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Phylogenetic relationships of glassfrogs (Centrolenidae) based on mitochondrial and nuclear genes

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ABSTRACT

Glassfrogs (family Centrolenidae) represent an exceptionally diverse group among Neotropical anurans, but their evolutionary relationships never have been assessed from a molecular perspective. Mitochondrial and nuclear markers were used to develop a novel hypothesis of centrolenid phylogeny. Ingroup sampling included 100 terminals, with 78 (53%) of the named species in the family, representing most of the phenotypic diversity described for the group. Thirty-five species representing taxa traditionally associated with glassfrogs were used as outgroups. Gene sampling consisted of complete or partial sequences of three mitochondrial (*12S*, *16S*, *ND1*) and three nuclear markers (*c-myc exon 2*, *RAG1*, *POMC*) for a total of ~4362 bp. Phylogenies were estimated using maximum parsimony, maximum likelihood, and Bayesian analyses for individual genes and combined datasets. The separate analysis of mitochondrial and nuclear datasets allowed us to clarify the relationships within glassfrogs; also, we corroborate the sister-group relationship between *Allophryne ruthveni* and glassfrogs. The new phylogeny differs significantly from all previous morphology-based hypotheses of relationships, and shows that hypotheses based on few traits are likely to misrepresent evolutionary history. Traits previously hypothesized as unambiguous synapomorphies are shown to be homoplastic, and all genera in the current taxonomy (*Centrolene*, *Cochranella*, *Hyalinobatrachium*, *Nymphargus*) are found to be poly- or paraphyletic. The new topology implies a South American origin of glassfrogs and reveals allopatric speciation as the most important speciation mechanism. The phylogeny profoundly affects the traditional interpretations of glassfrog taxonomy, character evolution, and biogeography—topics that now require more extensive evaluation in future studies.

1. Introduction

Anurans of the family Centrolenidae form a monophyletic group nested within Neobatrachia (Darst and Cannatella, 2004; Ford and Cannatella, 1993; Frost et al., 2006; Ruiz-Carranza and Lynch, 1991; Wiens et al., 2005; but see Haas, 2003). Currently, the family includes 147 species (AmphibiaWeb, 2006). Glassfrogs occur throughout the Neotropics and are nocturnal, epiphyllous, and arboreal. They have partially or completely transparent venters, and deposit their eggs on vegetation (leaves or branches) overhanging streams or on rocks above streams; tadpoles develop in streams (Ruiz-Carranza and Lynch, 1991).

To date, the most widely accepted taxonomy of centrolenids is that of Ruiz-Carranza and Lynch (1991, 1995, 1998), who recognized the genera *Centrolene*, *Cochranella*, and *Hyalinobatrachium*, and several infrageneric species groups. Their generic classification was based on the presence of two morphological characteristics—humeral spines in adult male *Centrolene*, and a white, bulbous liver in *Hyalinobatrachium*—and the absence of both of these features in frogs of the genus *Cochranella*. This arrangement implies that the evolutionary patterns of these derived characters (i.e., humeral spines and bulbous, white liver) are unequivocal, and that the frogs and the characters share a perfectly congruent evolutionary history. However, recent research has revealed a surprising amount of evolutionary lability in amphibian morphological traits previously thought to be conserved (e.g., Bossuyt and Milinkovitch, 2000; Manzano et al., 2007; Mueller et al., 2004; Parra-Olea and Wake, 2001; Wiens et al., 2003); the results of these studies suggest that phylogenies based solely on morphological characters

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should be tested with independent datasets. Several authors (Frost et al., 2006; Guayasamin et al., 2006) questioned the monophyly of the groups proposed by Ruiz-Carranza and Lynch (1991), but no alternative hypotheses based on comprehensive phylogenetic analyses have been proposed.

Herein, we present a molecular hypothesis of centrolenid relationships based on multiple mitochondrial and nuclear loci. We find that the molecular phylogeny of glassfrogs is incongruent with all previous hypotheses of relationships. Morphological traits that were hypothesized as unambiguous synapomorphies (i.e., humeral spine, white-bulbous liver) have complex evolutionary histories and are homoplastic. Our main biogeographic findings include the South American origin of glassfrogs and the identification of vicariance as the main mechanism promoting speciation. This comprehensive phylogeny is intended to provide a new evolutionary context for studies addressing the biology and systematics of this fascinating group of tropical anurans.

