Molecular Phylogenetics and Evolution 55 (2010) 871–882

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

An expanded phylogeny of treefrogs (Hylidae) based on nuclear and mitochondrial sequence data

John J. Wiens *, Caitlin A. Kuczynski, Xia Hua, Daniel S. Moen

Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY 11794-5245, USA

ARTICLE INFO

Article history:
Received 3 August 2009
Revised 11 February 2010
Accepted 9 March 2010
Available online 19 March 2010

Keywords:
Amphibians
Anura
Hylidae
Mitochondrial DNA
Nuclear DNA
Phylogeny

ABSTRACT

The treefrogs (Hylidae) make up one of the most species-rich families of amphibians. With 885 species currently described, they contain >13% of all amphibian species. In recent years, there has been considerable progress in resolving hylid phylogeny. However, the most comprehensive phylogeny to date (Wiens et al., 2006) included only 292 species, was based only on parsimony, provided only poor support for most higher-level relationships, and conflicted with previous hypotheses in several parts (including the monophyly and relationships of major clades of Hylinae). Here, we present an expanded phylogeny for hylid frogs, including data for 362 hylid taxa for up to 11 genes (4 mitochondrial, 7 nuclear), including 70 additional taxa and >270 sequences not included in the previously most comprehensive analysis. The new tree from maximum likelihood analysis is more well-resolved, strongly supported, and concordant with previous hypotheses, and provides a framework for future systematic, biogeographic, ecological, and evolutionary studies.

1. Introduction

Hylid frogs are one of the most species-rich families of amphibians. With 885 species and 57 genera currently recognized (Faivovich et al., 2005; Garda and Cannatella, 2007; Smith et al., 2007b; AmphibiaWeb, 2010), they contain ~13% of all 6629 amphibian species (AmphibiaWeb, 2010). Most hylid frogs are arboreal, and are known colloquially as treefrogs (Duellman and Trueb, 1985). Hylid frogs occur on all major continents except for Antarctica, but most species and genera occur in the New World tropics (AmphibiaWeb, 2010). They are also relatively diverse in Australia, but have only a limited number of species in North America, North Africa, Europe, and Asia (AmphibiaWeb, 2010).

The past 5 years have seen considerable progress in resolving hylid phylogeny. Faivovich et al. (2005) presented a phylogeny for 226 hylid species based on five nuclear and three mitochondrial genes, and presented an extensively revised classification for the family, which has been widely adopted. Wiens et al. (2005) presented a phylogeny for 169 hylid taxa, based on a partially overlapping set of taxa and character data from two nuclear genes, two mitochondrial genes, and morphology. These analyses agreed on many aspects of hylid phylogeny, especially many of the major clades. These clades include: (1) the three subfamilies of hylids (Hylinae, Pelodryadinae, Phyllomedusinae), (2) a clade consisting of Pelodryadinae and Phyllomedusinae, and (3) several major clades generally unrecognized in previous taxonomy but recognized as tribes within Hylinae by Faivovich et al. (2005) and informal clades by Wiens et al. (2005). These latter clades include the Cophomantini (*Boana* clade of Wiens et al. (2005)), Lophiohylini (*Phrynohyas* clade of Wiens et al. (2005)), Hylini (Middle American clade of Wiens et al. (2005)), and Dendropsophini (including *Scinax*, *Sphaenorhynchus*, *Xenohyla*, the genus *Dendropsophus* [formerly the 30-chromosome *Hyla*], and the former family Pseudidae [*Lysapsus*, *Pseudis*]). These trees also agreed in placing Cophomantini as basal within Hylinae, and in placing the Hylini and Lophiohylini as sister taxa. Finally, these studies agreed that the former hylid subfamily Hemiphractinae is only distantly related to other hylids, and should be recognized as either part of Leptodactylidae (Faivovich et al., 2005) or a separate family (Wiens et al., 2005; see also Wiens et al. (2007)).

Wiens et al. (2006) integrated the data of Faivovich et al. (2005) and Wiens et al. (2005) into a single combined matrix with 11 genes (along with data from Smith et al. (2005)), and included new data for 44 additional taxa for the 12S gene. This matrix included 292 hylid taxa, the most extensive phylogeny of hylids to date. However, many other recent phylogenetic studies have addressed various subclades within Hylidae, including many taxa not represented by Wiens et al. (2006). These include studies of the Australian pelodryadines (Young et al., 2005), Hylini (Smith et al., 2007a,b), Lophiohylini (Moen and Wiens, 2009), *Lysapsus-Pseudis* clade (Aguiar et al., 2007; Garda and Cannatella, 2007), and studies within some hylid genera, including *Agalychnis* (Gomez-Mestre et al., 2008), *Pseudacris* (Lemmon et al., 2007), and

* Corresponding author. Fax: +1 631 632 7626.
E-mail address: wiensj@life.bio.sunysb.edu (J.J. Wiens).

1055-7903/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2010.03.013

Hyla (Hua et al., 2009). In an on-line appendix, Moen and Wiens (2009) also re-analyzed some of the data of Wiens et al. (2006) using separate Bayesian analyses of the major South American clades to construct a supertree for evolutionary analyses.

Although the phylogeny presented by Wiens et al. (2006) was relatively extensive, many issues still remain. First, taxon sampling is still incomplete, including less than 50% of described hylid species. Second, there are many gaps in the sampling of genes, with many taxa represented by only one or a few genes. Third, due to computational limitations of likelihood and Bayesian methods available at the time of that study, the phylogeny for 292 hylids was analyzed using only parsimony. Wiens et al. (2006) also analyzed a subset of 124 taxa for 10 genes with Bayesian analysis. However, the parsimony tree for 292 taxa disagreed with the Bayesian tree for 124 taxa regarding relationships among some of the major clades, and with the other recent studies of hylid phylogeny (e.g., Faivovich et al., 2005; Wiens et al., 2005). For example, placement of Lophiohylini with Hylini was not supported in the 292-taxon tree, nor was monophyly of Dendropsophini (i.e., *Scinax* was not placed with the other genera). Furthermore, most relationships among the major clades were only weakly supported in the analysis of 292 taxa (bootstrap values <70%). Thus, the most extensive phylogeny of hylids to date is poorly supported and somewhat discordant with previous hypotheses.

In this study, we present a new and expanded phylogeny for hylid frogs. We begin with the 292-taxon matrix of Wiens et al. (2006), and add hundreds of sequences from dozens of taxa from recently published studies (e.g., Young et al., 2005; Smith et al., 2007a,b; Gomez-Mestre et al., 2008; Moen and Wiens, 2009; Hua et al., 2009). We also generate new sequence data for 28 taxa not included in previous studies, to bring the total to 362 hylid terminal taxa. We analyze this large data matrix using both parsimony and maximum likelihood, taking advantage of new fast and flexible software for likelihood analysis of large data sets (Stamatakis, 2006). Although some aspects of the phylogeny remain poorly supported, the new phylogeny is generally better supported and more consistent with previous hypotheses than the 292-taxon tree of Wiens et al. (2006).

2. Materials and methods

We began with the 325-taxon data set (292 ingroup taxa) from Wiens et al. (2006) as the baseline data matrix. This dataset includes eight genes sequenced by Faivovich et al. (2005), consisting of three mitochondrial genes (12S [ribosomal small subunit; ~1088 base pairs, bp], 16S [ribosomal large subunit; ~1646 bp], and cytochrome *b* [385 bp]) and five nuclear genes (RAG-1 [recombinase activating protein 1; 428 bp, exon], rhodopsin [316 bp, exon], SIA [sevenin-absentia, 397 bp, exon], tyrosinase [530 bp, exon], and 28S [887 bp]) for 276 taxa (228 hylids, 48 outgroups), although not all genes were sequenced in all taxa. The matrix also includes data from Wiens et al. (2005) from four genes, of which two are mitochondrial (12S [1088 bp] and ND1 [NADH dehydrogenase subunit 1, including adjacent transfer RNAs; ~1218 bp]) and two are nuclear (*c-myc* [proto-oncogene cellular myelocytomatosis, exons 2 and 3 combined for 832 bp] and POMC [proopiomelanocortin A; 550 bp, exon]). These data were obtained for some (e.g., POMC) or most (12S) of the 198 taxa (169 hylids, 29 outgroups). The two datasets overlap for the 12S gene. However, many species were sequenced by both Faivovich et al. (2005) and Wiens et al. (2005). When multiple genes from different studies were available for different individuals of the same species, data were combined so that each taxon was represented by a single individual (rather than multiple individuals with extensive missing data). As much as possible, data were checked to confirm that sequences from different genes for the same species all placed that species in the same phylogenetic neighborhood (e.g., same genus). However, we acknowledge that cryptic diversity within named species might cause problems in some cases, but only at the smallest phylogenetic scales. A total of 33 non-hylid outgroup taxa were also included, representing the major families of Hyloidea (including bufonids, centrolenids, dendrobatids, hemiphractids, and leptodactylids), the larger clade within which hylids are imbedded, as well as a few more distant outgroups (a pipid, pelobatid, ranid, and microhylid; for recent higher-level frog phylogenies, see Frost et al. (2006), Roelants et al. (2007), and Wiens (2007)). In addition to combining data from these two previous studies, Wiens et al. (2006) also added new 12S data for 44 hylid species.

