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Novel relationships among hyloid frogs inferred from 12S and 16S mitochondrial DNA sequences

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Abstract

Advanced frogs (Neobatrachia) are usually divided into two taxa, Ranoidea (the firmisternal frogs) and Hyloidea (all other neobatrachians). We investigated phylogenetic relationships among several groups of Hyloidea using 12S and 16S rRNA mitochondrial gene sequences and tested explicit relationships of certain problematic hyloid taxa using a sample of 93 neobatrachians. Parsimony, maximum likelihood, and Bayesian inference methods suggest that both the Ranoidea and Hyloidea are well-supported monophyletic groups. We reject three hypotheses using parametric bootstrap simulation: (1) Dendrobatidae lies within the Ranoidea; (2) The group containing Hylidae, Pseudidae, and Centrolenidae is monophyletic; and (3) *Brachycephalus* is part of Bufonidae.

Keywords: Anura; Parametric bootstrapping; Brachycephalidae; Centrolenidae; Dendrobatidae; Frogs; Hylidae; Hyloidea; Mitochondrial DNA; Neobatrachia; Phylogeny; Pseudidae; Ranoidea; Systematics; 12S; 16S

1. Introduction

The frogs and toads (Anura) include more than 4800 species in at least 26 families (Frost, 1985, 2002). Frogs were partitioned into Archaeobatrachia (''primitive'' frogs) and Neobatrachia (''advanced'' frogs) by Reig (1958) based on the presence of free ribs and the type of vertebrae in the ''primitive'' frogs; this arrangement was followed by Tihen (1965) and Duellman (1975). Based on morphological data, Cannatella (1985) and Ford and Cannatella (1993) argued that archaeobatrachians were paraphyletic with respect to Neobatrachia. In contrast, analyses based on DNA sequence data have supported the monophyly of Archaeobatrachia (Hay et al., 1995). The monophyly of Neobatrachia, however, was strongly supported by both molecular and morphological datasets.

The separation of the Neobatrachia into two units, Bufonoidea (more correctly, (Hyloidea Dubois, 1983)) and Ranoidea, has been accepted by most investigators of anuran classification since the mid-1800s (Lynch, 1973). The separation of hyloids and ranoids rests on morphological characters: shape of the vertebral centrum, pectoral girdle architecture, and conformation of thigh musculature (Ford and Cannatella, 1993; Lynch, 1973). Whereas morphological studies have suggested that hyloids are paraphyletic to ranoids (Ford, 1989; Kluge and Farris, 1969; Lynch, 1971, 1973), molecular analyses corroborate two monophyletic groups, Hyloidea and Ranoidea (Hay et al., 1995; Ruvinsky and Maxson, 1996; Vences et al., 2000). However, the placement of some basal neobatrachian clades (Heleophrynidae, Myobatrachidae, and Sooglossidae) remains uncertain. Given this, we here associate the name Hyloidea with a less inclusive and more stable clade, specifically the most recent common ancestor of Eleutherodactylini, Bufonidae, Centrolenidae, Phyllomedusinae, Pelodryadinae, and Ceratophryinae. This definition of Hyloidea is node-based (de Queiroz and Gauthier, 1992) and we elaborate upon our rationale in Section 4.

Within this more restricted clade Hyloidea, we address the relationships of certain taxa whose placement

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has been disputed. First, most morphological studies have proposed that Dendrobatidae, the poison frogs, be placed within Ranoidea based on the fusion of the epicoracoid cartilages (firmisterny) of the pectoral girdle (Duellman and Trueb, 1986; Ford, 1993; Ford and Cannatella, 1993; Griffiths, 1959), whereas molecular analyses have placed Dendrobatidae within Hyloidea (Hay et al., 1995; Ruvinsky and Maxson, 1996; Vences et al., 2000).

A second area of conflict is the relationships of the Hylidae, Pseudidae, and Centrolenidae. Pseudidae and Centrolenidae have traditionally been grouped together with the Hylidae based solely on the presence of intercalary elements, which are supernumerary skeletal elements between the distal and next-to-distal elements of the fingers and toes (Duellman and Trueb, 1986; Ford and Cannatella, 1993; Lynch, 1973). Molecular data, however, have placed Pseudidae sister to either Rhinodermatidae or Leptodactylidae (Hay et al., 1995; Ruvinsky and Maxson, 1996).

Brachycephalidae is also problematic. *Brachycephalus* was thought to be most closely related to *Atelopus* (Bufonidae) based on pectoral girdle similarities (Griffiths, 1959; Lynch, 1973; Noble, 1931). Later, McDiarmid (1971) placed *Brachycephalus* in its own family based mostly on lack of a Bidder's organ, which is otherwise found only in Bufonidae. Recently, however, Brachycephalidae has been suggested to have a close relationship to *Euparkerella* (Izecksohn, 1971, 1988), a leptodactylid of the tribe Eleutherodactylini. None of these phylogenetic hypotheses have been explicitly tested.

To address the phylogenetic relationships and test explicit phylogenetic hypotheses among the smaller hyloid families, we analyzed a 2.4 kb region spanning 12S and 16S rRNA mitochondrial genes and the intervening tRNA valine in 93 neobatrachian taxa. We address the following questions: (1) Is Dendrobatidae part of Ranoidea or Hyloidea? (2) Do Hylidae, Centrolenidae, and Pseudidae form an exclusive clade? (3) What is the relationship of *Brachycephalus* to other hyloideans?

2. Materials and methods

2.1. Taxa

We used 79 sequences from the ingroup (hyloid families Bufonidae, Dendrobatidae, Centrolenidae, Hylidae, Leptodactylidae, Brachycephalidae, and Pseudidae). The only families of hyloids not sampled were Rhinodermatidae (two species) and Allophrynidae (one species). Monophyly of the ingroup is based on published analyses (Ruvinsky and Maxson, 1996) as well as our unpublished data. Outgroup taxa consist of 14 sequences from Myobatrachidae, Heleophrynidae, and Ranoidea (Ranidae, Microhylidae, Rhacophoridae, and Hyperoliidae). Forty new sequences were added to taxa previously sequenced in the Cannatella lab (Basso and Cannatella, in prep.) to diversify taxon sampling so that relationships within Hyloidea could be estimated more accurately (Appendix A). The taxonomy generally follows Frost (2002) except that we retained the use of *Hylactophryne* (rather than *Eleutherodactylus*) and *Phrynomerus* (rather than *Phrynomantis*). Also, Eleutherodactylini is treated as a tribe rather than the subfamily Eleutherodactylinae (Frost, 2002; Laurent, 1986).

2.2. DNA amplification and sequencing

Genomic DNA was extracted from liver or muscle tissue using the Quiagen DNAeasy kit. The polymerase chain reaction (PCR) was used to independently amplify four overlapping DNA fragments spanning 2.4 kb of 12S and 16S mitochondrial rRNA genes and the intervening tRNA gene for valine, which corresponds to positions 2185–4574 in the complete mitochondrial sequence of *Xenopus laevis* (GenBank Accession No. NC 001573, derived from M10217; provisional reference sequence). Combinations of primers MVZ59, tRNAphe, tRNAval, MVZ50, 12L1, 16SH, 12SM, 16SA, 16SC, and 16SD were used (Goebel et al., 1999; Table 1). Standard PCR conditions (Palumbi, 1996) were used with the following thermal cycle profile: 2 min at 94 °C, followed by 35 cycles of: 94 °C for 30 s, 46 °C for 30 s, and 72 °C for 60 s. Annealing temperature and/or numbers of cycles were slightly modified as needed to improve the quality of the PCR product. This product was purified using the QIAquick Gel Extraction Kit. Cycle sequencing reactions were completed with ABI Prism BigDye Terminator chemistry (Versions 2 and 3; Applied Biosystems). Sequencing was performed on an ABI 3100 PRISM sequencer with the following conditions for 25 cycles: 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min.

