTTTAAGCAACTTTTAATACTACATTCCTTAAAATATTCTAGC |
| Ranidae | AAAATTATAAGACGAGAAGACCCCATGGAGCTTCAAACTCAT----CAT--GCAACTCTGTCCTCCATATCCCTTAATTC |
| Rhinodermatidae | AAACTTATAAGACGAGAAGACCCTATGGAGCTTTAAACAAATGAA-CAATTGCTATAAATATCC---AAATTTCAGAATT |
| *Smilisca* | AAGAATATAAGACGAGAAGACCCTATGGAGCTTTAAACTAAGTAACACCTGCTTTTAAACATTTTTAACTTCCGAGTACT |
| Sooglossidae | AAACCTATAAGACGAAAAGACCCTATGGAGCTTCAGATGAAATA-TTACATGCCCCAAAATTAACATACC--------- |
| Telmatobiinae | GTCTCTACAAGACGAGAAGACCCTTTGGAGCTTAAGACAAAATAAACACTTGCCAGCCTACACCAACCGTATACCCCCAC |

**FIG. 1**—*Continued*

rest of the proto-bufonoid range during the split of the Indian continent from Africa (153–148 million years ago (MYA); Smith *et al.*, 1994) and remained restricted to the northern part of India + Antarctica/Australia until the disintegration of this supercontinent around 120–130 MYA (Smith *et al.*, 1994). This scenario would involve either an assumption of extinction of members of this family in India and Madagascar or a continuous restriction of the sooglossids to the northern part of the Indian continent. Also, an older date for the separation of the Seychelles might be invoked.

Phylogenetic relationships of the Australian Myobatrachidae to the African Heleophrynidae and of these families to the rest of the bufonoids remain a phylogenetic enigma. Lynch (1973) placed the Cycloraninae (now the Limnodynastinae) together with the Heleo-