In the present study, we add new sequence data for 28 taxa for the 12S gene, including species of *Scinax* (11 species, one represented by two samples), *Phyllomedusa* (4 species), *Litoria* (10 species), *Cyclorana* (1 species), and *Nyctimystes* (1 species). Voucher and locality information are provided in on-line Appendix 1. Sequence data were obtained using standard methods and primers described by Smith et al. (2005) and Wiens et al. (2005). A previous analysis (Wiens et al., 2005) suggested that the support for placement of taxa in the combined analysis of multiple genes is significantly correlated with their level of support in the 12S gene alone, and not their overall level of completeness (see below).

We also integrated into this data matrix published sequences from other recent studies of hylids that were not included in Wiens et al. (2006). These added both new taxa as well as providing additional genes for species that were already represented in that matrix. Major sources of sequences included the following (in chronological order): Young et al. (2005; 12S and 16S data for 25 additional species of pelodryadines), Frost et al. (2006; various genes for some pelodryadines), Garda and Cannatella (2007; 12S and 16S data for *Lysapsus* and *Pseudis*), Smith et al. (2007a,b; new sequences and taxa for various nuclear and mitochondrial genes in Hylini, but excluding a few taxa for which only very short fragments were available)), Gomez-Mestre et al. (2008; multiple genes for *Agalychnis* and relatives), Moen and Wiens (2009; multiple genes for Lophiohylini), and Hua et al. (2009; various genes for *Hyla*). In total, relative to the matrix of Wiens et al. (2006), we added 283 sequences in 27 hylid genera, and 72 new hylid taxa in 18 genera. GenBank numbers are provided in on-line Appendix 2.

The completeness of taxa in the combined data matrix varied considerably (i.e., some taxa had many missing data cells). However, simulations (Wiens, 2003; Philippe et al., 2004) and analyses of empirical data sets (Philippe et al., 2004; Driskell et al., 2004; Wiens et al., 2005) suggest that highly incomplete taxa can be accurately placed in phylogenetic analyses, if the overall number of characters is large (e.g., thousands of characters, as in this study), despite recent simulation results based on limited numbers of characters (Lemmon et al., 2009). Perhaps most importantly, a previous study of hylid phylogeny (Wiens et al., 2005) showed that taxa with 12S data alone were placed in their expected genera with strong support in the context of a combined analysis of multiple genes (in both parsimony and Bayesian analyses). Further, they found that the support (parsimony bootstrapping and Bayesian posterior probabilities) for the placement of individual species in combined analyses of multiple genes was correlated with their support based on 12S data alone, and not based on their overall level of completeness. In other words, there was no relationship between the amount of missing data in a species and the support for its placement on the tree, and the 12S data were essential for placing taxa in the phylogeny with strong support. They also found that analyses based only on 12S, excluding the other nuclear and mitochondrial genes, gave somewhat problematic results (i.e., seemingly misplaced taxa), suggesting that the addition of other genes

besides 12S was very important despite the incomplete taxon sampling of these genes.

Alignment of protein-coding sequences was straightforward. DNA sequences were translated to amino acids to aid in alignment (using MacClade, version 4.0, Maddison and Maddison, 2000). The alignment for 28S from Wiens et al. (2006) was used (no new taxa added). For the 12S and 16S ribosomal genes, new sequences were added and concatenated and were re-aligned using MUSCLE (Edgar, 2004) with default parameters. Comparisons suggest that MUSCLE can provide superior alignments to those based on CLUSTAL (Edgar, 2004).

Most-parsimonious trees were sought using the parsimony ratchet (Nixon, 1999). Ten parsimony ratchet searches were conducted, utilizing the PAUPRat program of Sikes and Lewis (2001). Each ratchet search used 200 replicates. The resulting trees from all searches were then filtered to include only the shortest trees. Support for individual branches was evaluated using non-parametric bootstrapping (Felsenstein, 1985), with 200 bootstrap pseudoreplicates and 10 random-taxon-addition sequence replicates per bootstrap pseudoreplicate. Bootstrap values $\geqslant$70% were considered to be strongly supported, following Hillis and Bull (1993, but see their extensive caveats). All parsimony searches, including ratchet searches, utilized PAUP* 4.0b10 (Swofford, 2002).

Maximum likelihood trees were estimated using RAxML version 7.0.5 (Stamatakis, 2006). We conducted a series of searches that integrated 200 bootstrap replicates with 40 replicate searches for the best-fitting phylogeny (preliminary analyses using larger numbers of replicates gave similar results). RAxML uses the general-time reversible (GTR) model with a gamma parameter for variation in rates among sites (the author recommends using 25 gamma-rate categories to account for potentially invariant sites, rather than incorporating a parameter for the proportion of invariant sites). Therefore we did not do extensive analyses of model-fitting, which would be irrelevant. Furthermore, previous analyses (Wiens et al., 2006) showed that most genes best fit the GTR + I + Γ model, with only two exceptions (rhodopsin, SIA) that fit somewhat simpler substitution models (K80 + I + Γ and HKY + I + Γ, respectively). Previous analyses (Wiens et al., 2005, 2006) also suggested that all protein-coding genes should be partitioned by codon position and that stems and loops should be used as partitions within the 12S and 16S genes, based on comparisons of likelihood values from Bayesian analyses using the Bayes factor (e.g., Nylander et al., 2004; Brandley et al., 2005). Nucleotide positions in 12S and 16S were assigned to stems and loops based on models for *Pseudacris regilla* (12S) and *Rana temporaria* (16S) from the European ribosomal RNA database (http://oberon.fvms.ugent.be:8080/rRNA/). Previous comparisons suggest that the placement of stems and loops are highly conserved across anurans (Wiens et al., 2005). We also utilized partitions for putative stems and loops in the tRNAs adjacent to the ND1 gene (identified using Macey et al. (1997)).

We generated phylogenies based on both parsimony and likelihood. However, we emphasize the results from likelihood (for brevity, results from parsimony are presented in on-line Appendix 3 only). We generally prefer model-based methods (such as likelihood) over parsimony because these methods can potentially accommodate the complex evolutionary processes (e.g., the GTR + Γ model) and partitions within and between genes which seem to explain the evolution of these and many other DNA sequence datasets. Further, model-based methods may be less sensitive to the problem of long-branch attraction (Felsenstein, 2004). The data matrix and final parsimony and likelihood trees will be submitted to TreeBase upon final acceptance of the manuscript.

We recognize the potential advantages of species-tree methods that integrate data from multiple loci without concatenation (e.g., Edwards et al., 2007). However, such approaches were not practical for our study given that the sampling of genes among taxa was somewhat heterogeneous, and some taxa are represented by mtDNA only.

Finally, some additional clarifications are necessary regarding some aspects of the data matrix. First, we excluded the morphological data set of Wiens et al. (2005) because it applied to relatively few of the taxa (~80), could not be included in the likelihood analysis (at least using RAxML), and was shown to be potentially misleading (at least in part) by Wiens et al. (2005).

Second, following Hua et al. (2009), we used new sequences of *Hyla walkeri* (for 12S, ND1, c-myc, and POMC) and deleted all sequences for this species from Faivovich et al. (2005). The latter sequences are from a specimen from a pet store with no locality data; our genetic and morphological analyses suggest that this specimen is actually a misidentified *Hyla* from Asia (possibly *H. immaculata*), rather than the Central American species *Hyla walkeri* (Hua et al., 2009). Similarly, we used new sequences from Hua et al. (2009) for *Hyla gratiosa* for 12S, ND1, POMC, and c-myc and used those of Faivovich et al. (2005) for the other genes. The sequences for this species for these four genes (12S, ND1, POMC, c-myc) from Smith et al. (2005), also used by Wiens et al. (2006) and Smith et al. (2007a), seem to be from a museum tissue sample that was incorrectly labeled as *H. gratiosa* (Hua et al., 2009).

Third, we have followed various taxonomic changes that appeared subsequent to Wiens et al. (2006). Several species labeled as "*sp.*" by Faivovich et al. (2005; and subsequently by Wiens et al., 2006) have been formally described (Frost, 2010), including *Aplastodiscus eugeneioi* ("*A. sp. 1 aff. ehrhardti*"), *Bokermannohyla itapoty* ("*B. sp. 9 aff. alverangai*"), *Bokermannohyla oxente* ("*B. sp. 6 aff. pseudopseudis*"), *Hypsiboas curupi* ("*H. sp. 7 aff. semiguttatus*"), and *Hypsiboas nympha* ("*H. sp. 2*"). The sample of *Pseudacris feriarum* from Louisiana used by Moriarity and Cannatella (2004) and in subsequent papers (Wiens et al., 2005, 2006; Smith et al., 2005, 2007a) actually belongs to a newly described species, *P. foquettei* (Lemmon et al., 2008). We use *Hypsiboas cinerascens* to refer to the species previously referred to as *H. granosa* (Frost, 2010).

Fourth, we removed the 12S sequence of *Litoria peronii* and *L. rubella* from Wiens et al. (2005) and used the 12S sequences from Young et al. (2005) instead, which are accompanied by data from 16S for the same individuals. However, we also included the ND1 data from Wiens et al. (2005) for the latter species.