2.3. Sequence analysis

Contiguous sequences from eight completely overlapping fragments were constructed in Sequencher 4.1 (GeneCodes), and DNA sequences were aligned using Clustal X 1.8 under a variety of gap penalty weightings (Thompson et al., 1997). Using MacClade 4.0 (Maddison and Maddison, 2000), manual alignment adjustments were made to minimize informative sites under the parsimony criterion. Secondary structure models from the Gutell lab website (www.rna.icmb.utexas.edu) were used to help make decisions about ambiguous regions. Regions of the alignment for which homology of the sites could not be inferred were excluded from analysis.

Table 1
Primers used to amplify and sequence 12S, tRNA-val and 16S rRNA mitochondrial genes

Primer name	Primer sequence 5′ to 3′ (indicated by arrows)	Position[a]	Goebel No.[b]
MVZ59	ATAGCACTGAAAAYGCTDAGATG →	2153–2180	29
tRNAphe	GCRCTGAARATGCTGAGATGARCCC →	2161–2185	30
12L1	AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT →	2475–2509	46
12SM	GGCAAGTCGTAACATGGTAAG →	2968–2989	–
tRNAval	GGTGTAAGCGAGAGGCTT ←	3033–3059	73
MVZ50	TCTCGGTGTAAGCGAGAAACTT ←	3042–3063	72
16SH	GCTAGACCATKATGCAAAAGGTA ←	3282–3304	76
16SC	GTRGGCCTAAAAGCAGCCAC →	3623–3642	–
16SA	ATGTTTTTGGTAAACAGGCG ←	3956–3976	87
16SD	CTCCGGTCTGAACTCAGATCACGTAG ←	4549–4574	–

[a] As in Roe et al. (1985).
[b] Primers with no designated number were designed in the Cannatella lab, not modified from Goebel et al. (1999).

Parsimony analyses were performed with PAUP* 4.0b8 (Swofford, 2000) using heuristic searches under parsimony (all characters weighted equally, gaps were not scored as characters) with TBR branch swapping, and 1000 random addition sequence replicates. In order to obtain estimates of clade support, non-parametric bootstrapping was performed with heuristic searches of 1000 replicate datasets and 50 random addition sequences per dataset (Felsenstein, 1985).

For maximum likelihood analyses, a model of sequence evolution was estimated for the data set using MODELTEST (Posada and Crandall, 1998). Parameters were estimated from the most parsimonious trees and fixed for further analysis. Three independent maximum likelihood heuristic searches were performed with PAUP* 4.0b8 using random starting trees (rather than random-taxon addition). TBR branch swapping was used to swap to completion.

Bayesian analyses under the model determined by MODELTEST were performed with a beta version of MrBayes3b4 (Huelsenbeck and Ronquist, 2001) on Phylocluster, a NPACI Rocks cluster (www.rocksclusters.org) composed of one master node with eight slave nodes, each of which uses dual AMD 1533 MHz processors with 2 GB RAM. The Bayesian analysis uses Markov Chain Monte Carlo to estimate the target posterior probability distribution over tree topologies and evolutionary model parameters. Preliminary runs were performed to assess the appropriateness of the default Markov Chain proposal settings. For the final four independent runs, the γ-shape parameter and base frequency proposal distributions were changed to allow between 20 and 50% acceptance rate and therefore sample the target distribution more effectively. The default values of four Markov chains and the "temperature" parameter value of 0.2 were used to help avoid entrapment in local topological optima and to traverse tree space more broadly. The default priors were assumed: a uniform prior for topology, a uniform distribution (0,1) for proportion of invariant sites, a uniform distribution (0.1, 50) for the α-shape parameter, and a prior of exp(10) for branch lengths. A uniform dirichlet distribution (multinomial form of the beta distribution) was assumed for base frequencies and the rate matrix. The Markov chain length was 5,000,000 generations for two of the runs, 4,800,000 generations for a third, and 4,770,000 generations for the fourth. All chains were sampled every 100 generations. The first 5000 samples were discarded as burn-in; this value was found to be appropriate and conservative by plotting the likelihood and parameter values of the four runs to determine at what point the values had reached stationarity. The parameter values and bipartition posteriors were similar for the four independent runs; therefore all 175,515 post-burn-in trees were used. The proportion of the trees that contained each of the observed bipartitions was used as an estimate of the posterior probabilities (Larget and Simon, 1999).

2.4. Hypothesis testing

Three a priori hypotheses (H_0) were tested against the tree estimates obtained from the observed sequence data set: (1) Dendrobatidae is part of Ranoidea (Duellman and Trueb, 1986; Ford, 1993; Ford and Cannatella, 1993; Griffiths, 1959), (2) monophyly of Hylidae + Pseudidae + Centrolenidae (Duellman and Trueb, 1986; Ford and Cannatella, 1993; Lynch, 1973), and (3) *Brachycephalus* is part of Bufonidae (Griffiths, 1959; Lynch, 1973; Noble, 1931). We used the parametric bootstrap test to compare the best tree score from the observed data (H_A) to the best tree score obtained from a topology constrained to represent H_0 (Buckley, 2002; Goldman et al., 2000; Huelsenbeck et al., 1996). The observed dataset was used to calculate the difference (H_0–H_A) between the shortest tree score under the null hypothesis and the shortest tree score under the alternative hypothesis. A null distribution of tree length differences was generated by simulating 500 datasets (SeqGen, V. 1.2.5.) using the model of evolution which