| Taxon | 1040 |
|---|---|
| Discoglossidae | TTGTAATCAAGTGGACA-----TGACCCA-CTGTTTTCGGTTGGGGCGACCACGGAGAAAAACACATCCTCCGAGACGAA |
| Leiopelmatidae | ----------------------TGACTTT-CTGTCTTCGGTTGGGGCGACCACGGAGAAAAATA-ATCCTCCGAGCCGAA |
| Pelobatidae | CTTAACCAAGCAACGA------TGATCT--ATGTTTTCGGTTGGGGCGACCACGGAGAATAATAAATCCTCCTCGATGAA |
| Rhinophrynidae | AACCACTAGCTAAGCAGATCT-GGCTCCC-TTGTTTTCGGTTGGGGCGACCACGGAGAAAAAAAAATCCTCCATGAAGAA |
| Bufonidae | ACTTGAATTTTACTGGTATAC-TGACTAT-AAGTTTTTGGTTGGGGTGACCGAGGAGCATAATGCAACCTCCATGTTGAA |
| Centrolenidae | ATCTCCTTCACCTCTGCAGAA-TGGTTGC-CAGTTTTAGGTTGGGGTGACCGCGGAGAAAAAAC-ATCCTCCACATTGAA |
| *Colostethus* | CACTCTCTGAGCATTT------TTATTTC-TAATTTTAGGTTGGGGCGACCACGGAGCAAAATTAAACCTCCACGACGAA |
| *Dendrobates* | AAATATATCTCTAGCACTT---TAATTTC-TAGTTTTAGGTTGGGGTGACCACGGAGAACAAAATAACCTCCACGTTGAA |
| Heleophrynidae | CATTAAAACAAGCACGCA----TGACCAC-AAGTTTTTGGGTGGGGCGACCACGGAGAACAACTAAACCTGCGAGATGTA |
| Hemiphractinae | AATTCTTATTTTAGCCCTA---TGATTGT-TAGTTTTAGGTTGGGGTGACCGCGGAGTAAAAAACAACCTCCACAATGAA |
| *Hyla* | TTACACTTACTTTAGCCTCC--TGATTAT-TAGTTTTAGGTTGGGGTGACCGCGGAGTAAAAAATAACCTCCACATTGAA |
| Hyperoliidae | AA--------------------TGACTGA-CAGTTTTCGGTTGGGGCGACCCTGGAGAAAAACAAATCCTCCAAGCTGAA |
| Leptodactylinae | ACTTTACCCTGGC---TTAA--TAATTTT-TAGTTTTTGGTTGGGGTGACCACGGAGAAAAAAGAAACCTCCGCAATGAA |
| Limnodynastinae | AGCATTG---------------TGACTGC-TAGTTTTCGGTTGGGGCGACCACGGAGAATAACACATCCTCCGCGTTGAA |
| Mantellidae | TATGGAATC-------------TGCATTCTTGGTTTTAGGTTGGGGTGACCGCGGAGCACAATACAGCCTCCACGATGAA |
| Microhylidae | GTGGCCC---------------TGACTGC-TAGTTTTAGGTTGGGGTGACCGCGGAGCAAAACAAAACCTCCACGATGAA |
| Myobatrachinae | AATTTATCTAAGCATTA-----TGATAAT-AAGTTTTGGGTTGGGGTGACCGCGGAGTAAAAAACACCCTCCGCATTGAA |
| Pelodryadinae | ----------------------TGACCCA-TAGTTTTCGGTTGGGGCGACCACGGAGAAAAACCCACCCTCCACGCTGAA |
| *Phobobates* | TATTTTTATTTAAGCATTA---TTATTTC-TAGTTTTAGGTTGGGGTGACCACGGAGCAAAACTAAACCTCCATGAAGAA |
| Phyllomedusinae | AATTTACTTCAATATTA-----TGCCTGT-TAGTTTTCGGTTGGGGTGACCACGGAGTAAAATTCACCCTCCGCAATGAA |
| Pseudidae | TA--------------------TGCCCAA-TAGTTTTAGGTTGGGGCGACCGCGGAGTAAAACTTAGCCTCCATGACAAA |
| Ranidae | AAGAGATG--------------TGCATGT-TAGTTTTGGGTTGGGGGGACCTCGGAGTATAACTTAACCTCCAAAACAAA |
| Rhinodermatidae | ATAAAACTTATCTTTCGCAATTTGATTAC-TAGTTTTAGGTTGGGGTGACCGCGGAGTAAAAGTAACCTCCACATTGAA |
| *Smilisca* | CAACAATAATTTAGCTTGC--TGATTAC-TAGTTTTAGGTTGGGGTGACCGCGGAGCATAAAACAACCTCCGCGTTAAA |
| Sooglossidae | ----------------------TACATAT-TCATCTTCGGTTGGGGTGACCACGGAGAAAAACAAACCCTCCACGACAAA |
| Telmatobiinae | AACATCA---------------TGTTTAT-AAGTCTTAGGTTGGGGCAACCGCGGAGTAAAAACAAACCTCCAAGCTGAA |