2. Materials and methods

2.1. Taxonomy and terminology

Throughout this work, we use the name Centrolenidae as originally defined by Taylor (1951; i.e., exclusive of *Allophryne ruthveni*). When referring to the current taxonomy of centrolenid frogs, we follow the generic and infrageneric classifications proposed by Ruiz-Carranza and Lynch (1991, 1995, 1998), with the addition of the genus *Nymphargus* (Cisneros-Heredia and McDiarmid, 2007a). For each species included in the analysis, we examined key morphological traits (i.e., presence/absence of humeral spines, color and shape of liver, and hand webbing) to verify the correct generic assignment. Family and genus of outgroups are as summarized by Frost (2007), except for the placement of *Allophryne ruthveni*, for which we maintain the use of Allophrynidae (Guayasamin and Trueb, 2007). Museum abbreviations follow Frost (2007), with additions noted below (Appendix A).

2.2. Ingroup and outgroup taxon sampling

We obtained molecular data for 100 terminals, including 78 recognized and 11 undescribed centrolenid species (Appendix A). Species with dubious identifications are indicated by adding cf. (=*confer*) between the genus and the specific epithet; putative new species are indicated by adding aff. (=*affinis*) or sp. (=*species*) after the genus. The ingroup sampling thus represents 53.1% of the known species diversity of Centrolenidae, including representatives from all currently recognized genera and infrageneric groups, and all major ecoregions in which these anurans occur.

Traditionally, amphibian systematists have considered Centrolenidae to be closely related to Hylidae (Duellman, 1975, 2001; Ford and Cannatella, 1993; Lynch, 1973) because frogs of both families have an intercalary element between the ultimate and penultimate phalanges. Additionally, several species of glassfrogs and hylids have green bones and a white ventral parietal peritoneum. However, recent studies based on molecular and/or morphological data (Austin et al., 2002; Burton, 2004; Faivovich et al., 2005; Frost et al., 2006; Grant et al., 2006; Wiens et al., 2005) support the hypothesis that the monotypic Allophrynidae is the sister species of Centrolenidae. Other groups proposed to be closely related to Centrolenidae are Leptodactylidae, Dendrobatidae, and Bufonidae (Biju and Bossuyt, 2003; Darst and Cannatella, 2004; Heinicke et al., 2007; Roelants et al., 2007). In our analyses, we include 35 species as outgroups to represent clades that have been associated with centrolenid frogs (Appendix B). We used *Xenopus laevis* and *Spea bombifrons* as more distant outgroups to root the phylogeny.

2.3. Data collection

Tissue samples were obtained from specimens listed in Appendix A. Additional sequences were downloaded from GenBank (NCBI; Appendix B). We included relatively fast-evolving mitochondrial loci for resolution of recent divergences, as well as more slowly evolving nuclear loci to illuminate relationships among older clades. The genes chosen for this study are the mitochondrial 12S rRNA, 16S rRNA, NADH Dehydrogenase Subunit 1 (*ND1*), and portions of the nuclear proto-oncogene cellular myelocytomatosis (*c-myc*), proopiomelanocortin A gene (*POMC*), and recombination activating gene 1 (*RAG1*).

Genomic DNA was extracted from frozen, Laird's buffer (Laird et al., 1991), or ethanol-preserved tissues with the DNeasyTissue extraction kit (Qiagen Inc.) or using standard phenol–chloroform extraction protocols (Sambrook et al., 1989). Primers and Polymerase Chain Reaction (PCR) amplification protocols are presented in Tables 1 and 2, respectively. PCR products were visualized in agarose gels, and unincorporated primers and dNTPs were removed from PCR products using ExoSap purification (ExoSap-it, GE Healthcare). Cycle sequencing reactions were completed using the corresponding PCR primers and BigDye Terminator 3.1 chemistry (Applied Biosciences), with a standard cycle sequencing profile (96 °C/3 min; 35 cycles of 96 °C/10 s, 50 °C/15 s, 60 °C/3 min; and 72 °C/7 min). Reaction products were purified with CleanSEQ magnetic beads (Agencourt) and run in an ABI Prism 3100 Genetic Analyzer (Applied Biosciences) or purified using ethanol precipitation and run in an ABI 3730xl. Data from heavy and light strands were compared to generate a consensus sequence for each DNA fragment using Sequencher 4.1 (Gene Codes Corp., 2000). Sequences were initially aligned in CLUSTAL_X (Thompson et al., 1997) and adjusted by hand in MacClade 4.07 (Maddison and Maddison, 2000). Manual adjustments were particularly important in protein coding genes to maintain reading frames. In some cases (*Centrolene altitudinale*, *C. prosoblepon*, *C. venezuelense*, *Cochranella granulosa*, *C. oyampiensis*, *Hyalinobatrachium* aff. *mondolfii*; Appendix A), incomplete sequences from different individuals of the same species were joined to construct a single complete composite sequence for the combined analyses to reduce the number of terminals and simplify search space. We only applied this approach after confirming that the genetic distances between the shared DNA fragments were minimal (nucleotide divergence <1%). GenBank accession numbers for all individual sequences generated in this study are listed in Appendices A and B. All alignments are available from TreeBase (http://www.treebase.org/treebase/index.html; accession numbers: S2047, M3830, M3831, M3832).