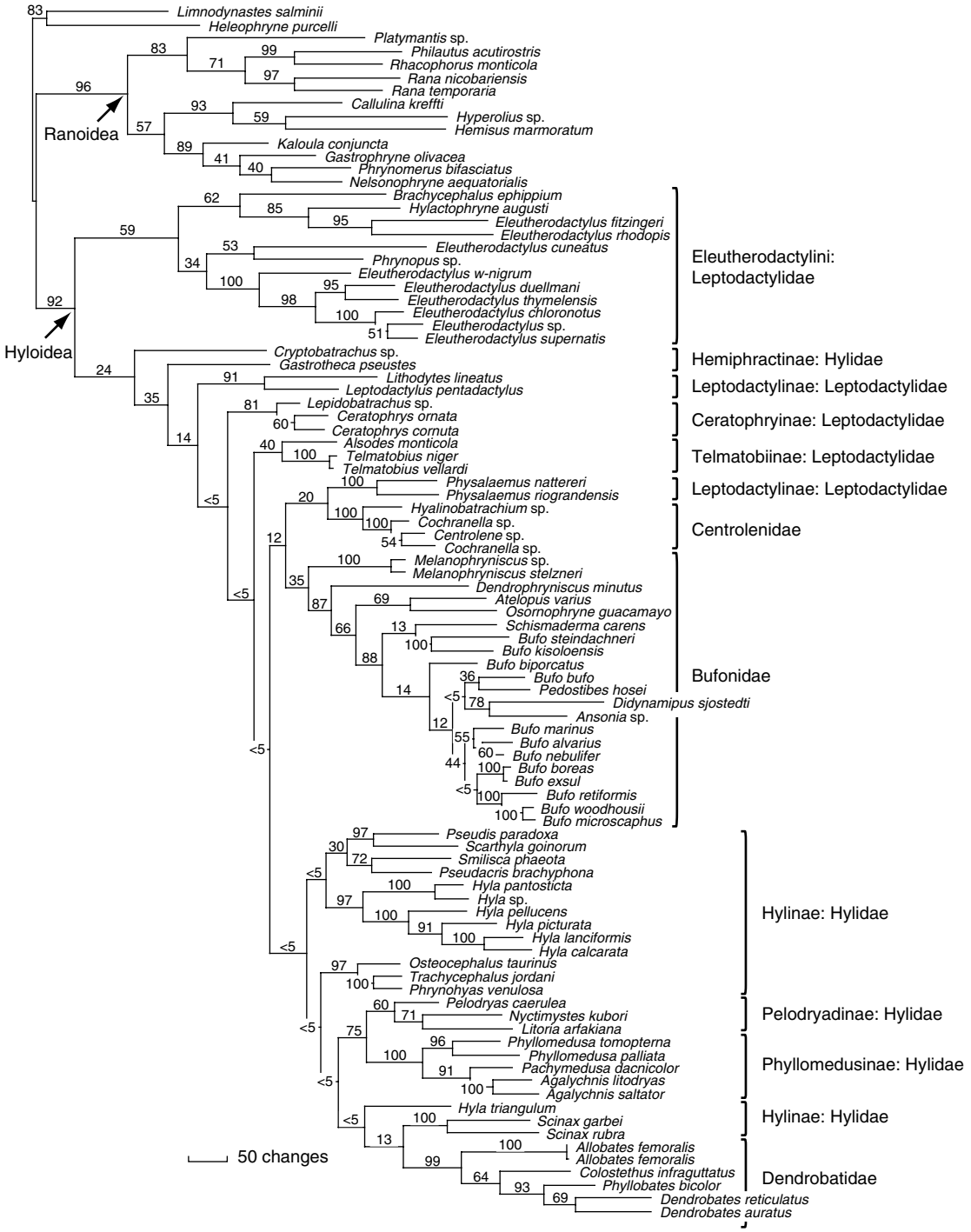

Fig. 1. Maximum parsimony phylogram rooted with *Limnodynastes salminii* (Myobatrachidae) and *Heleophryne purcelli* (Heleophrynidae). Numbers above branches indicate non-parametric bootstrap values based on 1000 pseudoreplicates. Hyloid clades are labeled with family, subfamily, or tribe name. Families included are Brachycephalidae, Leptodactylidae (includes subfamilies: Telmatobiinae [including the tribe Elutherodactylini], Leptodactylinae, and Ceratophryinae), Centrolenidae, Bufonidae, Pseudidae, and Hylidae (includes subfamilies Hemiphractinae, Hylinae, Pelodryadinae, and Phyllomedusinae).

best described the observed sequence data under the null hypothesis. For each simulated data set, the difference in tree scores under H_0 and H_A was calculated. These 500 differences comprised the expected difference to which the observed difference was then compared. If the observed difference was greater than 95% of the 500 differences computed from the simulated data sets, then the observed difference was judged to be significantly different from the null distribution, and therefore, the null hypothesis was rejected.

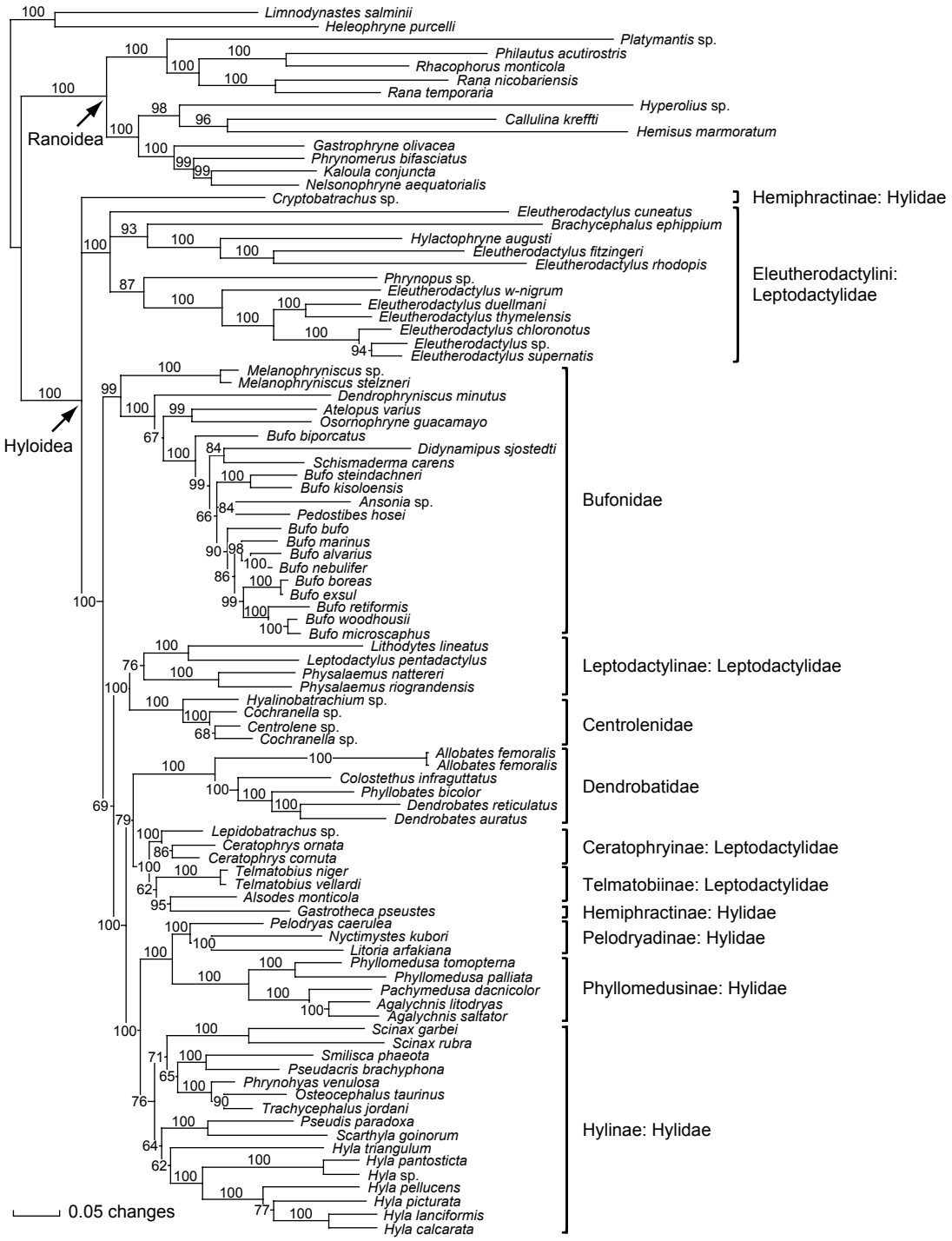

Fig. 2. Maximum likelihood phylogram under a GTR + Γ + I model of evolution. Numbers above branches indicate posterior probabilities recovered from the Bayesian analysis. Hyloid clades are labeled as in Fig. 1.