| Taxon | 1120 |
|---|---|
| Discoglossidae | AGGGC-----ATACACCCTTAACCTAGAGCCACTACTCCAA-GTAATAGAACCTCTAACT--CCA-TGATCCAATCTT-- |
| Leiopelmatidae | CGGGGCACACACCACCCCTTAACCAAGAGCCACAATTCCAA-GTAATAGAACCTCTAACGG-AGTCTGATCCAAACTAA- |
| Pelobatidae | TGA--------AACAATTTAAACCAAGAGCTACAGCTCTAA-GTATTAGAATATCTAACAT-ACATTGATCCAATCCTA- |
| Rhinophrynidae | AGGA-----AATCTCTCCTAAACCAAGAACCACATTTCTAA-GTATCAGAACTTCTGACTA-TAATTGATCCAGTCTGA- |
| Bufonidae | AGAT------ACTTATTCTTAGCTAAGACCTACCAGTCTAA-GCATCAATA-TATTGACAT-CCATTGACCCAATAAAA- |
| Centrolenidae | AGGGG-----CTTACCCCTTAGCCAAGAGCCACACCTCCAC-GCACCGAAGAAATGGACATTTAATTGACCCAAGATA-- |
| *Colostethus* | GGAGACTCCTT----CTCTTAGCTAAAAGCTACTCCTTTAA-GCATCAGCA-AACTGACTT-CTTTTGACCCAATAAA-- |
| *Dendrobates* | CGAG------CCCTCCTCTTAGCAAAAAGTCACAACTTCAT-GCATCAACA-CATTGACTT-CCATTGACCCAATTTT-- |
| Heleophrynidae | TAGAGTAT-----TACCCTAAGCCAAAAGCCACCGCTTTAA-GCATCAACA-CCTTGACAT-ACATTGACCCATTTA--- |
| Hemiphractinae | TAGGGCCCT-----CCCTTAAGCAAAAAGCCACAACTTTAC-GCATCAACAAAATTGACAT-TAATTGACCCAATATT-- |
| *Hyla* | TGGGAA----AATTCCCCTGAGCCGTGAACTACAACTCTAA-GCACCAATA-AATTGACAT-CAATTGACCCAATATA-- |
| Hyperoliidae | CGATTA----AAATAATCTAATGCAAGAACAACAACTCAAA-CAATCAAAA-TATTGACA--AAA-TGACCCAGAATACA |
| Leptodactylinae | AGA-----CTCTCCTTCTTAATTTTAGGACTACTTTCCTAA-ACATCAATA-AATTGACAT-ATATTGACCCAACAACG- |
| Limnodynastinae | AGAATTCT-----ATTCTTAA-CTAAAAGCTACACCTTTAA-GTATCAACA-CCTTGACAT-CCATTGACCCAATAAAA- |
| Mantellidae | CGGG-----ATTACCCCCTTATCTAAGAGCTACACCTCTAA-GAATTAGCA-TTCTAACAT-AAAATGATCCGACAA--- |
| Microhylidae | CGA------CCTCCTCTCTAATCCGAGAGCCACCACTCAAA-GAATCAAAA-AATTGACAC-ACATTGACCCAAATTATT |
| Myobatrachinae | CAAGA-----TTATTTTTTTAGCTAAGAGCTACAACTCTAA-GCATCAATA-AATTGACAT--TATTGACCCAATATT-- |
| Pelodryadinae | TGACCCCCA-TCTTAGCCCAAAGCCACAGCTTCAAGCATCA-GCATC-------CTGACAC-CCCTTGACCCAATTAA-- |
| *Phobobates* | ATG-------AATATTTTTAAGCCACAAACTACTTTTTTAA-GCATCAACA-AATTGACCT-TCATTGACCCAATATA-- |
| Phyllomedusinae | TAAATT--------ATTTTAAGCAATAAACTACAATTTTAT-GCATCAAAA-AATTGACAC--TATTGACCCAACACT-- |
| Pseudidae | AGG-------ACATTTCCTAATCCAAGAAAGACATTTCTAA-GAATCACTA-ACCTGATGT-TTCATGACCCGACTA--- |
| Ranidae | TGGGTTA-----ACACCTTTATCCGCGAGAAACACCTCTAA-GAATTATTA-AACTAATGC-TTTTTGATCCGATAA--- |
| Rhinodermatidae | AGAA------ATACTTTCTAAACAAAAAACTACAACTTTAT-GTATCAATA-AATTGACAT-CAATTGACCCAATATTT- |
| *Smilisca* | CGGG------GACCCCCCTGAGCGATGAGCTACAACTCTAT-GCACCAATA-TATTGATTT-TCATTGACCCAATATTT- |
| Sooglossidae | ------------------CAAGCCTAAACACACAACTTAAAAGCACCAATA-ATTTAACAC----TTGACCCAATTACAA |
| Telmatobiinae | CGG-------ATACTTCCTAAACAAAAAGCAACAACTTTAT-GTATCATAA--ACTGACAT-AATTTGACCCAATATACT |