2.4. Phylogenetic analysis

Phylogenetic analyses were conducted using maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses (BA) for individual genes, as well as for a combined dataset. Parsimony analyses were performed in PAUP*4b10 (Swofford, 2002) using heuristic searches (10,000 stepwise random additions with TBR branch-swapping) and clade support was estimated via 500 bootstrap pseudo-replicates with 10 random additions (Felsenstein, 1985). Maximum likelihood analyses were run in RAxML (Randomized Axelerated Maximum Likelihood for High Performance Computing 2.2.0; Stamatakis, 2006; available at http://icwww.epfl.ch/~stamatak/index-Dateien/Page443.htm), which uses a GTR + CAT model of nucleotide substitution (GTR with per-site rate categories) as an approximation to GTR + Γ, and allows for data partitioning (Stamatakis, 2006), a feature that is still not implemented in other likelihood programs. We partitioned the dataset by gene (*12S*, *16S*) or by gene and codon position in protein coding genes (*ND1*, *c-myc*, *POMC*, *RAG1*, and combined datasets).

Table 1
Genes and primers used in this study

Genes and primers	Sequence (5′ → 3′)	Source
Mitochondrial *12S*		
t-Phe-frog	ATAGCRCTGAARAYGCTRAGATG→	Wiens et al. (2005); modified "MVZ 59" from Graybeal (1997)
t-Val-frog	TGTAAGCGARAGGCTTTKGTTAAGCT←	Wiens et al. (2005)
Mitochondrial *16S*		
16SC	GTRGGCCTAAAAGCAGCCAC→	Darst and Cannatella (2004)
16Sbr-H	CCGGTCTGAACTCAGATCACGT←	Palumbi et al. (1991)
Mitochondrial *ND1*		
16S-frog	TTACCCTRGGGATAACAGCGCAA→	Wiens et al. (2005)
tMet-frog	TTGGGGTATGGGCCCAAAAGCT←	Wiens et al. (2005)
Nuclear *c-myc exon 2*		
cmyc1U	GAGGACATCTGGAARAARTT→	Crawford (2003)
cmyc-ex2 R	TCATTCAATGGGTAAGGGAAGACC←	Wiens et al. (2005)
Nuclear *POMC*		
POMC-1	GAATGTATYAAAGMMTGCAAGATGGWCCT→	Wiens et al. (2005)
POMC-2	TAYTGRCCCTTYTTGTGGGCRTT←	Wiens et al. (2005)
Nuclear *RAG1*		
R1-GFF	GAGAAGTCTACAAAAAVGGCAAAG→	Faivovich et al. (2005)
R1-GFR	GAAGCGCCTGAACAGTTTATTAC←	Faivovich et al. (2005)

The arrow indicates primers located in the forward (→) or in the reverse (←) strand.