Fifth, we added several new sequences from the study of *Pseudis* and *Lysapsus* by Garda and Cannatella (2007). These authors treated various subspecies of *Lysapsus limellus* and *Pseudis paradoxus* as if they were potentially distinct species, a practice which we also followed (see also Aguiar et al., 2007). However, other previous studies of hylid phylogeny did not identify the subspecies to which their specimens belonged. In order to integrate data from multiple genes for these two species, we assigned the sequences from previous studies (e.g., Faivovich et al., 2005; Wiens et al., 2005) to the "type" subspecies of each of these two species. Garda and Cannatella (2007) also recognized *Pseudis cardosoi* and *P. minutus* as a separate genus (*Podonectes*), a move which we tentatively follow here. Aguiar et al. (2007) also studied relationships among species of *Lysapsus* and *Pseudis*, but their sampling of species and genes generally overlapped with those of Garda and Cannatella (2007) and previous studies, and they did not make their sequences available on GenBank.

Sixth, we excluded a few taxa with very limited sampling of genes that were included in other studies, particularly if they lacked 12S data (see above), including *Isthmohyla lancasteri* and *Ptychohyla salvadorensis* (the latter should probably be assigned to *Duellmanohyla*; Smith et al., 2007a).

Seventh, we note that for some of the outgroup taxa, the study of Wiens et al. (2006) combined genes from Wiens et al. (2005) with genes from Faivovich et al. (2005) from congeneric species

or other related species for distant outgroup taxa (e.g., the microhyline microhylids *Kaloula* and *Gastrophyne*; the myobatrachine myobatrachids *Pseudophryne* and *Uperoleia*; the limnodynastine myobatrachids *Limnodynastes* and *Notaden*). We also included two additional outgroup taxa (*Phrynopus*) not included by Wiens et al. (2006).

3. Results

3.1. Higher-level relationships

Higher-level relationships based on maximum likelihood (ln = −358,019.695) are similar to those postulated in previous studies (Fig. 1). We find strong support for the monophyly of Hylidae, for each of the three subfamilies of Hylidae, and for a sister group relationship between Pelodryadinae and Phyllomedusinae (e.g., Faivovich et al., 2005; Wiens et al., 2005, 2006). Our results do not support those of Roelants et al. (2007), which suggested that phyllomedusines and pelodryadines are not closely related to other hylids (but with weak support and limited taxon sampling). Further, we do not support the related suggestion by Bossuyt and Roelants (2009) that Pelodryadinae and Phyllomedusinae should be recognized as separate families.

There is strong support for the monophyly of most tribes within Hylinae, including Cophomantini, Hylini, and Lophiohylini. However, the monophyly of Dendropsophini is only weakly supported (bs [bootstrap support] <50%). The basal placement of Cophomantini is very strongly supported. There is moderate support (bs = 60%) for placing Lophiohylini and Hylini as sister taxa.

Higher-level relationships based on parsimony (see on-line Appendix 3) are similar to those from the parsimony analysis of Wiens et al. (2006). We found a single tree of length 88,526 steps. Again, the monophyly and relationships of the three subfamilies are strongly supported. Most tribes are strongly supported as monophyletic, but the Dendropsophini is not monophyletic, given that *Scinax* is only distantly related to the other genera. Furthermore, all relationships among the hyline tribes are weakly supported (e.g., support for basal placement of Cophomantini is only 52%) and some are discordant with the maximum likelihood results of this study and results from previous studies (e.g., Faivovich et al., 2005; Wiens et al., 2005). *Scinax* is placed as the sister taxon to all tribes above the Cophomantini. The remaining Dendropsophini are placed as the sister group to Hylini rather than the Lophiohylini. For the sake of brevity, we focus on results from likelihood in all subsequent sections.

3.2. Pelodryadinae

Previous studies of hylid phylogeny have included relatively few pelodryadine species (only 17 in Wiens et al. (2006)). Here we include 51. Our results (Fig. 2) show that the genus *Litoria* is paraphyletic with respect to the other two pelodryadine genera (*Cyclorana*, *Nyctimystes*). This was suggested in previous studies (Faivovich et al., 2005; Wiens et al., 2005, 2006), based on more limited sampling of species, and Frost et al. (2006) proposed placing *Cyclorana* and *Nyctimystes* in the synonymy of *Litoria*. Our results strongly support this taxonomic change, as we describe below.

We show that pelodryadines are divided into two strongly supported clades (Fig. 2). The first clade includes 21 sampled species of *Litoria* (species groups below follow Tyler and Davies, 1978; Frost, 2010), including species of the *rubella* group (*L. dentata*, *L. rubella*), *peronii* group (*L. rothii, L. amboinensis*, *L. peronii*), *dorsalis* group (*L. microbelos*), *beckii* group (*L. modica*), *arfakiana* group (*L. arfakiana*), *thesaurensis* group (*L. thesaurensis*), *bicolor* group (*L. bicolor*, *L. fallax*), *booroolongensis* group (*L. booroolongensis*), *latopalmata* group (*frecyneti*, *L. inermis*, *L. nasuta*, *L. pallida*, *L. tornieri*, *L. watjulumensis),* and *coplandi* group (*L. coplandi*). The tree confirms the monophyly of some of these groups (e.g., *peronii* group, *rubella* group), but suggests that the *bicolor* group is paraphyletic with respect to the *booroolongensis* group, and that the *latopalmata* group is paraphyletic with respect to the *coplandi* group. The placement of *L. wollastoni* with the biogeographically distant *L. watjulumensis* with very little divergence between them most likely represents an error.

The second clade is subdivided into two strongly supported subclades (Fig. 2). One subclade includes *Litoria infrafrenata* and the genus *Nyctimystes*. The other subclade includes 15 sampled species of *Litoria*, the genus *Cyclorana*, and one species of *Nyctimystes* (*N. dayi*), which is sister to *Litoria nannotis*. Interestingly, *N. dayi* is the only species of *Nyctimystes* occurring in Australia, and it occurs in the rainforests near Cairns, Queensland, as does *L. nannotis* (Cogger, 1992). This second subclade includes species of the *citropa* group (*L. phyllocroa*, *L. subglandulosa*), *caerulea* group (*L. caerulea*, *L. gilleni*, *L. splendida*), *chloris* group (*L. chloris*, *gracilenta*, *L. xanthomera*), *eucnemis* group (*L. eucnemis*, *L. genimaculata*), *lesueurii* group (*L. lesueurii*), *nannotis* group (*L. nannotis*), *infrafrenata* group (*L. infrafrenata*), and *aurea* group (*L. aurea*, *L. cyclorhyncha*, *L. dahlii*). The tree supports monophyly of the *citropa*, *caerulea*, and *chloris* groups, but shows the *aurea* group to be paraphyletic (with respect to *Cyclorana*) and does not support placement of *L. genimaculata* in the *L. eucnemis* group.

3.3. Phyllomedusinae

Our likelihood tree (Fig. 2) places *Cruziohyla* and *Phrynomedusa* as relatively basal within phyllomedusines, although the relationships of these taxa are somewhat uncertain. The remaining phyllomedusines are divided into two strongly supported clades. One clade includes *Hylomantis*, *Pachymedusa*, and *Agalychnis*. Our results suggest that *Hylomantis* is not monophyletic. Instead, the Brazilian taxon *H. granulosa* is more closely related to *Pachymedusa* and *Agalychnis* than is the primarily Central American *H. lemur*. The monotypic *Pachymedusa* is strongly supported as the sister group to *Agalychnis*, which is strongly supported as monophyletic. *Phasmahyla* and *Phyllomedusa* are strongly supported as sister taxa. These relationships are similar to those postulated by Faivovich et al. (2005; but who lacked data for *Phrynomedusa*), but differ from those postulated by Wiens et al. (2006) who placed *Phasmahyla* as basal (Bayesian analysis) or in a clade with *Cruziohyla*, *Hylomantis*, *Pachymedusa*, and *Agalychnis* (parsimony).

3.4. Hylinae

Within Hylinae, the tribe Cophomantini is placed as sister group to other hylines with strong support, and is strongly supported as monophyletic in our likelihood tree (Fig. 1). Furthermore, relationships among genera within this clade (Fig. 3) are generally strongly supported and congruent between studies (*Myersiohyla* (*Hyloscirtus* (*Bokermannohyla* (*Aplastodiscus* + *Hypsiboas*)))). However, neither our likelihood analyses nor the parsimony analyses of Wiens et al. (2006) support the monophyly of *Myersiohyla* (although it is supported in our parsimony analysis here; on-line Appendix 3).

Our likelihood results offer only weak support for monophyly of Dendropsophini (Fig. 1). However, we do find strong likelihood support for some subclades within this tribe (Fig. 4), including placement of *Xenohyla* with *Dendropsophus* and placement of *Scarthyla* with *Pseudis* and *Lysapsus*. We also find moderately strong support (bs = 68%) for a clade including these five genera. We place *Sphaenorhynchus* with *Scinax* with only weak support.