**FIG. 1**—*Continued*

phryninae (Heleophrynidae) as a basal lineage of the Bufonoidea, suggesting that it gave rise to the Leptodactylidae which, in turn, served as a source of a vast bufonoid radiation in the Neotropics. At the same time, he proposed that the Myobatrachinae + Sooglossidae gave rise to the ranoids. Lynch's (1973) polyphyletic family, Myobatrachidae, was created to distinguish between the Old World and the New World leptodactyloids. Tyler (1979) argued that no factors other than geographic distribution were used to erect the Myobatrachidae. Accordingly, he referred both the Old World and New World taxa to the Leptodactylidae, insisting that more diagnostic characters were needed for the recognition of an independent Myobatrachidae. Duell-

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                                                                                                          1200
Discoglossidae    -TTGATCAACGAACCAAGTTACCCTGGGGATAACAGCGCAATCCATTTTAAGAGTCCATATCGACAAATGGGTTTACGAC
Leiopelmatidae    -TTGATCAACGAACCAAGTTACCCTGGGGATAACAGCGCAATCCACTCCGAGAGTCCATATCGACGAGCGGGTTTACGAC
Pelobatidae       -TTGATCAACGAACCAAGTTACCCTGGGGATAACAGCGCAATCCATTTCAAGAGTCCATATCGACAAATGGGTTTACGAC
Rhinophrynidae    -CTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCTATTTTAAGAGTTCATATCGACAAATAGGTTTACGAC
Bufonidae         -TTGAACAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAGAGCTCCTATCGACAAGTGGGTTTACGAC
Centrolenidae     -TTGATCATCGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTTAAGAGCCCCTATCGACAAGTGGGTTTACGAC
Colostethus       -TTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCTACTTCAAGAGCTCATATCGACAAGTAGGTTTACGAC
 Dendrobates      -TTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCTACTTCGAGAGTTCTTATCGACAAGTAGGTTTACGAC
Heleophrynidae    -TTGACCAACGAACCAAGTTACCCTGGGGATAACAGCGCAAGTCATTTCTAGAGNCCNTATCGACAGATGGGTTTACGAC
Hemiphractinae    -TTGATCAACGAAACAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAGAGCCCGTATCGACAAGTGGGTTTACGAC
Hyla              -TTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCGCTTTAAGAGCCCCTATCGACAAGCGGGTTTACGAC
Hyperoliidae      TCTGATCAACGAACCAAGTTACCCTGGGGATAACAGCGCAATCCATTTCAAGAGCCCCTATCGACAAATGGGTTTACGAC
Leptodactylinae   -TTGATCAATGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGAC
Limnodynastinae   -TTGATCAACGAACCTAGTTACCCTGGGGATAACAGCGCAATCCATTTCAAGAGCCCCTATCGACAAATGGGTTTACGAC
Mantellidae       -TCGATCAACGAACCAAGTTACCCTGGGGATAACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGAC
Microhylidae      -TTGATCAACGAACCAAGTTACCCTGGGGATAACAGCGCAATCCATTTCAAGAGCTCCTATCGACAAATGGGTTTACGAC
Myobatrachinae    -TTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAGAGCTCCTATCGACAAGTGGGTTTACGAC
Pelodryadinae     -TTGATCAACGAACCAAGTTACCCCGGGGATAACAGCGCAATCTACTTCAAGAGCTCCTATCGACAAGTGGGTTTACGAC
Phobobates        -TTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCTACTTCAAGAGCTCATATCGACAAGTAGGTTTACGAC