Table 2
Thermocycling conditions used to amplify mitochondrial and nuclear genes using the polymerase chain reaction (PCR)

Gene	Protocol
12S, *16S*	1 cycle: 2 min 94 °C, 30 s 42 °C, 1 min 72 °C 9 cycles: 30 s 94 °C, 30 s 42 °C, 1 min 72 °C 30 cycles: 30 s 94 °C, 30 s 50 °C, 1 min 72 °C; 1 cycle: 5 min 72 °C
ND1	1 cycle: 2 min 94 °C, 30 s 50 °C, 1 min 72 °C 10 cycles: 30 s 94 °C, 30 s 50 °C, 1 min 72 °C 29 cycles: 30 s 94 °C, 30 s 58 °C, 1 min 72 °C; 1 cycle: 5 min 72 °C
c-myc exon 2	1 cycle: 2 min 96 °C 45 cycles: 20 s 96 °C, 45 s 54 °C, 90 s 72 °C 1 cycle: 7 min 72 °C
POMC, *RAG1*	1 cycle: 2 min 96 °C 45 cycles: 20 s 95 °C, 25 s 52 °C, 2 min 72 °C 1 cycle: 7 min 72 °C

Protocols were developed by J.W. Fetzner in J.J. Wiens lab (see Wiens et al., 2005).

We performed a total of 100 runs to reduce the probability of inferring a suboptimal likelihood solution. Node support was assessed via 1000 bootstrap replicates.

For Bayesian analyses, we implemented the model of nucleotide substitution selected as the best fit for every particular dataset (partition) according to the Akaike Information Criterion (AIC) in ModelTest 3.7 (Posada and Crandall, 1998; Table 3). Bayesian analyses of each mitochondrial and nuclear gene and the combined datasets were performed in Mr Bayes 3.1 (Ronquist and Huelsenbeck, 2003). The combined datasets were analyzed by partitioning the data as for the ML analyses (14 partitions). The analysis for each gene consisted of a minimum of 5 million generations and four Markov chains with default heating values. The prior used for the rate matrix was a uniform Dirichlet and no prior information on topology was incorporated. Trees were sampled every 1000 generations; stationarity was assessed by examining the standard deviation of split frequencies and by plotting the $-\ln L$ per generation using Tracer 1.2.1 (Rambaut and Drummond, 2005), and trees generated before stationarity were discarded as "burn-in." For the combined dataset, runs were as described above, but consisted of 20 million generations. Two independent runs were performed each for the combined mitochondrial, combined nuclear, and complete dataset to assess if the resulting topologies and posterior probabilities were congruent.

2.5. Topological congruence and combinability

Topologies resulting from each gene were compared by eye to detect areas of incongruence that were strongly supported by non-parametric bootstrap values and/or posterior probabilities (Wiens, 1998). We did not employ the Incongruence Length Difference (ILD) test as it has been shown to be a poor check of the compatibility of separate data partitions (Hipp et al., 2004). Bootstrap values $\geqslant$70% are considered to indicate strong support (Hillis and Bull, 1993). Clades with posterior probabilities $\geqslant$0.95 are considered strongly supported, but we caution that relatively high posterior probabilities for short internodes (particularly those with low bootstrap values) may be over-estimates of confidence (Alfaro et al., 2003; Erixon et al., 2003).

2.6. Statistical testing of alternative phylogenies

There are several probabilistic approaches to testing phylogenetic hypotheses, including parametric ML tests (Goldman et al., 2000; Huelsenbeck and Bull, 1996; Huelsenbeck et al., 1996; Swofford et al., 1996), non-parametric ML tests (Shimodaira and Hasegawa, 1999), and Bayesian posterior probabilities (Huelsenbeck and Ronquist, 2001; Larget and Simon, 1999; Li et al., 2000; Mau et al., 1999; Rannala and Yang, 1996; Yang and Rannala, 1997). Buckley (2002) demonstrated that parametric ML tests tend to produce Type-I errors because of model misspecification coupled with branch-length heterogeneity, a result also mentioned by Huelsenbeck et al. (1996). In contrast, the non-parametric ML Shimodaira–Hasegawa (SH) test was observed to be much more conservative, even under high substitution rate and branch-length heterogeneity (Buckley, 2002). The SH test takes multiple comparison corrections into consideration and allows evaluation of a priori and a posteriori hypotheses (Goldman et al., 2000; Shimodaira and Hasegawa, 1999), but it also requires simultaneous comparison of all reasonable topologies to ensure that the true topology is available for any bootstrap data set (Goldman et al., 2000). Buckley (2002) suggested that the number of candidate topologies should be minimized through the application of prior knowledge. In the case of our study, restricting the set of possible topologies to those that