3. Results

3.1. Parsimony analysis

Unweighted parsimony analysis of the 2001 included characters (of which 1040 were parsimony-informative; 498 ambiguous sites were excluded from the analysis) yielded three most-parsimonious reconstructions each with a score of 11,763 steps, CI = 0.198 and RI = 0.436 (Fig. 1). All three trees supported a monophyletic Hyloidea (Hylidae, Leptodactylidae, Bufonidae, Centrolenidae, Pseudidae, and Brachycephalidae), and monophyletic Ranoidea (''Ranidae,'' Microhylidae, Hyperoliidae, and Rhacophoridae), with high non-

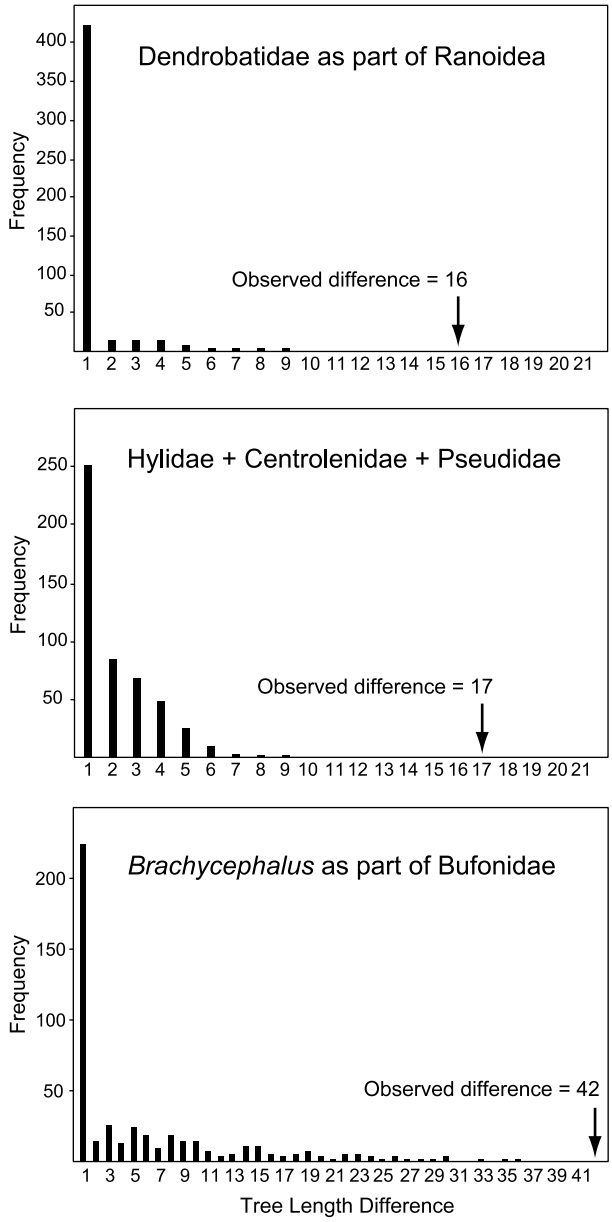

Fig. 3. Null distributions for the parametric bootstrap test. All observed tree length differences fall outside of their respective null distribution and are therefore significant at $P < 0.002$.

parametric bootstrap values (bp) of 92 and 96, respectively (Fig. 1). Between Hyloidea and Ranoidea, uncorrected sequence divergence varied from 15 to 27%, and within-Hyloidea sequence divergence reached 23%. Non-parametric bootstrap resampling revealed that no interfamilial relationships within Hyloidea have support values greater than 50%. Three monophyletic hyloid families were recovered: Dendrobatidae, Bufonidae, and Centrolenidae (bp = 99, 35, and 100).

Although relationships within Ranoidea are not the focus of these analyses, our limited taxon sampling recovered three major clades: one with ranine ranids, platymantine ranids, and Rhacophoridae; another with brevicipitine microhylids, Hyperoliidae, and *Hemisus*; and a third composed of the remaining microhylids. This renders Microhylidae non-monophyletic.

Hylidae is polyphyletic. Pseudidae, as represented by *Pseudis paradoxa*, is most closely related to the hyline *Scarthyla goinorum* (bp = 97). The two representatives of the hylid subfamily Hemiphractinae, *Cryptobatrachus* sp. and *Gastrotheca pseustes*, are the sequential sister-groups to the clade containing all hyloids except *Brachycephalus* and the eleutherodactylines, but this relationship is poorly supported (bp < 50).

Brachycephalidae, as represented by *Brachycephalus ephippium*, is most closely related to a clade of Mexican and Central American members of the leptodactylid tribe Eleutherodactylini, including *Hylactophryne augusti*, *Eleutherodactylus fitzingeri*, and *E. rhodopis* (bp = 62). The clade containing *Brachycephalus* and all members of Eleutherodactylini appears as the sister group to the rest of Hyloidea (bp = 59). This renders Leptodactylidae polyphyletic; the family is represented on the parsimony tree by five clades.

3.2. Maximum likelihood and Bayesian inference analyses

MODELTEST determined that the best-fit model for our data was GTR + Γ + I. Under this model, the following parameter values were estimated from one of the most parsimonious trees: rate matrix AC 2.71, AG 8.41, AT 3.88, CG 0.57, CT 22.15, GT 1.0; nucleotide frequencies A 0.41, C 0.22, G 0.13, T 0.24; proportion of invariant sites 0.275, γ distribution shape parameter 0.646.

Maximum likelihood analyses recovered exactly the same topology as was estimated using Bayesian methods, with the exception of one basal hyloid polytomy. Bayesian analyses recovered a polytomy at the most basal hyloid node: (*Cryptobatrachus* sp., *Brachycephalus ephippium* + Eleutherodactylini, the remaining Hyloidea) (Fig. 2). As in the parsimony analyses, both likelihood and Bayesian methods recovered a monophyletic Hyloidea and Ranoidea, both with Bayesian posterior probabilities (pp) of 100% (Fig. 2). Again, three major clades of ranoids were recovered, although relationships within these differ slightly from the parsimony results.

Support for the monophyly of the hyloid families Centrolenidae and Dendrobatidae is also 100%. Support for a monophyletic Bufonidae is 99%. As under parsimony, Hylidae is found to be polyphyletic under likelihood and Bayesian analyses, due to the unclear relationships of *Cryptobatrachus* and *Gastrotheca*. Bayesian analyses recovered *Cryptobatrachus* in a polytomy with the clade containing Eleutherodactylini + *Brachycephalus* and the rest of Hyloidea. The likelihood tree placed *Cryptobatrachus* as the sister

group to Eleutherodactylini + *Brachycephalus*. *Gastrotheca* appears most closely related to the leptodactylid *Alsodes monticola* (pp = 95%). Again, *Pseudis paradoxa* is most closely related to the hyline *Scarthyla goinorum* (pp = 100%).

The relationship of *Brachycephalus ephippium* and Mexican and Central American eleutherodactylines is strongly supported (pp = 100%). Specifically, *Brachycephalus* is supported as the sister taxon of the Mexican eleutherodactylines (pp = 93%). In addition to the Eleutherodacylini, Leptodactylidae is represented by two clades, one of which includes *Gastrotheca*.

3.3. Hypothesis testing

Parametric bootstrap analyses revealed that the three hypotheses—the placement of Dendrobatidae in Ranoidea, monophyly of Hylidae + Pseudidae + Centrolenidae, and *Brachycephalus* as part of Bufonidae—were rejected by the observed sequence data at $P < 0.002$ (Fig. 3).

4. Discussion

4.1. Phylogenetic taxonomy

Our phylogenetic definition of Hyloidea provides a stable name for a strongly supported clade. This definition excludes *Heleophryne*, Myobatrachidae, Limnodynastidae, and Sooglossidae from the definition of Hyloidea. A re-analysis of the data from Ruvinsky and Maxson (1996) and Hay et al. (1995), as well as our unpublished results, indicate that the relationships among these basal neobatrachian clades are not stable.

We here associate the name Hyloidea with a less inclusive and more stable clade, specifically the most recent common ancestor of Eleutherodactylini, Bufonidae, Centrolenidae, Phyllomedusinae, Pelodryadinae, and Ceratophryinae. Because all our analyses indicate high confidence in this slightly more restricted clade, and other analyses have also found it to be well supported (Hay et al., 1995; Ruvinsky and Maxson, 1996; Vences et al., 2000), we recognize this clade formally. If *Heleophryne*, Sooglossidae, Myobatrachidae, or Limnodynastidae are later found to be nested within Hyloidea, then the definition of Hyloidea will not change.