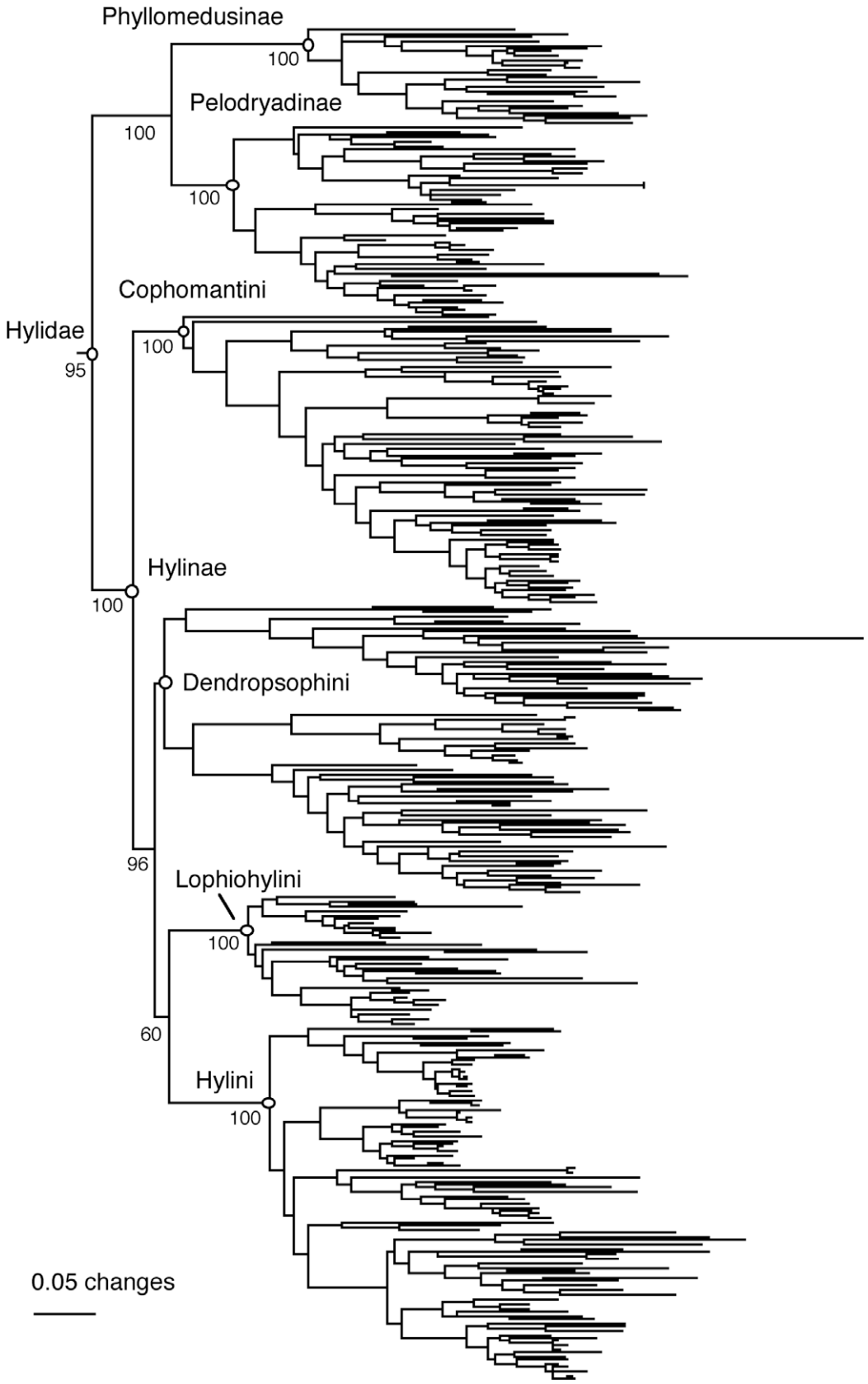

Fig. 1. Higher-level phylogeny and taxonomy of hylid frogs based on maximum likelihood analysis of combined nuclear and mitochondrial genes. Numbers adjacent to nodes indicate bootstrap values $\geqslant$50%, but only values for named clades are shown. Species names and bootstrap values within named clades are shown in subsequent figures.

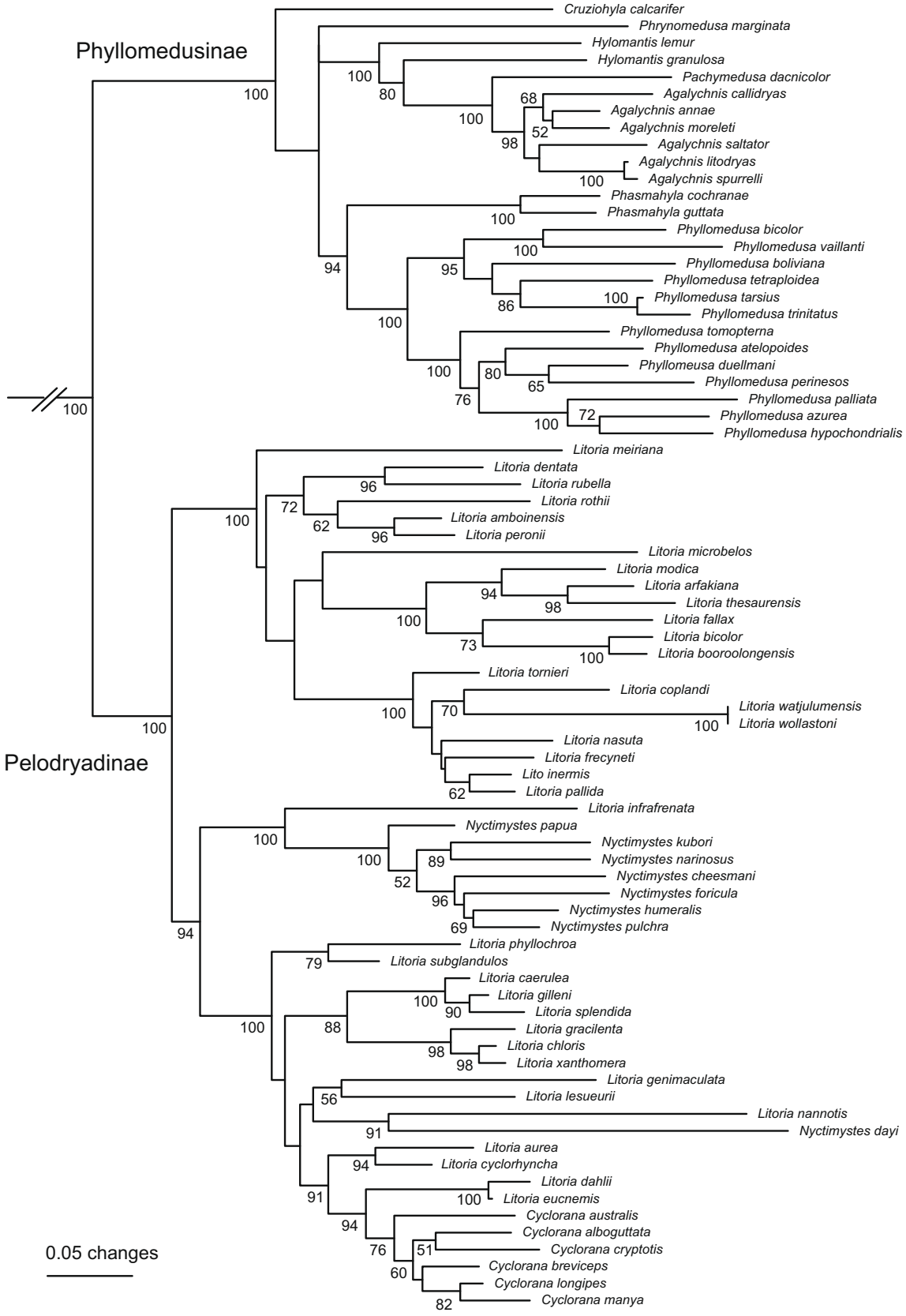

Fig. 2. Phylogeny of Pelodryadinae and Phyllomedusinae based on maximum likelihood analysis of combined nuclear and mitochondrial genes. Numbers adjacent to nodes indicate bootstrap values $\geqslant 50\%$.