Table 3
Estimated parameters for Bayesian analyses

Gen	Best-fit model	AIC score	−ln likelihood	I	Γ	Rate matrix						Base frequency			
						AC	AG	AT	CG	CT	GT	A	C	G	T
12S	GTR + I + G	44091.0	22035.5	0.2793	0.5343	5.7982	12.2505	4.7613	1.2135	47.0203	1.000	0.3926	0.2104	0.1357	0.2613
16S	GTR + I + G	32128.3	16054.1	0.3245	0.5572	3.6088	10.3639	3.1151	0.2059	30.2198	1.000	0.4105	0.2206	0.1299	0.2390
ND1, 1st position	GTR + I + G	15685.5	7832.7	0.4010	0.6263	0.3046	4.2541	0.8228	0.0937	5.8679	1.000	0.3591	0.2881	0.1294	0.2304
ND1, 2nd position	TVM + I + G	4715.1	2348.6	0.5129	0.4237	4.4685	10.5478	3.4721	5.6068	10.5478	1.000	0.1644	0.3031	0.0985	0.4340
ND1, 3rd position	GTR + G	51013.4	25497.7	0	1.2935	1.9603	36.3408	0.8957	1.3152	14.3190	1.000	0.4024	0.2697	0.0572	0.2707
RAG1, 1st position	TIM + G	1485.0	785.5	0	0.2780	1.000	1.0350	1.5637	1.5637	2.9152	1.000	0.3706	0.1952	0.2516	0.1826
RAG1, 2nd position	K81uf + I	1165.6	575.8	0.7578	Equal	1.000	6.7376	3.8195	3.8195	6.7376	1.000	0.3627	0.2168	0.1865	0.2339
RAG1, 3rd position	SYM + G	5191.1	2589.6	0	2.3261	1.7948	7.4332	1.7302	0.6656	10.6561	1.000	Equal frequencies			
c-myc exon 2, 1st position	TVM + G	1772.4	878.2	0	0.2680	0.5166	1.8694	1.1017	0.1485	1.8694	1.000	0.2407	0.2809	0.3009	0.1775
c-myc exon 2, 2nd position	TVMef + G	1454.2	722.1	0	0.3437	5.3706	3.0273	0.444	1.2301	3.0273	1.000	Equal frequencies			
c-myc exon 2, 3rd position	TVM + G	5865.7	2924.9	0	0.8099	0.3732	3.2190	0.8676	0.4508	3.2190	1.000	0.1356	0.3501	0.2983	0.2160
POMC, 1st position	K81uf + I + G	3007.8	1496.9	0.3195	0.5752	1.0000	2.0651	0.5691	0.5691	2.0651	1.000	0.4123	0.1898	0.2534	0.1445
POMC, 2nd position	HKY + I + G	2733.2	1360.6	0.3261	0.8427	Ti/Tv ratio 1.2296						0.4230	0.1959	0.1960	0.1851
POMC, 3rd position	TVM + G	9151.1	4567.5	0	1.5530	1.9864	9.1007	1.7524	0.7377	9.1007	1.000	0.2203	0.3144	0.1980	0.2673

Parameters were calculated using ModelTest 3.7 (Posada and Crandall, 1998). AIC = Akaike information criterion; I = proportion of invariable sites; Γ = Gamma distributed rate variation among sites.