Ford and Cannatella (1993) defined Ranoidea as "the common ancestor of hyperoliids, rhacophorids, ranids, dendrobatids, *Hemisus*, arthroleptids, microhylids, and all of its descendants." In retrospect, their inclusion of Dendrobatidae in the definition of Ranoidea was unfortunate because its relationships were historically labile. Based on our analysis, two actions are possible: (1) adherence to the original definition, which would drastically expand the content of Ranoidea to include another 3100 species, because the last ancestor of Ranoidea as originally defined now subtends a much larger clade; (2) re-define the name Ranoidea, using reference taxa that provide a more stable definition. In expectation of a more extensive analysis of ranoids, we choose a third option and defer from re-defining the name Ranoidea.

Alternatives to naming the entire clade as Ranoidea should be considered. Our analysis and that of Emerson et al. (2000) indicate three well-supported clades: (1) one of rhacophorids, Mantellinae, and traditional "ranids" such as *Rana* and *Platymantis*; (2) one of most groups of microhylids; and (3) one of Arthroleptidae, Hyperoliidae, *Hemisus* (in Hemisotidae), and brevicipitine microhylids. The oldest available Linnean superfamily name for the clade of ranids, mantellines, and rhacophorids is Ranoidea. The oldest available Linnean superfamily name for the clade of microhylids excluding Brevicipitinae is Microhyloidea. There seems to be no available superfamily name for the third clade; the oldest available genus name in this clade is *Breviceps* Merrem 1920. Thus, the superfamily name would be Brevicipitoidea; its author and date would derive from Brevicipitinae Bonaparte 1850.

4.2. Hypothesis testing

Our tests yielded new insights into long-standing controversies in anuran systematics. The position of Dendrobatidae has long been debated. Noble (1926, 1931) suggested that dendrobatids were associated with the hylodine leptodactylids based on the presence of digital dermal scutes and the morphology of the pectoral girdle. Lynch (1971, 1973) also strongly supported this hypothesis. Griffiths (1959) proposed placing Dendrobatidae with the ranoids based mostly on features of the pectoral girdle and thigh musculature. The dendrobatid-ranoid hypothesis was further fueled by Duellman and Trueb (1986), Ford and Cannatella (1993), and Ford (1993). Three molecular studies found Dendrobatidae to be associated with hyloid families and excluded from the cluster of ranoid families (Hay et al., 1995; Ruvinsky and Maxson, 1996; Vences et al., 2000). With a fourfold increase in non-dendrobatid neobatrachian taxa, our placement of Dendrobatidae is concordant with previous molecular analyses.

Using parametric bootstrap simulation we rejected the placement of Dendrobatidae within Ranoidea, $P < 0.002$. However, the systematic affinities of Dendrobatidae within Hyloidea are still unresolved. Parsimony placed Dendrobatidae closest to the hyline *Scinax*, whereas Bayesian and maximum likelihood placed it as the sister group to a clade of some telmatobiine leptodactylids and *Gastrotheca*. Haas (2003) found dendrobatids to be closely related to hylodine

leptodactylids, but we had no molecular sequences of hylodines.

Biogeographically, the placement of dendrobatids with hyloids seems more in accord with the observation that hyloids are primarily Neotropical, whereas under the "dendrobatids as ranoids" hypothesis, Dendrobatidae was the only large radiation of firmisternal frogs in the Neotropics, aside from the lesser invasion of the Neotropics by *Rana* from North America.

Pseudis (Pseudidae) was formerly placed in the Hylidae or Leptodactylidae until it was elevated to family level by Savage and de Carvalho (1953) based on the presence of a large intercalary element in each digit. Lynch (1973), Duellman and Trueb (1986), and Ford and Cannatella (1993) used this character to unite the hylids, centrolenids, and pseudids. Hay et al. (1995), however, found Pseudidae to be the sister taxon to a clade including Dendrobatidae, Rhinodermatidae, Bufonidae, Hylidae, and Centrolenidae. Upon adding eight new neobatrachian taxa to the Hay et al. (1995) data matrix, Ruvinsky and Maxson (1996) found Pseudidae and Rhinodermatidae in a weakly supported trichotomy with Pelodryadinae + Phyllomedusinae. At $P < 0.002$, we were able to reject the monophyly of the clade containing Hylidae, Pseudidae, and Centrolenidae.

Both parsimony and Bayesian analyses recovered *Pseudis paradoxa* as most closely related to the hyline *Scarthyla goinorum* (bp = 97; pp = 100%). Like pseudids, this hylid (originally *S. ostinodactyla*) has ossified intercalary elements between the penultimate and distal phalanges (Duellman and de Sá, 1988). As in our analyses, da Silva (1998: Figure II-7) placed *Scarthyla* as the sister-taxon of (*Pseudis* + *Lysapsus*), nested within hylines. However, his morphological data indicate that the presence of calcified intercalary elements is not a synapomorphy for *Scarthyla* + Pseudidae; rather, this character appears deeper in his tree and is homologous among pseudids, *Scarthyla*, some *Sphaenorhynchus*, and *Pseudacris*.

Based on da Silva (1998); Duellman (2001) argued that pseudid frogs should be recognized as a subfamily of Hylidae, and he figured Pseudinae as the sister taxon to Hylinae (Duellman, 2001: Figure 331). However, da Silva (1998) intimated that pseudids should be placed within Hylinae (rather than in Pseudinae), given that Pseudinae was nested within hylines, but he stopped short of a formal taxonomic change. Because our results place *P. paradoxa* within Hylinae, ranking pseudids as either family or subfamily (Pseudidae or Pseudinae) still renders Hylidae or Hylinae paraphyletic, which is inconsistent with the principles of phylogenetic taxonomy (de Queiroz and Gauthier, 1992). Therefore, within the Linnean framework, we consider the names Pseudidae and Pseudinae to be junior subjective synonyms of Hylidae.

Brachycephalus and *Psyllophryne* (Brachycephalidae) are endemic to the Atlantic forest of southeastern Brazil and are characterized by their tiny size and reduced number of phalanges in the hands and feet. *Brachycephalus* has generally been considered to be related to hyloids, specifically bufonids (Griffiths, 1959; Noble, 1926, 1931). McDiarmid (1971) removed *Brachycephalus* from Bufonidae based on the absence of a Bidder's organ and elevated the genus to its own family, Brachycephalidae. Izecksohn (1971, 1988) hypothesized a close relationship of *Euparkerella* to *Brachycephalus* and *Psyllophryne*. *Euparkerella* is a diminutive member of the leptodactylid tribe Eleutherodactylini, which like *Brachycephalus* and *Psyllophryne*, lives in leaf litter in the forests of southeastern Brazil.

Using parsimony, maximum likelihood, and Bayesian analysis, we recovered a close association between *Brachycephalus ephippium* and Eleutherodactylini, especially those species in Mexico and Central America. It is surprising that *Brachycephalus* is allied to Central American and Mexican species rather than to South American species; however, our sample of eleutherodactylines is limited.

We were able to reject the null hypothesis that *Brachycephalus* is a bufonid using parametric bootstrap analysis ($P < 0.002$). Our results strongly support Izecksohn's (1988) hypothesis that *Brachycephalus* is most closely related to Eleutherodactylini. Inclusion of *Brachycephalus* in Eleutherodactylini would nest a family (Brachycephalidae) within a tribe, which is inconsistent with Linnean taxonomy. This arrangement also forces Eleutherodactylini to be paraphyletic and is inconsistent with the principles of phylogenetic taxonomy (de Queiroz and Gauthier, 1992). Therefore, continued recognition of a family-group name based on the type-genus *Brachycephalus* is unwarranted. However, the nomenclatural implications of synonymization of Brachycephalidae are extensive and will be treated elsewhere (Cannatella and Darst, in prep.).