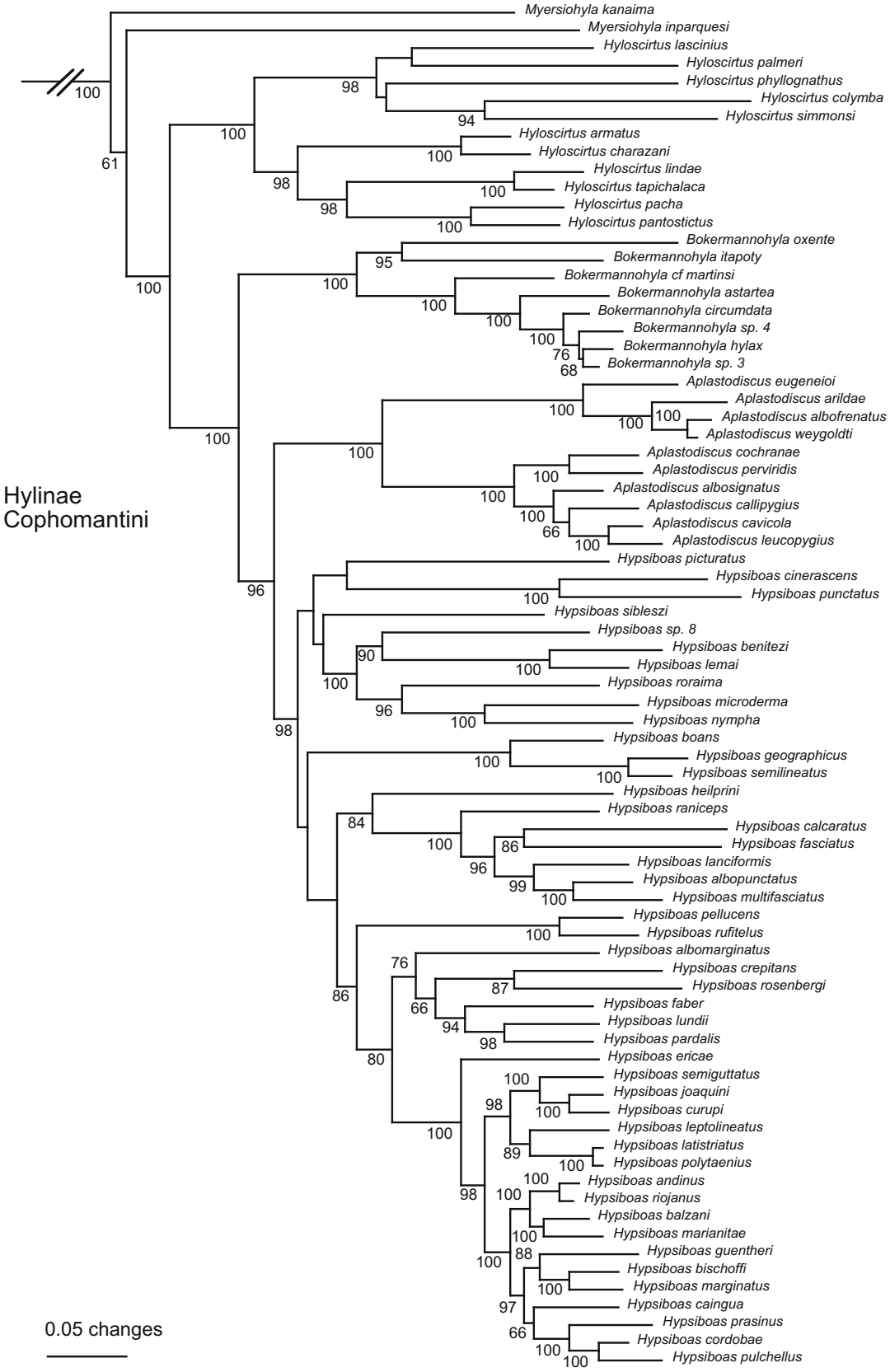

Fig. 3. Phylogeny of the hyline tribe Cophomantini based on maximum likelihood analysis of combined nuclear and mitochondrial genes. Numbers adjacent to nodes indicate bootstrap values $\geqslant 50\%$.

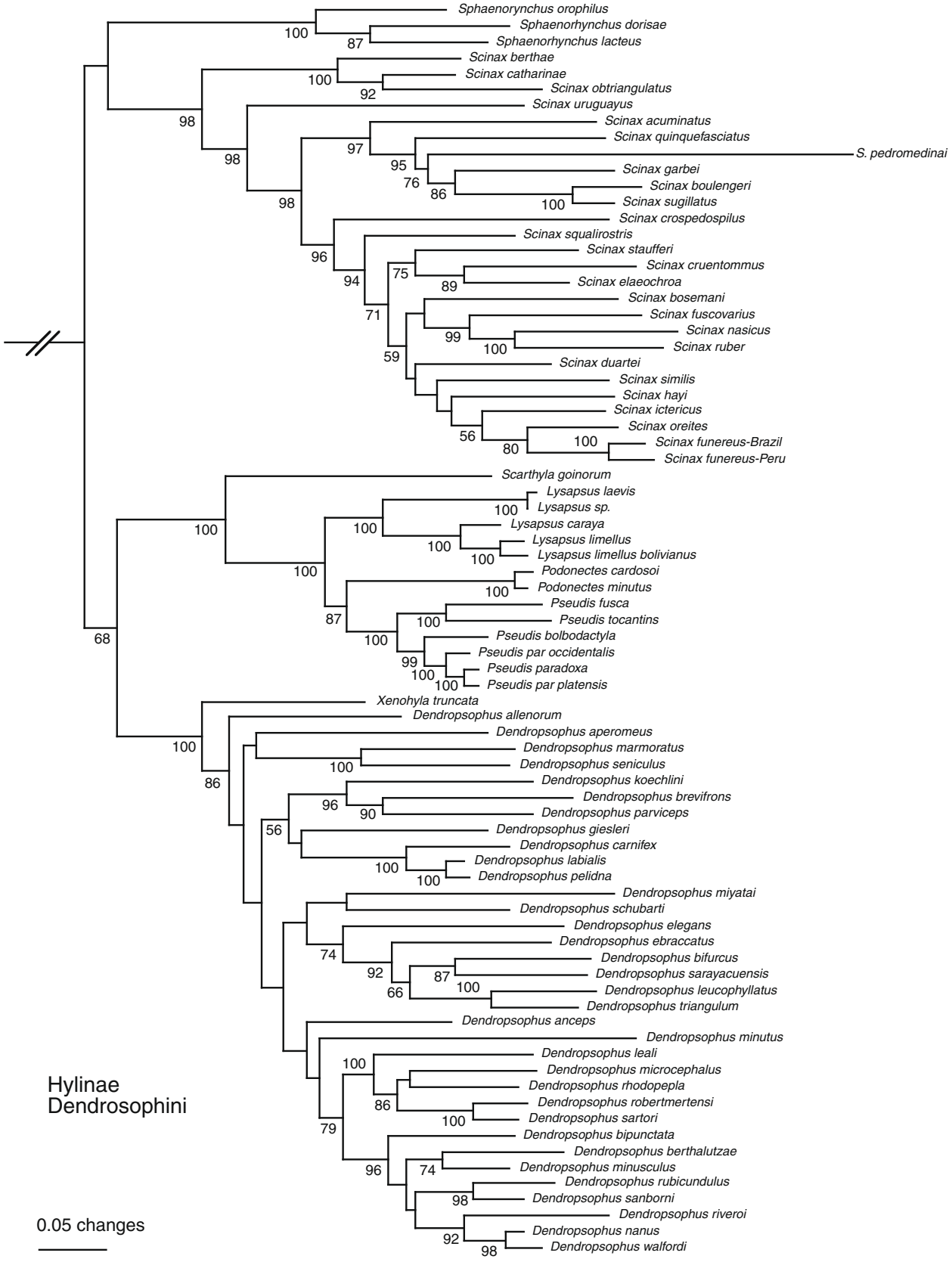

Fig. 4. Phylogeny of the hyline tribe Dendropsophini based on maximum likelihood analysis of combined nuclear and mitochondrial genes. Numbers adjacent to nodes indicate bootstrap values $\geqslant 50\%$.

Contrary to the results of Garda and Cannatella (2007), our results do not suggest that *Pseudis* is paraphyletic with respect to *Lysapsus*. Our results support the monophyly of their genus *Podonectes*, but they also suggest that recognition of this genus is unnecessary to maintain the monophyly of *Pseudis*. Similarly, Aguiar et al. (2007) found weak support for non-monophyly of *Pseudis* with respect to *Lysapsus*, and suggested that *Lysapsus* should be placed in the monophyly of *Pseudis*. Our results suggest that such a change is unnecessary.

Our phylogeny includes many species of *Scinax* not included in previous analyses. We strongly support the *catharinae* group (represented by *S. berthae*, *S. catharinae*, and *S. obtriangulatus*) as the sister group to other *Scinax*. Our phylogeny supports the *Scinax ruber* clade recognized by Faivovich et al. (2005) for the remaining species. We strongly support *S. uruguayus* as sister taxon to all other species of this clade. Our data strongly support *S. acuminatus* and *S. quinquefasciatus* as successive outgroups to the distinctive *rostratus* group, represented here by *S. boulengeri*, *S. garbei*, *S. pedromedinai*, and *S. sugillatus*.

Our phylogeny within *Dendropsophus* (Fig. 4) supports some of the species groups recognized by Faivovich et al. (2005), including the sampled species of the *labialis* group (*D. labialis*, *D. pelidna*) and *marmoratus* group (*D. marmoratus*, *D. seniculus*). However, our phylogeny does not support monophyly of the *leucophyllatus* group, given the failure of *D. anceps* to cluster with *D. elegans*, *D. ebraccatus*, *D. bifurcus*, *D. sarayacuensis*, *D. leucophyllatus*, and *D. triangulum*. Our phylogeny does not support monophyly of the *parviceps* group, given the failure of *D. allenorum* and *D. schubarti* to cluster with *D. koechlini*, *D. brevifrons*, and *D. parviceps*. Our phylogeny does not support monophyly of the *D. minimus* group (represented by *D. aperomeus*, *D. miyatai*, and *D. riveroi*). We do support a clade that includes all of the sampled species of the *microcephalus* group including *D. berthalutzae*, *D. bipunctatus*, *D. leali*, *D. microcephalus*, *D. minusculus*, *D. nanus*, *D. rhodopeplus*, *D. robertmertensi*, *D. rubicundulus*, *D. sartori*, *D. sanborni*, and *D. walfordi*. However, *D. riveroi* of the *minimus* group is also placed in this clade.