represent prior taxonomic hypotheses is relatively simple (Fig. 1). However, incorporating all the possible topologies that are compatible with these a priori hypotheses was impractical given the large number of species. With this limitation in mind, we used our complete dataset and searched for the best tree compatible with each prior hypothesis (Fig. 1) with the program RAxML. Then, we performed a SH test including our best tree as estimated by RAxML and the best trees compatible with the following prior hypotheses (for each of the described hypotheses, the species composition of the genera may vary according to the corresponding taxonomic arrangement: for complete description of the hypotheses see references): (1) monophyletic *Centrolene*, monophyletic *Cochranella*, monotypic Teratohyla (sensu Taylor, 1949, 1951; Fig. 1A); (2) monophyletic Centrolenella with three species groups, monotypic *Centrolene* (sensu Savage, 1967; Fig. 1B); (3) monophyletic *Centrolene*, monophyletic *Hyalinobatrachium*, unresolved *Cochranella* (sensu Ruiz-Carranza and Lynch, 1991; Fig. 1C); (4) monophyletic *Centrolene*, monophyletic *Hyalinobatrachium*, unresolved *Cochranella*, monophyletic *Centrolene* + *Cochranella* (sensu Ruiz-Carranza and Lynch, 1991; as modified by Bolívar et al., 1999; Fig. 1D); (5) monophyletic *Centrolene*, monophyletic *Hyalinobatrachium*, unresolved *Cochranella*, with their respective species groups (sensu Ruiz-Carranza and Lynch, 1991, 1995, 1998; Fig. 1E); (6) monophyletic *Centrolene*, monophyletic Centrolenella, monophyletic *Hyalinobatrachium*, unresolved *Cochranella* (sensu Ruiz-Carranza and Lynch, 1991; modified by Savage, 2002; Fig. 1F); (7) monophyletic *Centrolene*, monophyletic *Hyalinobatrachium*, unresolved *Cochranella*, with their corresponding species groups (sensu Ruiz-Carranza and Lynch, 1991, 1995, 1998; modified by Duellman and Señaris, 2003; Señaris and Ayarzagüena, 2005; Cisneros-Heredia and McDiarmid, 2006a,b; Fig. 1G); (8) monophyletic *Centrolene*, unresolved *Cochranella*, monophyletic *Hyalinobatrachium*, monophyletic *Nymphargus*, with their respective species groups (asterisks denote uncertain placement of *eurygnathum*, *parvulum*, and *uranoscopum*; sensu Ruiz-Carranza and Lynch, 1991, 1995, 1998; modified by Cisneros-Heredia and McDiarmid, 2007a; 1H). Also, given that the cladistic proposal of Ruiz-Carranza and Lynch (1991; Fig. 1E) is based on the unambiguous evolution of the humeral spine in the genus *Centrolene* and the white-bulbous liver in the genus *Hyalinobatrachium*, the SH test assessed the statistical support for a single origin of both character states.

From a Bayesian perspective, we evaluated the support for the alternative hypotheses (Fig. 1) based on posterior probabilities. From all possible trees found during the MCMC search, the probability of a particular hypothesis being correct was calculated as the proportion of the trees in agreement with the hypothesis, using the filter command in PAUP* with a constraint describing the hypothesis.

3. Results

3.1. Molecular data and models of evolution

For most of the species in Centrolenidae, we obtained a total of ~4362 bp from the following markers: mitochondrial *12S rRNA* (~974 bp), fragment of the mitochondrial *16S rRNA* (~895 bp), mitochondrial *ND1* (~973 bp), and portions of the nuclear *POMC* (~634 bp), nuclear *c-myc exon 2* (~430 bp), and nuclear *RAG1* (~456 bp). See Appendix A for genes sequenced for each individual. Parameter value estimates for best-fit models for each gene and codon position are summarized in Table 3. As expected, mitochondrial genes presented more variability across taxa than nuclear genes (Table 4). For some data partitions in the ML analysis, we used a slightly more parameter-rich model than that estimated in ModelTest 3.7 (Table 3) because of constraints of the software used to perform the ML analysis (RAxML). However, we expect that this