Phyllomedusinae   -TTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCATCTCAAGAGCTCATATCGACAGATGGGTTTACGAC
Pseudidae         -TCGATCAACGAACCAAGTTACCCTGGGGATAACAGCGCAATCCATTTTGAAAGCTCCTATCGACAAATGGGTTTACGAC
Ranidae           -TCGATCAATGGACCAAGTTACCCTGGGGATAACAGCGCAATCTACTTCAAGAGTTCATATCGACAAGTGGGTTTACGAC
Rhinodermatidae   -TTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCTACTTCAAGAGCCCATATCGACAAGTAGGTTTACGAC
Smilisca          -TTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCGCTTCAAGAGCCCCTATCGACAAGCGGGTTTACGAC
Sooglossidae      -TTGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCATTTCAAGAGCCCATATCAACAAATGGGTTTACGAC
Telmatobiinae     -TTGATTAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCACTTCAAGAGTTCATATCGAAAAGTGGGCTTACGAC
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                                                                   16s1> 1258
Discoglossidae    CTCGATGTTGGATCAGGGTGTCCTAGTGGTGCAGCCGCCACTAAGGG-TTCGTTTGTT
Leiopelmatidae    CTCGATGTTGGATCAGGACCCCCTAATGGTGAAGCCGCTATTAACGTGTTCGTTTGTT
Pelobatidae       CTCGATGTTGGATCAGGGCATCCCAGTGGTGCAGCCGCTACTAAAGG-TTCGTTTGTT
Rhinophrynidae    CTCGATGTTGGATCAGGGCCCCCCAGTGGTGCAGCCGCTACTAACGG-TTNGTTTGTT
Bufonidae         CTCGATGTTGGATCAGGGTATCCCAGTGGTGCAGCCGCTACTAAAGG-TTCGTTTGTT
Centrolenidae     CTCGATGTTGGATCAGGGTATCCCGGTGGTGCAGCCGCTACCAAAGG-TTCGTTTGTT
Colostethus       CTCGATGTTGGATCAGGGTATCCTAGTGGTGCAGCCGCTACTAATGG-TTCGTTTGNN
Dendrobates       CTCGATGTTGGATCGGGGTATCCTGGTGGTGCAGCCGCTACTAAAGG-TTCGTTTGTT
Heleophrynidae    CTCGATGTTGGATCAGGGCATCCGGGTGGTGNAGGCGCTACCGACGG-TTCGTTTGTT
Hemiphractinae    CTCGATGTTGGATCAGGGTACCCCAGTGGTGCAGCCGCTACNNNNNNNNNNNNNNNNN
Hyla              CTCGATGTTGGATCAGGGTATCCCAGTGGTGCAGCCGCTACTAAAGG-TTCGTTTGTT
Hyperoliidae      CTCGATGTTGGATCGGGATATCCTAGCAGCGCAGCAGTTGCTCAAGG-TTCGTNNNNN
Leptodactylinae   CTCGATGTTGGATCAGGGTATCCCAGTGGTGCAGCCGCTACTAAAGG-TTCGTTTGTT
Limnodynastinae   CTCGATGTTGGATCAGGGTATCCCAGTGGTGCAGCCGCTACTAAAGG-TTCGTTTNNN
Mantellidae       CTCGATGTTGGATCAGGGTATCCTAGTGGTGCAGCAGCTACTAAAGNNNNNNNNNNNN
Microhylidae      CTCGATGTTGGATCAGGGTATCCCAGTGGTGCAGCCGCTACTAAAGG-TTCGTTTGTT
Myobatrachinae    CTCGATGTTGGATCAGGGTATCCCAGTGGTGCAGCCGCTANTAAAGG-TTCGTTTGTT
Pelodryadinae     CTCGATGTTGGATCAGGGTA-CCAAGTGGTGCAGCCGCTACTAAAGG-TTCGTTTGTT
Phobobates        CTCGATGTTGGATCAGGGTATCCTAGTGGTGCAGCCGCTACTAATGG-TTCGTTTGTT
Phyllomedusinae   CTCGATGTTGGATCAGGGTACCCCAGTGGTGCAGCCGCTNNNNNNNNNNNNNNNNNNN
Pseudidae         CTCGATGTTGGATCAGGATACCCAGGTGGTGCAGNNNNNNNNAAAGG-TTTGTTTGTT
Ranidae           CTCGATGTTGGATCAGGGTATCCTGGTGGTGCAACCGCTACTAATGG-TTCGTTTGTT
Rhinodermatidae   CTCGATGTTGGATCAGGGTACCCCAGTGGTGCAGCCGCTACTAACGG-TTCGTTTGTT
Smilisca          CTCGATGTTGGATCAGGGTACCCCAGTGGTGCAGCCGCTACTAAAGG-TTCGTTTGTT
Sooglossidae      CTCGATGTTGGATCAGGGTATCCTAGTGGTGCAACCGCTACTAAAGG-TTCGTTTGTT
Telmatobiinae     CTCGATGTTGGATCAAGGTACCCTGGTGGTGCAGCCGCTACCAAAGG-TTCGTTTGTT
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**FIG. 1**—*Continued*