Our likelihood phylogeny strongly supports monophyly of Lophiohylini, but relationships among some of the genera are somewhat uncertain (Fig. 5). For example, our results show weak support for placing *Phyllodytes auratus* with *Itapotihyla langsdorffii*, rather than with the other sampled species of *Phyllodytes*. Interestingly, *Phyllodytes auratus* occurs in Trinidad and Tobago, distantly removed from the other species of *Phyllodytes* in southeastern Brazil (IUCN 2009). Based on an analysis of mtDNA sequences only for Lophiohylini (almost all data from Faivovich et al. (2005)), Jowers et al. (2008) suggested that *Phyllodytes auratus* should be recognized as a monotypic genus (*Phytotriades*). However, such a move seems premature given that only one other named species of *Phyllodytes* was included in their analysis and in the present study (and that our parsimony analysis and that of Wiens et al. (2006) actually support monophyly of *Phyllodytes*). We find some support (bs = 56%) for a clade linking *Trachycephalus* with a clade consisting of (*Corythomantis greeningi* (*Aparasphenodon brunoi* (*Argenteohyla siemersi*, *Nyctimantis rugiceps*))). These relationships are similar to those postulated by Faivovich et al. (2005) and Wiens et al. (2006; Bayesian), but differ in placement of *Aparasphenodon* and *Argenteohyla*.

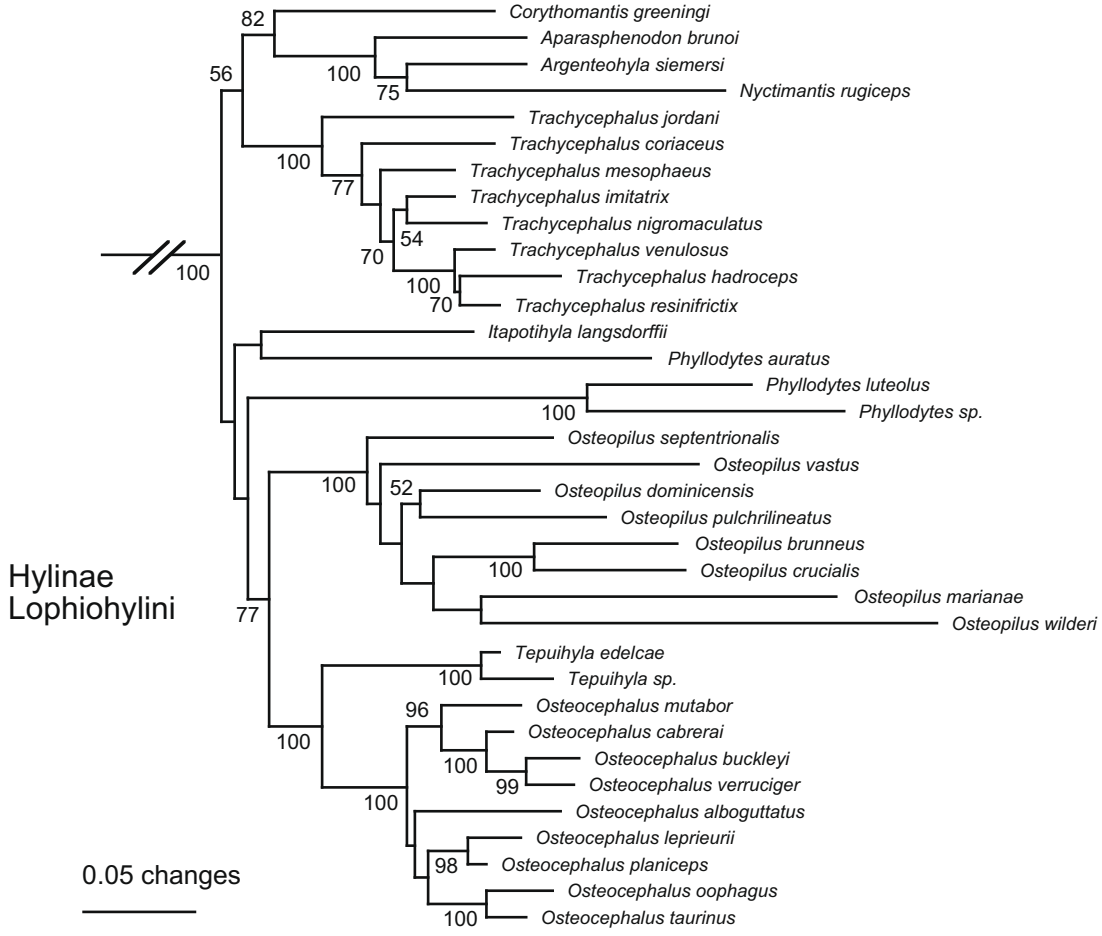

Fig. 5. Phylogeny of the hyline tribe Lophiohylini based on maximum likelihood analysis of combined nuclear and mitochondrial genes. Numbers adjacent to nodes indicate bootstrap values ⩾50%.

We find moderately strong support for a clade uniting *Osteopilus*, *Tepuihyla*, and *Osteocephalus* (bs = 77%), and strong support for a clade consisting of *Osteocephalus* and *Tepuihyla* (bs = 100%). These clades were also found by Faivovich et al. (2005), the Bayesian analysis of Wiens et al. (2006), and Moen and Wiens (2009). We note that relationships among some lophiohylines (e.g., *Osteopilus*) are more strongly supported in the study of Moen and Wiens (2009), which included additional genes too narrowly sampled to include here. Many relationships among lophiohyline genera were unresolved in the parsimony analysis of Wiens et al. (2006).

We find strong support for the monophyly of Hylini, or Middle American Clade. Within this clade (Fig. 6), most relationships among the genera are strongly supported, as is the monophyly of the genera. We find strong support for a series of successively derived clades consisting of: (a) *Acris* and *Pseudacris*, (b) *Exerodonta* and *Plectrohyla*, (c) *Bromeliohyla*, *Duellmanohyla*, *Ecnomiohyla*, and *Ptychohyla*, (d) *Charadrahyla* and *Megastomatohyla*, and (e) the seven remaining genera (*Anotheca*, *Diaglena*, *Hyla*, *Isthmohyla*, *Smilisca*, *Tlalocohyla*, *Triprion*). Within this latter clade, relationships are weakly supported among *Hyla*, *Isthmohyla*, and the *Smilisca* clade (*Anotheca*, *Diaglena*, *Smilisca*, *Triprion*). Relationships within the Hylini are generally similar to those postulated by previous authors (especially Smith et al., 2007a), but with some exceptions. Most notably, Faivovich et al. (2005) did not place *Charadrahyla* and *Megastomatohyla* as sister taxon. Wiens et al. (2006) placed them as sister taxa, but they also placed them collectively as the sister group to the *Ptychohyla* clade (*Bromeliohyla*, *Duellmanohyla*, *Ecnomiohyla*, *Ptychohyla*). Our results suggest that *Ecnomiohyla* may not be monophyletic, but this is not strongly supported.

4. Discussion

In this study, we present an expanded phylogeny for hylid frogs based primarily on maximum likelihood analysis of 362 putative hylid species, including all hylid genera. Prior to this study, the most comprehensive phylogeny for hylids was a parsimony analysis of 292 species (Wiens et al., 2006). However, that phylogeny was, in several parts, weakly supported (e.g., relationships among tribes within Hylinae), unresolved (e.g., relationships among many lophiohyline genera), and discordant with previous hypotheses (e.g., non-monophyly of Dendropsophini, placement of most Dendropsophini with Hylini). Our new likelihood-based phylogeny is generally more well-resolved, well-supported, and congruent with previous hypotheses (e.g., Faivovich et al., 2005; Wiens et al., 2005; and the smaller, Bayesian tree of Wiens et al. (2006)).

These improvements in support and congruence in the present study (relative to the large parsimony tree of Wiens et al. (2006)) seem to come from the application of model-based methods (i.e., likelihood), rather than new data. In fact, parsimony analysis of these data (on-line Appendix 3) yields similar trees to the parsimony trees from Wiens et al. (2006). These parsimony trees share many of the same weaknesses (e.g., poor support and resolution in parts, incongruence with previous hypotheses).

Our results provide an interesting contrast with those of Faivovich et al. (2005). Our data set contains essentially all of the data of Faivovich et al. (2005), but supplemented with many additional taxa and genes. Yet, parsimony analyses of this combined data set yield results that are somewhat incongruent with those of Faivovich et al. (2005). In some ways, our likelihood tree is more similar to the parsimony tree of Faivovich et al. (2005) than is the parsimony tree from our study. In addition, many intergeneric relationships are only weakly supported by parsimony bootstrapping in our study (on-line Appendix 3). In contrast, nearly every node of the Faivovich et al. (2005) is strongly supported by parsimony jackknifing (255 of 272 nodes have values >75% and 245 nodes have values >90%). Paradoxically, despite the added data, there seems to be generally weaker support in both our parsimony and likelihood trees (although it is not clear if bootstrap and jackknife support values are directly comparable).