4.3. Other relationships

All phylogenetic methods recovered a monophyletic Hyloidea and Ranoidea. We found, however, topological and nodal support incongruences between parsimony and model-based methods for basal hyloid relationships. The weak bootstrap support for the deep hyloid divergences most probably comes from a combination of apparent short divergence times on internal branches (Fig. 2) with possible substitutional saturation.

Bayesian analyses estimated much higher support values than did parsimony. Bootstrap proportions are known to be highly conservative (Hillis and Bull, 1993), whereas the higher levels of support seen in posterior probabilities reflect a closer measure of phylogenetic accuracy (Wilcox et al., 2002; but see Suzuki et al., 2002). However, the support values from non-parametric bootstrapping and Bayesian analyses are not strictly comparable because bootstrap values were

calculated under parsimony whereas the Bayesian analyses used a likelihood function.

5. Conclusions

Our analysis of 12S, tRNA-valine, and 16S rRNA mitochondrial genes from 93 neobatrachian taxa provides statistically significant support for a monophyletic Hyloidea and Ranoidea. Some new patterns of hyloid phylogenetic relationships were uncovered. First, monophyly of Centrolenidae, Bufonidae, and Dendrobatidae, is strongly supported by parsimony, maximum likelihood, and Bayesian analyses. Also, we explicitly rejected the hypothesis that the Dendrobatidae is most closely related to ranoid taxa. Second, Hylidae is polyphyletic. Specifically, *Cryptobatrachus* sp. and *Gastrotheca pseustes* (Hemiphractinae) do not appear closely related to each other, nor to other hylids; greater taxon sampling is needed. Third, a clade of Hylidae, Pseudidae, and Centrolenidae was not recovered and we explicitly rejected the monophyly of this clade using parametric bootstrapping. Using both parsimony and Bayesian analysis, Centrolenidae appears to be most closely related to leptodactyline leptodactylids. *Pseudis paradoxa* and the hylid *Scarthyla goinorum* form a well-supported clade. This position of *P. paradoxa* within Hylinae supports synonymization of Pseudidae (and Pseudinae). Lastly, we rejected the hypothesis that *Brachycephalus* is most closely related to Bufonidae. Rather, it is most closely related to the leptodactylid tribe Eleutherodactylini, especially species from Central America and Mexico.

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Appendix A

List of specimens examined. ICN, Instituto de Ciencias Naturales, Universidad Nacional de Colombia; KU, University of Kansas; MVZ, Museum of Vertebrate Zoology; PNM/CMNH, Philippines National Museum/Cincinnati Museum of Natural History; QCAZ, Quito-Católica-Zoología; TNHC, Texas Natural History Collection; USNM, United States National Museum; USP, Universidade de São Paulo; UTACV, University of Texas at Arlington Collection of Vertebrates.

Family	Species	Field number	Museum number	GenBank Accession number	Locality
Brachycephalidae	*Brachycephalus ephippium*	DMH #2	Not Available (NA)	AY326008	Brazil
Bufonidae	*Ansonia* sp.	H1473	PNM/CMNH	AY325992	Philippines: Mindanao: S. Cotobat Province, Municipality of Kiamba, Mt. Busa
	Atelopus varius	AG 36	MVZ 223279	AY325996	Costa Rica: South of Las Alturas
	Bufo alvarius	DCC 2906	TNHC 61247	AY325984	Arizona: Just north of Tucson
	Bufo biporcatus	DCC 2914	TNHC 61079	AY325987	No data
	Bufo boreas	RDS 239	NA	AY325983	No data
	Bufo bufo	DMH 89-13	TNHC 56744	AY325988	USSR: Latvian Republic, Riga
	Bufo exsul	FC12574	MVZ 142947	AY325990	California: Inyo: 0.8 mi S. Deep Springs College, Bucklehorn Spring, Deep Springs Valley

Appendix A (*continued*)

Family	Species	Field number	Museum number	GenBank Accession number	Locality
	Bufo kisoloensis	AG 46	MVZ 223361	AY325995	Uganda: Buhoma, Bwindi Forest Reserve
	Bufo marinus	WED 55596	KU 205236	AY325994	Peru: Madre de Dios: Cusco Amazónico
	Bufo microscaphus	RDJ 865	NA	AY325989	New Mexico: Catron: Bull Pass Tank, 5 mi N, 35.5 mi W of Winston; T10S, R14W, Sec 27
	Bufo retiformis	AG 125	MVZ 222506	AY325982	Arizona: Pima: 12 mi N of Quijotoa, Indian Route 15
	Bufo steindachneri	AG 61	MVZ 223373	AY325981	Kenya: Arobuko Sokoka forest, sand quarry
	Bufo nebulifer	DCC 3107	TNHC 62000	AY325985	Texas: San Saba: Colorado Bend State Park
	Dendrophryniscus minutus	USNM-FS 189767	USNM 520905	AY326000	Peru: Loreto: Rio Lagarto Cocha, Aguas Negras
	Didynamipus sjostedti	AG 259	NA	AY325991	Cameroon
	Melanophryniscus sp.	RMB 4125	TNHC 62494	AY325998	No data
	Melanophryniscus stelzneri	AG 87	NA	AY325999	No data
	Osornophryne guacamayo	AGG 220	QCAZ 4580	AY326036	Ecuador: Napo: Lago Sumaco, Volcán Sumaco
	Pedostibes hosei	JAM 1159	NA	AY325993	Malaysia: Pahang: Krau Wildlife Reserve, Pehang main research field station, ~13 km NW Kuala Krau at confluence Krau and Lompat Rivers
	Schismaderma carens	DCC 3172	TNHC 62001	AY325997	Tanzania: Dodoman
Centrolenidae	*Cochranella* sp.	WED 53034	KU 202801	AY326025	Ecuador: Carchi: ~5 km W La Gruel, 2340 m
	Centrolene sp.	WED 52978	KU 202796	AY326022	Ecuador: Napo: 18 km E Santa Bárbara
	Cochranella sp.	AGG 507	QCAZ 10801	AY326023	No data
	Hyalinobatrachium sp.	RMB 4126	TNHC 62495	AY326024	No data
Dendrobatidae	*Allobates femoralis*	WED 55470	KU 205291	AY326026	Peru: Madre de Dios: Cusco Amazónico
	Allobates femoralis	WED 55560	KU 205292	AY326027	Peru: Madre de Dios: Cusco Amazónico
	Colostethus infraguttatus	AGG 504	QCAZ 10812	AY326028	Ecuador: Manabí: 12 km al norte de Puerto Cayo
	Dendrobates auratus	DCC 2895	TNHC 62487	AY326036	No data
	Dendobates reticulatus	DCC 3155	TNHC 61143	AY326029	Peru
	Phyllobates bicolor	DCC 2907	TNHC 62488	AY326031	No data
Heleophrynidae	*Heleophryne purcelli*	DMH #15	NA	AY326072	South Africa
Hemisotidae	*Hemisus marmoratum*	DCC 3047	TNHC 62489	AY326070	Tanzania: Arusha near Mt. Kilamanjaro
Bufonidae	*Bufo woodhousii*	TJL 686	TNHC 60511	AY325986	Texas: King Co.: FM 193, 11.9 mi w us Hwy 83

Appendix A (*continued*)