man and Trueb (1986) placed a taxon composed of the Myobatrachidae + Sooglossidae and the Heleophrynidae at the base of the neobatrachian radiation. Ford and Cannatella (1993) identified the Myobatrachinae and Sooglossidae as sister taxa (excluding the Limnodynastinae) on the basis of "at least five shared derived characters" of morphology. Based on our data, it is tempting to argue that the Heleophrynidae, Limnodynastinae, and Myobatrachinae (with the former two clustering) constitute a monophyletic group equal to the Myobatrachidae (*sensu* Lynch). However, given the degree of support for the appropriate nodes on the tree (Fig. 2), we can claim only that these three taxa are bufonoids ($P_C = 0.96$) but are likely to be excluded from

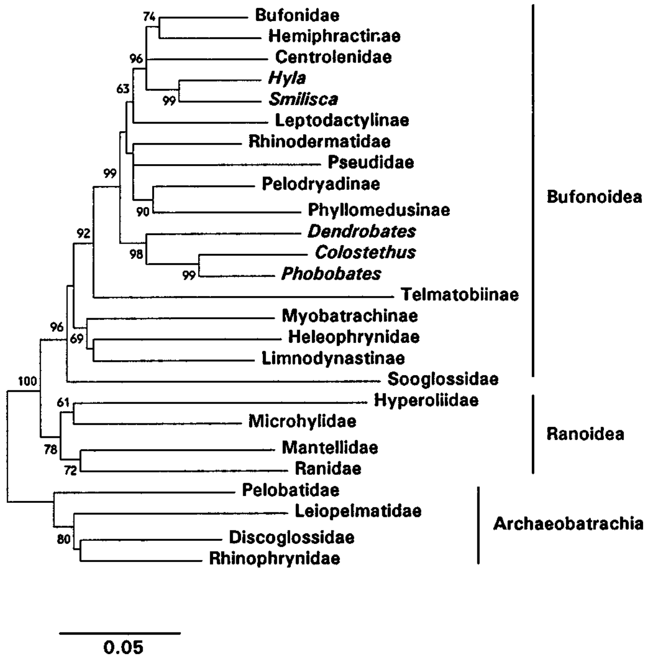


**FIG. 2.** Phylogenetic relationships of the Neobatrachia based on a neighbor-joining analysis of combined mitochondrial 12S and 16S rRNA gene sequences (Jukes-Cantor distances, pairwise deletion; 899 aligned sites, 465 variable). The Archaeobatrachia is used as an outgroup. The following bufonoid families were represented by more than one sample: Dendrobatidae (*Colostethus*, *Dendrobates*, and *Phobobates*), Hylidae (Hemiphractinae, Hylinae (*Hyla* and *Smilisca*), Pelodryadinae, and Phyllomedusinae), Leptodactylidae (Leptodactylinae and Telmatobiinae), and Myobatrachidae (Limnodynastinae and Myobatrachinae). Numbers on the tree represent confidence value expressed as percentages from the interior-branch test (values below 50% are not shown). The distance scale is drawn below the tree.

the neotropical radiation ($P_C = 0.92$). Their relationships with the Sooglossidae also are unclear. Based on the paleogeographic data (Smith *et al.*, 1994), speciation events giving rise to the three separate myobatrachid (*sensu* Lynch) lineages could have taken place 145–150 MYA, when Antarctica/Australia separated from Africa, thus determining the present-day distribution of these taxa. This date is in a good agreement with the one for the divergence of the Sooglossidae, considering their proximity on the tree.