What explains this discrepancy? One potential explanation is that Faivovich et al. (2005) utilized direct optimization (Wheeler, 1996) as implemented in POY (Wheeler et al., 2002), a method by which alignment and phylogeny are estimated simultaneously. Whether POY offers a better or worse method for aligning sequences is debated (e.g., Kjer et al., 2007; Ogden and Rosenberg 2007; Lehotonen, 2008). However, there are clearly troubling aspects to this approach, at least as it is commonly implemented. For example, in a broader study of amphibian phylogeny, these same authors (Frost et al., 2006) generated >15,000 characters from ~4700 base pairs of sequence data in their "implied alignment" from POY ("implied" because an actual alignment is not generated), suggesting that the overwhelming majority of the characters generated by this method were from indels. It is unclear how many characters the tree of Faivovich et al. (2005) is actually based on, and what kinds of changes these are, as this information was not published. Further, Faivovich et al. (2005) noted that their support values are based on the implied alignment, and that "this implies that the parsimony jackknife values could be overestimated" (p. 48). Also, Faivovich et al. (2005) conducted their primary analyses assuming that insertions and deletions have the same weight as substitutions and that all length changes are independent of each other (e.g., a 10 bp deletion is equivalent to 10 substitutions, even though a single evolutionary change may have generated this 10 bp gap). Faivovich et al. (2005) briefly assessed the sensitivity of their results to this assumption, and found that only a few clades shifted position (e.g., *Lysapsus*, *Pseudis*, *Scarthyla*; their Fig. 4, p. 56). However, their support for the original placement of these clades is also very strong. In other words, the support values for clades in the tree of Faivovich et al. (2005) are not necessarily indicative of the robustness of these clades, even using identical data and similar methods. In summary, although alignments from POY may have some advantages, there is cause for some concern about the underlying characters and potentially misleading support values in the results of Faivovich et al. (2005).

Much additional work is still needed on hylid phylogeny. For example, the monophyly and interrelationships of the Dendropsophini are still somewhat uncertain (Fig. 1). Our results also suggest the possibility that some genera may not be monophyletic, including *Ecnomiohyla*, *Hylomantis*, *Myersiohyla*, and *Phyllodytes* (and *Duellmanohyla* and *Ptychohyla*; see Smith et al. (2007a)). However, the non-monophyly of these genera is only moderately well-supported by likelihood, and some genera are actually supported in the parsimony analyses (*Ecnomiohyla*, *Myseriohyla*, *Phyllodytes;* on-line Appendix 3). In addition, even if non-monophyly were strongly supported, subdivision of these genera may require more extensive taxon sampling to define new generic limits.

Clearly, the major area for future work in hylid phylogeny is to include the >400 hylid species that have yet to be sampled. These unsampled species are spread throughout the phylogeny; only a handful of non-monotypic genera have complete taxon sampling (e.g., *Agalychnis*, *Osteopilus*, *Tlalocohyla*). Further, for taxa that are included, it would be useful to have multiple loci sequenced for all species. Given this, some might argue that it is not useful to publish an expanded phylogeny until more species and genes are added. Even if it is not the last word on hylid phylogeny, we have nevertheless provided an improved estimate of phylogeny that can be used as a foundation for future systematic studies and for use in comparative evolutionary, ecological, and biogeographic studies that utilize hylids as a model system (e.g., Young et al., 2005; Smith et al., 2005, 2007a,b; Wiens et al., 2006; Gomez-Mestre et al., 2008; Moen and Wiens, 2009; Moen et al., 2009; Hua and Wiens, 2010).

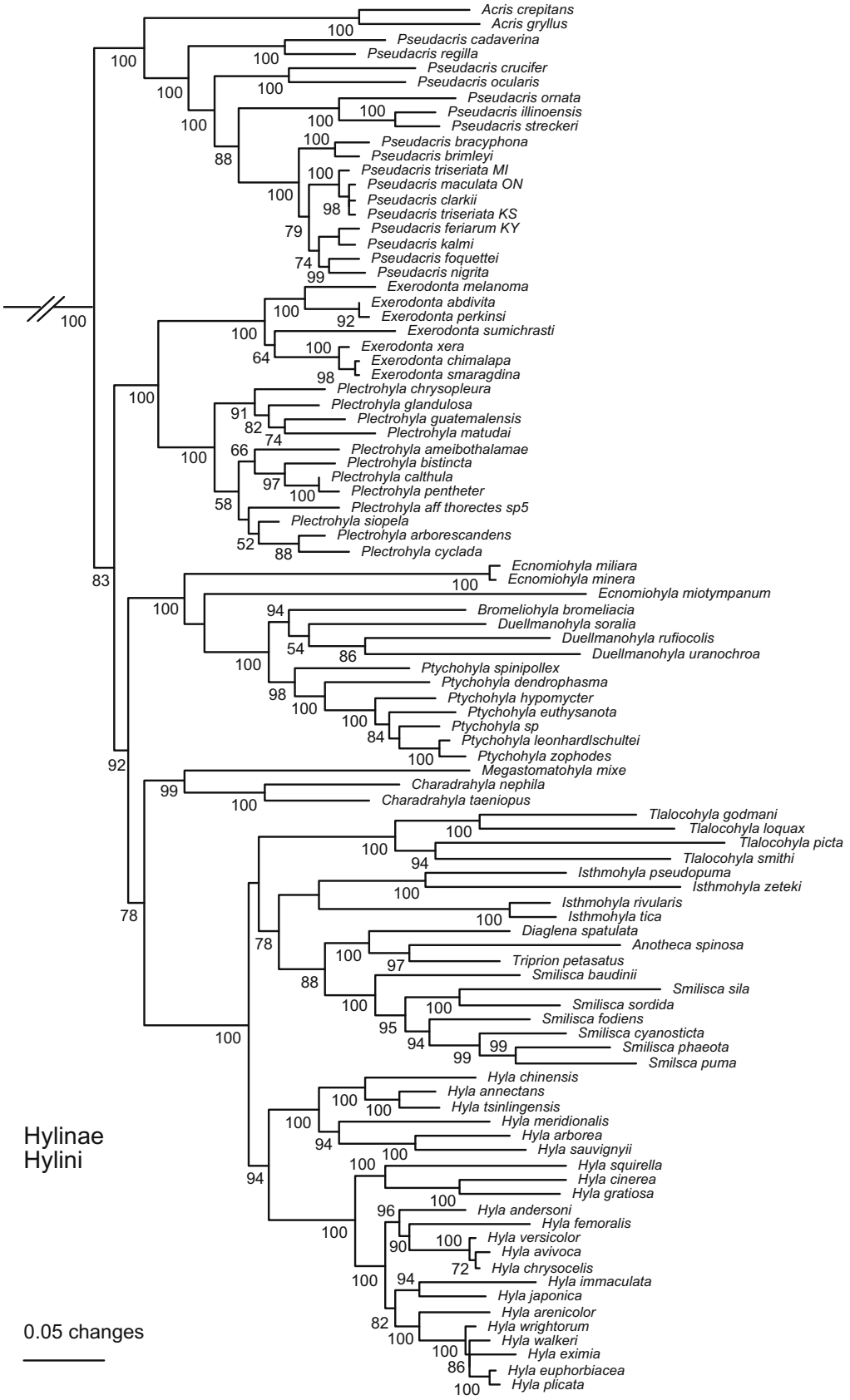

Fig. 6. Phylogeny of the hyline tribe Hylini based on maximum likelihood analysis of combined nuclear and mitochondrial genes. Numbers adjacent to nodes indicate bootstrap values $\geqslant$50%.

Acknowledgments

We thank the many individuals and institutions who provided tissue samples that were used in this and our previous phylogenetic analyses of hylids, including J. Campbell and E. Smith (Univ. Texas, Arlington), W.R. Heyer and K. de Queiroz (U.S. National Museum), E. Greenbaum, W.E. Duellman and J. Simmons (Univ. Kansas), S.B. Hedges, K. Lips, I. De La Riva, A. Nieto Montes de Oca, B.P. Noonan, T.W. Reeder, J. Skejic, and D. Wake (Museum of Vertebrate Zoology, Univ. California, Berkeley). We thank A. Kathriner for assistance in the laboratory. We are grateful to our collaborators on previous papers on hylid phylogeny, who provided tissues and sequence data, including W.E. Duellman, C. Fu, I. Gomez-Mestre, J. Li, T.W. Reeder, and S. Smith. We thank A. Nieto Montes de Oca and T.W. Reeder for assistance with fieldwork in Mexico, which was supported by grants from the Netting and O'Neill funds of the Carnegie Museum of Natural History. For financial support we thank U.S. National Science Foundation Grant (EF 0334923) to J.J.W. and a National Science Foundation Graduate Research Fellowship to D.S.M. We thank J. Schulte and two anonymous reviewers for comments on the manuscript.