Family	Species	Field number	Museum number	GenBank Accession number	Locality
Hylidae	*Agalychnis litodryas*	CP13217	QCAZ 13217	AY326043	Ecuador
	Agalychnis saltator	DCC 2132	MVZ 203768	AY326044	Costa Rica: Heredia: Starkey's Woods, 1.5–3.0 km E Rio Frio rd at 1 km NW entrance to Estación Biológica La Selva
	Cryptobatrachus sp.	JDL 14865	ICN	AY326050	Colombia: Santander: Municipio San Gil: 7 km by road SW San Gil
	Gastrotheca pseustes	DMH 90E-19	TNHC 62492	AY326051	Ecuador: Chimborazo: 3.3 km S Tixán, 2990 m
	Hyla calcarata	WED 54086	KU 202911	AY326056	Ecuador: Napo: Misahualli, 600 m
	Hyla lanciformis	WED 54081	KU 202724	AY326054	Ecuador: Pastaza: 5.6 km N Puyo, 1150 m
	Hyla pantosticta	WED 52976	KU 202732	AY326052	Ecuador: Napo: 18 km E Santa Barbara
	Hyla picturata	WED 53656	KU 202737	AY326055	Ecuador: Pichincha: Tinalandia, 15.5 km SE Santo Domingo de Colorados, 700 m
	Hyla sp.	WED 53493	KU 202760	AY326057	Ecuador: Azuay 2.0 km SSE Palmas, 2340 m
	Hyla triangulum	WED 54094	KU 202745	AY326053	Ecuador: Napo: Misahualli, 600 m
	Hyla pellucens	WED 53621	KU 202734	AY326058	Ecuador: Pichincha: 1.8 km SSE San Juan, 3420 m
	Litoria arfakiana	CCA 503	TNHC 51936	AY326039	Papua New Guinea: Madang: ~10 km NW Simbai, Kaironk Village, 2000 m
	Nyctimystes kubori	CCA 496	TNHC 51924	AY326037	Papua New Guinea: Madang: ~10 km NW Simbai, Kaironk Village, 2000 m
	Osteocephalus taurinus	WED 55452	KU 205406	AY326041	Peru: Madre de Dios: Cusco Amazónico
	Pachymedusa dacnicolor	FC12110	MVZ 164906	AY326047	Mexico: Michoacán: Capirio, Río Tepalcatepec
	Pelodryas caerulea	DMH	NA	AY326038	No data
	Phrynohyas venulosa	DCC 3069	TNHC 62490	AY326048	Ecuador
	Phyllomedusa palliata	WED 55638	KU 205420	AY326046	Peru: Madre de Dios: Cusco Amazónico
	Phyllomedusa tomopterna	WED 55380	KU 205428	AY326045	Peru: Madre de Dios: Cusco Amazónico
	Pseudacris brachyphona	ECM 41	TNHC 62304	AY326049	Alabama: Tallapoosa Co.
	Scarthyla goinorum	WED 55411	KU 205763	AY326035	Peru: Madre de Dios: Cusco Amazónico
	Scinax garbei	WED 54071	KU 202764	AY326033	Ecuador: Chimborazo: 6.7 km E Riobamba, 2550 m

Appendix A (*continued*)

Family	Species	Field number	Museum number	GenBank Accession number	Locality
	Scinax rubra	WED 56265	KU 207622	AY326034	Peru: Madre de Dios: Cusco Amazónico
	Smilisca phaeota	DMH 86-115	NA	AY326040	Costa Rica: Limón: Estación Experimental La Lola
	Trachycephalus jordani	DCC 2917	TNHC 61092	AY326042	Ecuador
Hyperoliidae	*Hyperolius* sp.	DCC 3159	TNHC 61197	AY326069	Tanzania
Leptodactylidae	*Alsodes monticola*	NB #2	NA	AY326016	Chile
	Ceratophrys cornuta	WED 55587	KU 202561	AY326014	Peru: Madre de Dios: Cusco Amazónico
	Ceratophrys ornata	DMH A6	NA	AY326013	No data
	Eleutherodactylus chloronotus	WED 52959	KU 202325	AY326007	Ecuador: Napo: 3.5 km E Santa Barbara
	Eleutherodactylus cuneatus	SBH 172809	NA	Y10944	Cuba: Cienfuegos Province, Soledad
	Eleutherodactylus duellmani	WED 53050	KU 202404	AY326003	Ecuador: Carchi: ~5 km W La Gruel, 2340 m
	Eleutherodactylus fitzingeri	DMH 86-112	NA	AY326001	Costa Rica: Limón: Estación Experimental La Lola
	Eleutherodactylus rhodopis	JAC 8492	UTACV A-12957	AY326006	Mexico: Hidalgo: 4.5 km NE Tlanchinol
	Eleutherodactylus sp.	WED 52979	KU 202623	AY326002	Ecuador: Napo: 18 km E Santa Barbara
	Eleutherodactylus supernatis	WED 52961	KU 202432	AY326005	Ecuador: Napo: 3.5 km E Santa Barbara
	Eleutherodactylus thymelensis	WED 53004	KU 202519	AY326009	Ecuador: Carchi: 12 km W Tufino, 3520 m
	Eleutherodactylus w-nigrum	WED 53045	KU 205076	AY326004	Ecuador: Carchi: ~5 km W La Gruel, 2340 m
	Hylactophryne augusti	JAC 8191	UTACV A-12980	AY326011	Mexico: Jalisco: 2.4 km NW Tapalpa
	Lepidobatrachus sp.	DCC 2915	TNHC 62497	AY326019	No data
	Leptodactylus pentadactylus	FC13095	MVZ 233238	AY326017	Costa Rica: Limón: Río Pentencia, 2 mi N Tortuguero
	Lithodytes lineatus	N. Basso	USP 968438	AY326012	Brazil: Apiacás
	Phrynopus sp.	WED 52998	KU 202652	AY326010	Ecuador: Carchi: 13.6 km W El Carmelo, 3080 m
	Physalaemus nattereri	AJC 95-267	NA	AY326020	Brazil: São Paulo: Luiz Antonio
	Physalaemus riograndensis	AJC 95-233	NA	AY326021	Brazil: Rio Grande do Sul: El Dorado
	Telmatobius niger	DMH 90E-36	TNHC 62493	AY326015	Ecuador: Azuay: 48.8 km WNW Cuenca, 3380 m
	Telmatobius vellardi	WED 53381	KU 202679	AY326018	Ecuador: Azuay: 10 km NE Girón, 2750 m
Microhylidae	*Callulina kreffti*	DCC 3162	TNHC 62491	AY326068	Tanzania: Mazumbai
	Gastrophryne olivacea	DCC 3106	TNHC 61952	AY326066	Texas: San Saba: Colorado Bend State Park

Appendix A (*continued*)

Family	Species	Field number	Museum number	GenBank Accession number	Locality
	Kaloula conjuncta	RMB 2252	PNM/CMNH	AY326064	Philippines: Negros Island: city of Dumaguete
	Nelsonophryne aequatorialis	WED 53386	KU 202919	AY326067	Ecuador: Loja: 3.7 km S Saraguro, 2800 m
	Phrynomerus sp.	DCC 2901	TNHC 61077	AY326065	No data
Myobatrachidae	*Limnodynastes salminii*	DCC 2898	TNHC 61075	AY326071	No data
Pseudidae	*Pseudis paradoxa*	DCC 3284	NA	AY326032	Brazil: São Paulo: Fazenda Santa Helena, ~18 km S Luiz Antonio
Ranidae	*Platymantis* sp.	JF 0131	NA	AY326061	Solomon Islands
	Rana nicobariensis	RMB 2086	TNHC 59856	AY326062	Indonesia: Jawa Barat: Java Is.: Desa Cikopo; 6° 40′19″S, 106 ° 52′42″E
	Rana temporaria	DMH	NA	AY326063	No data
Rhacophoridae	*Philautus acutirostris*	RMB 589	TNHC 59857	AY326059	Philippines: Davao City Prov.: Mindanao Is.: Eagle Foundation Inc. (PEFI) Malagos Eagle camp
	Rhacophorus monticola	RMB 1236	NA	AY326060	Indonesia: Sulawesi Is.: S. Sulawesi: Mt. Lompo Batang: 1580 m

References

Buckley, T.R., 2002. Model misspecification and probabilistic tests of topology: evidence from empirical data sets. Syst. Biol. 51, 509–523.