Our data suggest an extensive bufonoid radiation with an apparent center in South America. Somewhat in accord with the morphology-based proposal (Lynch, 1973) we see a member of the Leptodactylidae at the base of this event. However, it is a representative of the Telmatobiinae (*Eleutherodactylus cuneatus*) which, according to Lynch's (1971) scheme, was deeply nested within the family tree and not basal to the rest of the neotropical leptodactylids as shown by our data (Fig. 2). The second member of this family which is included in the present analysis, *Lithodytes lineatus* (Leptodactylinae), was placed within the neotropical bufonoids, not with the Telmatobiinae, suggesting a polyphyletic Leptodactylidae. Since the majority of the bufonoid radiation is restricted to South America and because the bufonoid diversity is the highest in this region, it is likely that initial radiation took place there after both Africa and Australia became separated. The present-day distribution of the cosmopolitan bufonids and hylines can be explained as a series of secondary dispersal events (see comments on the Pelodryadinae below).

Antarctica/Australia became disjoined from South America around 153 MYA (Smith *et al.*, 1994). They were reconnected by a narrow land bridge between 140 and 130 MYA. The area of contact between South America and Africa was more extensive; they achieved a substantial degree of separation around 125 MYA (Smith *et al.*, 1994) and became completely disjoined before 100 MYA. The several multifurcating nodes on our tree (Fig. 2) are likely a reflection of an explosive radiation taking place among the Neotropical bufonoids soon after they became restricted to South America, thus dating the event around 110–120 MYA. More extensive sampling of diverse leptodactylid lineages as well as bufonids and hylids from different localities in the Old and New Worlds will be needed to test this biogeographic hypothesis.

The Pelodryadinae is a biogeographic oddity. While the three other hylid subfamilies are predominantly South American (Hylinae is cosmopolitan), pelodryadines are restricted to Australia and New Guinea. Savage (1973) raised this subfamily to familial level, suggesting a leptodactyloid ancestry of unspecified affinity. Tyler (1979) argued for the recognition of the Australo-Papuan tree frogs as a subfamily of the Hylidae. Molecular (Maxson, 1976) and morphological (reviewed in Tyler, 1979) studies aimed at identifying a sister group of the Pelodryadinae found no apparent association between the members of this subfamily and any other bufonoid lineage tested. Our present data suggest the South American Phyllomedusinae as the most likely candidate for such association. Following the line of paleogeographic considerations above, a divergence between these two lineages had to take place immediately after or during the extensive bufonoid radiation in South America, but at or before the time of the separation of Australia. Otherwise, it would be necessary to postulate an unlikely dispersal event either across the Drake Passage or the Pacific. Considering the short internal nodes in our tree, it is possible that an ancestral phyllomedusine diverged from an ancestral pelodryadine within a few million years of the beginning of a large-scale bufonoid diversification in the Neotropics. Thus the timing of this speciation can be roughly placed at 110–120 MYA. According to the paleogeographic data (Smith *et al.*, 1994) an Antarctica/Australia supercontinent remained connected to South America from 140 until 130 MYA, after which there was an archipelago link until approximately 100–105 MYA. Therefore, the inferred dates of major land mass rearrangements are within a time frame allowing all necessary speciations to occur. However, this model would require an assumption of an explosive radiation as well as rapid expansion of the ranges occupied by different lineages and, perhaps, a dispersal event through an archipelago linking South America with Antarctica/Australia. Some estimates suggest that the average temperatures in the latitudes where colonization and initial radiation are postulated to have taken place could have been about 20°C higher than at present (Barron, 1983), thus making Antarctica habitable to frogs. A split of Australia from Antarctica around 80 MYA allows ample time for the range expansion. An estimated divergence between the major groups of the Pelodryadinae (55 million years; Hutchinson and Maxson, 1987) lies well within this date.