Appendices 1–3. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.03.013.

References

Aguiar Jr., O., Bacci Jr., M., Lima, A.P., Rossa-Feres, D.C., Haddad, C.F.B., Recco-Pimentel, S.M., 2007. Phylogenetic relationships of *Pseudis* and *Lysapsus* (Anura, Hylidae, Hylinae) inferred from mitochondrial and nuclear gene sequences. Cladistics 23, 455–463.

AmphibiaWeb, 2010. Information on amphibian biology and conservation (web application). AmphibiaWeb, Berkeley, California. <http://amphibiaweb.org/> (accessed 8.02.2010).

Bossuyt, F., Roelants, K., 2009. Anura. In: Hedges, S.B., Kumar, S. (Eds.), Timetree of Life. Oxford University Press, New York, pp. 357–364.

Brandley, M.C., Schmitz, A., Reeder, T.W., 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Syst. Biol. 54, 373–390.

Cogger, H.A., 1992. Reptiles and Amphibians of Australia. Cornell University Press, Ithaca, New York.

Driskell, A.C., Ané, C., Burleigh, J.G., McMahon, M.M., O'Meara, B.C., Sanderson, M.J., 2004. Prospects for building the Tree of Life from large sequence databases. Science 306, 1172–1174.

Duellman, W.E., Trueb, L., 1985. Biology of Amphibians. McGraw-Hill, New York, NY.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

Edwards, S.V., Liu, L., Pearl, D.K., 2007. High resolution species trees without concatenation. Proc. Natl. Acad. Sci. USA 104, 5936–5941.

Faivovich, J., Haddad, C.F.B., Garcia, P.C.A., Frost, D.R., Campbell, J.A., Wheeler, W.C., 2005. Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. Bull. Am. Mus. Nat. Hist. 294, 1–240.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Felsenstein, J., 2004. Inferring Phylogenies. Sinauer Associates, Sunderland, MA.

Frost, D.R., 2010. Amphibian Species of the World: An Online Reference, version 5.3. American Museum of Natural History, New York, USA. <http://research.amnh.org/herpetology/amphibia/> (accessed 5.02.2010).

Frost, D.R. et al., 2006. The amphibian tree of life. Bull. Am. Mus. Nat. Hist. 297, 1–370.

Garda, A.A., Cannatella, D.C., 2007. Phylogeny and biogeography of paradoxical frogs (Anura, Hylidae, Pseudae) inferred from 12S and 16S mitochondrial DNA. Mol. Phylogenet. Evol. 44, 104–114.

Gomez-Mestre, I., Wiens, J.J., Warkentin, K.M., 2008. Evolution of adaptive plasticity: risk-sensitive hatching in neotropical leaf-breeding treefrogs (*Agalychnis*: Hylidae). Ecol. Monogr. 78, 205–224.

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.

Hua, X., Wiens, J.J., 2010. Latitutudinal variation in speciation mechanisms in frogs. Evolution 64, 429–443.

Hua, X., Fu, C., Li, J., Nieto Montes de Oca, A., Wiens, J.J., 2009. A revised phylogeny of Holarctic treefrogs (genus *Hyla*) based on nuclear and mitochondrial DNA sequences. Herpetologica 65, 246–259.

Jowers, M.J., Downie, J.R., Cohen, B.L., 2008. The Golden Tree Frog of Trinidad, *Phyllodytes auratus* (Anura: Hylidae): systematic and conservation status. Stud. Neotrop. Faun. Environ. 43, 181–188.

Kjer, K.M., Gillespie, J.J., Ober, K.A., 2007. Opinions on multiple sequence alignment, and an empirical comparison of repeatability and accuracy between POY and structural alignments. Syst. Biol. 56, 133–146.

Lehotonen, S., 2008. Phylogeny estimation and alignment via POY versus Clustal + PAUP*: a response to Ogden and Rosenberg (2007). Syst. Biol. 57, 653–657.

Lemmon, E.M., Lemmon, A.R., Collins, J.T., Lee-Yaw, J.A., Cannatella, D.C., 2007. Phylogeny-based delimitation of species boundaries and contact zones in the trilling chorus frogs. Mol. Phylogenet. Evol. 44, 1068–1082.

Lemmon, E.M., Lemmon, A.R., Collins, J.T., Cannatella, D.C., 2008. A new North American chorus frog species (Amphibia: Hylidae: *Pseudacris*) from the south-central United States. Zootaxa 1675, 1–30.

Lemmon, A.R., Brown, J.M., Stanger-Hall, K., Moriarty Lemmon, E., 2009. The effect of ambiguous data on phylogenetic estimates obtained by maximum likelihood and Bayesian inference. Syst. Biol. 58, 130–145.

Macey, J.R., Larson, A., Ananjeva, N.B., Papenfuss, T.J., 1997. Replication slippage may cause parallel evolution in the secondary structures of mitochondrial transfer RNAs. Mol. Biol. Evol. 14, 30–39.

Maddison, D.R., Maddison, W.P., 2000. MacClade 4.0. Sinauer Associates, Sunderland, MA.

Moen, D.S., Wiens, J.J., 2009. Phylogenetic evidence for competitively-driven divergence: body-size evolution in Caribbean treefrogs (Hylidae: *Osteopilus*). Evolution 63, 195–214.

Moen, D.S., Smith, S.A., Wiens, J.J., 2009. Community assembly through evolutionary diversification and dispersal in Middle American treefrogs. Evolution 63, 3228–3247.

Moriarity, E.C., Cannatella, D.C., 2004. Phylogenetic relationships of North American chorus frogs (genus *Pseudacris*), from 12S and 16S mtDNA. Mol. Phylogenet. Evol. 30, 409–420.

Nixon, K.C., 1999. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics 15, 407–414.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J.L., 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53, 47–67.

Ogden, T.H., Rosenberg, M.S., 2007. Alignment and topological accuracy of the direct optimization approach via POY and traditional phylogenetics via ClustalW + PAUP*. Syst. Biol. 56, 182–193.

Philippe, H., Snell, E.A., Bapteste, E., Lopez, P., Holland, P.W.H., Casane, D., 2004. Phylogenomics of eukaryotes: impact of missing data on large alignments. Mol. Biol. Evol. 21, 1740–1752.

Roelants, K., Gower, D.J., Wilkinson, M., Loader, S.P., Biju, S.D., Guillaume, K., Bossuyt, F., 2007. Patterns of diversification in the history of modern amphibians. Proc. Natl. Acad. Sci. USA 104, 887–892.

Sikes, D.S., Lewis, P.O., 2001. PAUPRat: PAUP Implementation of the Parsimony Ratchet. Beta Software, version 1. Distributed by the authors, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs.

Smith, S.A., Stephens, P.R., Wiens, J.J., 2005. Replicate patterns of species richness, historical biogeography, and phylogeny in Holarctic treefrogs. Evolution 59, 2433–2450.

Smith, S.A., Nieto Montes de Oca, A., Reeder, T.W., Wiens, J.J., 2007a. A phylogenetic perspective on elevational species richness patterns in Middle American treefrogs: why so few species in lowland tropical rainforests? Evolution 61, 1188–1207.

Smith, S.A., Arif, S., Nieto Montes de Oca, A., Wiens, J.J., 2007b. A phylogenetic hotspot for evolutionary novelty in Middle American treefrogs. Evolution 61, 2075–2085.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.

Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony* v. 4.0b10. Sinauer, Sunderland, MA.

Tyler, M.J., Davies, M., 1978. Species-groups within the Australopapuan hylid frog genus *Litoria* Tschudi. Aust. J. Zool. 63, 1–47 (Supplemental Series).

Wheeler, W.C., 1996. Optimization Alignment: the end of multiple sequence alignment in phylogenetics? Cladistics 12, 1–9.

Wheeler, W.C., Gladstein, D.S., De Laet, J., 2002. POY, version 3.0. Available from: <ftp://amnh.org/pub/molecular/poy> (current version 3.0.11).

Wiens, J.J., 2003. Missing data, incomplete taxa, and phylogenetic accuracy. Syst. Biol. 52, 528–538.

Wiens, J.J., 2007. Global patterns of species richness and diversification in amphibians. Am. Nat. 170, S86–S106.

Wiens, J.J., Fetzner, J.W., Parkinson, C.L., Reeder, T.W., 2005. Hylid frog phylogeny and sampling strategies for speciose clades. Syst. Biol. 54, 719–748.

Wiens, J.J., Graham, C.H., Moen, D.S., Smith, S.A., Reeder, T.W., 2006. Evolutionary and ecological causes of the latitudinal diversity gradient in hylid frogs: treefrog trees unearth the roots of high tropical diversity. Am. Nat. 168, 579–596.

Wiens, J.J., Kuczynski, C., Duellman, W.E., Reeder, T.W., 2007. Loss and re-evolution of complex life cycles in marsupial frogs: can ancestral trait reconstruction mislead? Evolution 61, 1886–1899.

Young, J.E., Christian, K.A., Donnellan, S., Tracy, C.R., Parry, D., 2005. Comparative analysis of cutaneous evaporative water loss in frogs demonstrates correlation with ecological habits. Physiol. Biochem. Zool. 78, 847–856.