Cannatella, D.C., 1985. A phylogeny of primitive frogs (archaeobatrachians). Ph.D. Dissertation., The University of Kansas, Lawrence.

da Silva, H.R., 1998. Phylogenetic relationships of the family Hylidae with emphasis on the relationships within the subfamily Hylinae (Amphibia: Anura). Ph.D. Dissertation., The University of Kansas, Lawrence.

De Queiroz, K., Gauthier, J., 1992. Phylogenetic taxonomy. Ann. Rev. Ecol. Syst. 23, 449–480.

Dubois, A., 1983. Classification et nomenclature supragénérique des amphibiens anoures. Bull. Soc. Linn. Lyon 52, 270–276.

Duellman, W.E., 1975. On the classification of frogs. Occas. Pap. Mus. Nat. Hist. Univ. Kansas 42, 1–14.

Duellman, W.E., 2001. Hylid Frogs of Middle America. vol. 2. Society for the Study of Reptiles and Amphibians. New York.

Duellman, W.E., de Sá, R.O., 1988. A new genus and species of South American hylid frog with highly modified tadpole. Trop. Zool. 1, 117–136.

Duellman, W.E., Trueb, L., 1986. Biology of Amphibians. McGraw-Hill, New York.

Emerson, S.B., Richards, C., Drewes, R.C., Kjer, K.M., 2000. On the relationships among ranoid frogs: a review of the evidence. Herpetologica 56, 209–230.

Felsenstein, J., 1985. Confidence limits on phylogenies: a justification. Evolution 39, 783–791.

Ford, L.S., 1989. The phylogenetic position of poison-dart frogs (Dendrobatidae): reassessment of the neobatrachian phylogeny with commentary on complex character systems. Ph.D. Dissertation. The University of Kansas, Lawrence.

Ford, L.S., 1993. The phylogenetic position of the dart-poison frogs (Dendrobatidae) among anurans: an examination of the competing hypotheses and their characters. Ethol. Ecol. Evol. 5, 219–231.

Ford, L.S., Cannatella, D.C., 1993. The major clades of frogs. Herpetol. Monogr. 7, 94–117.

Frost, D.R., 1985. Amphibian Species of the World. Allen Press, Lawrence.

Frost, D.R., 2002. Amphibian Species of the World: An Online Reference.V2.21 (15 July 2002). Electronic database available at research.amnh.org/herpetology/amphibia/index.html.

Goebel, A.M., Donnelly, J.M., Atz, M.E., 1999. PCR primers and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase I, and cytochrome *b* in bufonoids and all other frogs, an overview of PCR primers which have amplified DNA in amphibians successfully. Mol. Phylogenet. Evol. 11, 163–199.

Goldman, N., Anderson, J.P., Rodrigo, A.G., 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49, 652–670.

Griffiths, I., 1959. The phylogeny of *Sminthillus limbatus* and the status of Brachycephalidae (Amphibia, Salientia). Proc. Zool. Soc. London 132, 457–487.

Haas, A., 2003. Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). Cladistics 19, 23–89.

Hay, J.M., Ruvinsky, I., Hedges, S.B., Maxson, L.R., 1995. Phylogenetic relationships of amphibian families inferred from DNA sequences of mitochondrial 12S and 16S ribosomal RNA genes. Mol. Biol. Evol. 12, 928–937.

Hillis, D.M., Bull, J.J., 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42, 182–192.

Huelsenbeck, J.P., Hillis, D.M., Nielsen, R., 1996. A likelihood-ratio test of monophyly. Syst. Biol. 45, 546–558.

Huelsenbeck, J.P., Ronquist, F.R., 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Izecksohn, E., 1971. Novo gênero e nova especie de Brachycephalidae do Estado do Rio de Janeiro, Brasil (Amphibia-Anura). Bol. Mus. Nac. Rio de Jan. 280, 1–12.

Izecksohn, E., 1988. Algumas considerações sobre o gênero *Euparkerella*, com a descrição de tres novas especies (Amphibia, Anura, Leptodactylidae). Rev. Brasil. Biol. 48, 59–74.

Kluge, A.G., Farris, J.S., 1969. Quantitative phyletics and the evolution of anurans. Syst. Zool. 18, 1–32.

Larget, B., Simon, D., 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol. Biol. Evol. 16, 750–759.

Laurent, R.F., 1986. Sous classe des lissamphibiens. Systématique, In: Grassé, P.-P., Delsol, M. (Eds.), Traité de Zoologie. Masson, Paris, pp. 594–797.

Lynch, J.D., 1971. Evolutionary relationships, osteology, and zoogeography of leptodactylid frogs. Misc. Publ. Mus. Nat. Hist. Univ. Kansas 53, 1–238.

Lynch, J.D., 1973. The transition from archaic to advanced frogs. In: Vial, J.L. (Ed.), Evolutionary Biology of the Anurans: Contemporary Research on Major Problems. University of Missouri Press, pp. 133–182.

Maddison, W.P., Maddison, D.R., 2000. MacClade: Analysis of Phylogeny and Character Evolution. Version 4.0. Sinauer, Sunderland, MA.

McDiarmid, R.W., 1971. Comparative morphology and evolution of frogs on the Neotropical genera *Atelopus, Dendrophryniscus, Melanophryniscus* and *Oreophrynella*. Sci. Bull. Nat. Hist. Mus. Los Angeles Co. 12, 1–66.

Noble, G.K., 1926. The pectoral girdle of the brachycephalid frogs. Am. Mus. Novitates 230, 1–14.

Noble, G.K., 1931. The Biology of the Amphibia. McGraw-Hill, New York.

Palumbi, S.R., 1996. Nucleic acids II: The polymerase chain reaction. In: Hillis, D.M., Mable, B.K., Moritz, C. (Eds.), Molecular Systematics. second ed., Sinauer, Sunderland, MA, pp. 205–247.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Reig, O.A., 1958. Proposiciones para una nueva macrosistemática de los anuros. Nota preliminar. Physis 21, 109–118.

Roe, B.A., Ma, D.P., Wilson, R.K., Wong, J.F., 1985. The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. J. Biol. Chem. 260, 9759–9774.

Ruvinsky, I., Maxson, L.R., 1996. Phylogenetic relationships among bufonoid frogs (Anura: Neobatrachia) inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 5, 533–547.

Savage, J.M., de Carvalho, A.L., 1953. The family position of the Neotropical frogs currently referred to the genus *Pseudis*. Zoologica 38, 193–200.

Suzuki, Y., Glazko, G.V., Nei, M., 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. Proc. Natl. Acad. Sci. USA 99, 16138–16143.

Swofford, D.L., 2000. PAUP* Phylogenetic Analysis Using Parsimony (*And Other Methods), Version 4.0b.8a. Sinauer, Sunderland, MA.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal_Xwindows interface: flexible strategies for multiple sequence alignment by quality analysis tools. Nucleic Acid Res. 25, 4876–4882.

Tihen, J.A., 1965. Evolutionary trends in frogs. Am. Zool. 5, 309–318.

Vences, M., Kosuch, J., Lötters, S., Widmer, A., Jungfer, K.-H., Köhler, J., Veith, M., 2000. Phylogeny and classification of poison frogs (Amphibia: Dendrobatidae), based on mitochondrial 16S and 12S ribosomal RNA gene sequences. Mol. Phylogenet. Evol. 15, 34–40.

Wilcox, T.P., Zwickl, D.J., Heath, T.A., Hillis, D.M., 2002. Phylogenetic relationships of the dwarf boas and a comparison on Bayesian and bootstrap measures of phylogenetic support. Mol. Phylogenet. Evol. 25, 361–371.