The only other well-defined cluster ($P_C = 0.96$) within the Bufonoidea consists of the representatives of three currently recognized families: Bufonidae, Centrolenidae, and Hylidae. A significant pairing of the two hylines (*Hyla* and *Smilisca*) presents little surprise. Most workers also associate the Hylidae and Centrolenidae (Lynch, 1973; Duellman and Trueb, 1986; Ford and Cannatella, 1993). On the other hand, the suggestion of an association of the Bufonidae with the hylid subfamily Hemiphractinae is totally unexpected. We predict that wider taxonomic sampling of both the Hylidae and the Bufonidae—including additional representatives of both Old World and New World genera—will clarify relationships among these lineages. We further predict that such analyses will still show that hylid and bufonid lineages are closer to one another than they are to lineages of other bufonoid families, as already indicated in Fig. 2.

## CONCLUSIONS

Our analysis of portions of mitochondrial 12S and 16S rRNA genes for additional representatives of the Neobatrachia revealed some new patterns of phylogenetic relationships within the suborder. There is additional evidence for a deep split between the Bufonoidea and Ranoidea. We are able to assign the Sooglossidae to the bufonoids; however, its sister taxon is not identified. A weakly supported group including the Australian Myobatrachidae and African Heleophrynidae is placed basally to the rest of the Bufonoidea, supporting morphology-based classifications. The leptodactylid Telmatobiinae appears to lie at the base of bufonoid radiation in the Neotropics. This radiation may have served as a source for the main portion of the rich South American anuran fauna and later provided initial stocks for expansion into North America and the rest of the world. The Dendrobatidae is monophyletic and within the neotropical Bufonoidea. The Pelodryadinae and Phyllomedusinae cluster, as do the Hylinae, the Centrolenidae, the Bufonidae, and the Hemiphractinae. These arrangements suggest that the Hylidae, the Leptodactylidae, and the Myobatrachidae may not be monophyletic families. Based on the phylogenetic relationships advocated here and the paleogeographic data, we propose a scenario in which a series of vicariant events divided species ranges, resulting in speciation and accounting for the present-day species distribution. Thus, this study provides a new phylogenetic scheme for the Neobatrachia and identifies areas where additional work needs to be done. We also support our earlier assertion that extensive species sampling and an increased amount of sequence data will be required to resolve enigmatic and controversial phylogenetic relationships among the Anura.

## APPENDIX

**Species for which rRNA Gene Sequences Were Obtained**

| Family | Species | Locality | Specimen |
|---|---|---|---|
| Dendrobatidae | *Colostethus pratti* | Panama | LM 1143-A |
| | *Phobobates trivittatus* | Peru, Panguana | LM 739-A |
| Hylidae | | | |
| Hemiphractinae | *Gastrotheca riobambae* | Ecuador, San Rafael | LM 3176 |
| Hylinae | *Smilisca phaeota* | Ecuador, Esmeraldas Province | LM 2504 |
| Pelodryadinae | *Litoria cyclorhynchus* | Western Australia, Angerup | LM 3175 |
| Phyllomedusinae | *Phyllomedusa palliata* | Peru, Cuzco Amazonico | LM 2010 |
| Leptodactylidae | | | |
| Leptodactylinae | *Lithodytes lineatus* | Peru | LM 269 |
| Myobatrachidae | | | |
| Myobatrachinae | *Pseudophryne guentheri* | Western Australia, Mt. Margaret | LM 2725 |

*Note.* LM, frozen tissue collection of LRM. Voucher specimens are available for LM 3176 (UNIMNH 94580), LM 3175 (SAMA R20141), LM 2010 (KU 205420), and LM 2725 (WAM 101218). Collections are from the University of Illinois, Museum of Natural History (UIMNH), South Australian Museum (SAMA), University of Kansas (KU), and Western Australian Museum (WAM).

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