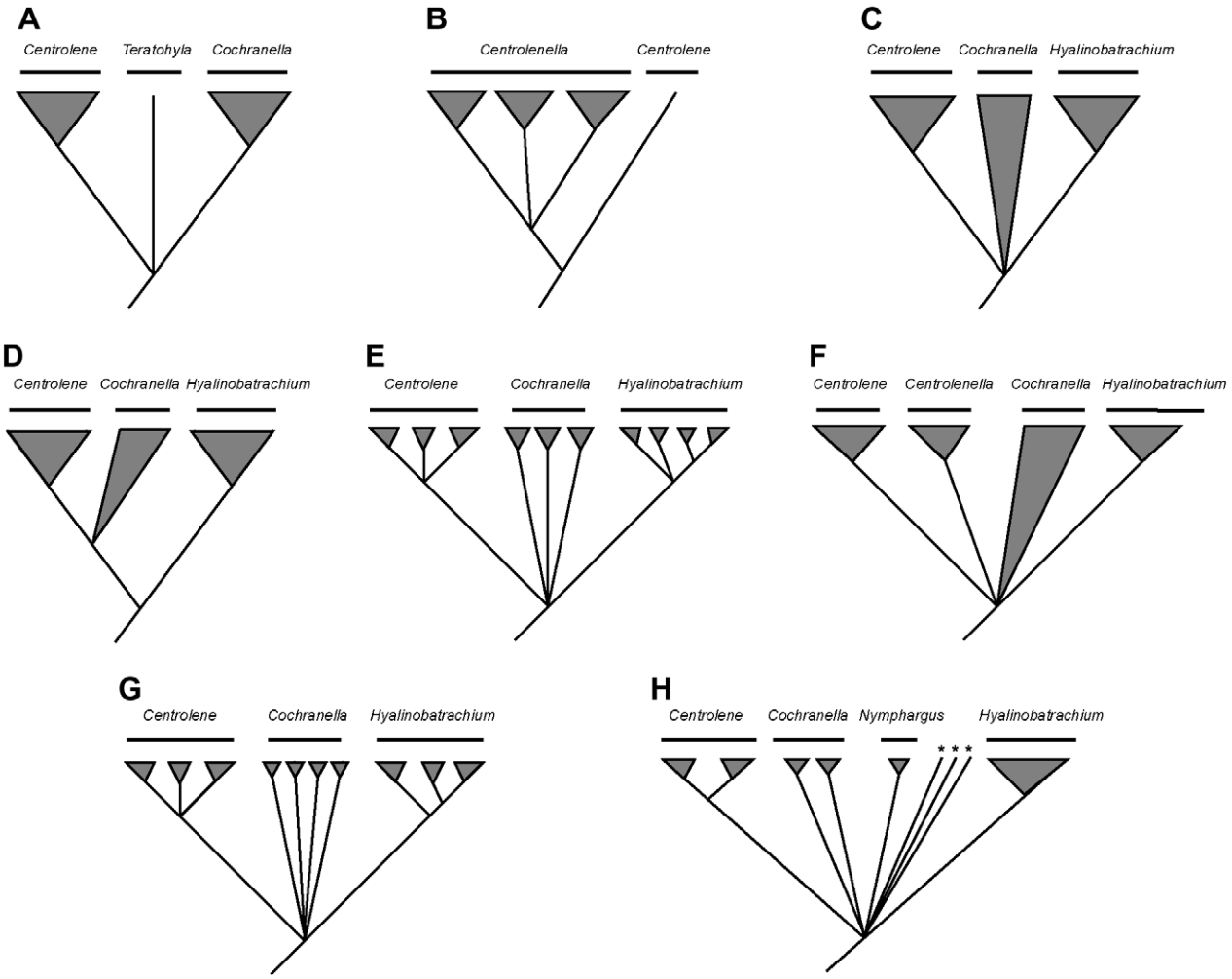

Fig. 1. Previous taxonomic hypotheses for centrolenid frogs. (A) Taxonomy sensu Taylor (1949, 1951). (B) Taxonomy sensu Savage (1967). (C) Generic arrangement by Ruiz-Carranza and Lynch (1991). (D) Hypothesis of relationships sensu Ruiz-Carranza and Lynch (1991), as modified by Bolívar et al. (1999). (E) Hypothesis of relationships sensu Ruiz-Carranza and Lynch (1991, 1995, 1998). (F) Hypothesis of relationships sensu Ruiz-Carranza and Lynch (1991), as modified by Savage (2002). (G) Hypothesis of relationships sensu Ruiz-Carranza and Lynch (1991, 1995, 1998), as modified by Duellman and Señaris (2003), Señaris and Ayarzagüena (2005), and Cisneros-Heredia and McDiarmid (2006a, 2006b). (H) Hypothesis of relationships sensu Ruiz-Carranza and Lynch (1991, 1995, 1998), as modified by Cisneros-Heredia and McDiarmid (2007a); asterisks denote uncertain placement of *eurygnathum*, *parvulum*, and *uranoscopum*.

Table 4
Proportion of parsimony informative (PI) and invariable characters

Gen	Alignment positions	No. of PI	Proportion of PI	No. of invariable sites	Proportion of invariable sites
12S	974	472	0.485	366	0.376
16S	895	376	0.420	384	0.429
ND1	973	533	0.548	370	0.380
c-myc exon 2	430	141	0.328	141	0.481
RAG1	456	152	0.333	152	0.533
POMC	634	251	0.396	251	0.481

overparameterization has little influence on the resulting topology (Lemmon and Moriarty, 2004; Kelchner and Thomas, 2007).

3.2. Relationships of glassfrogs and other anurans

For the individual genes, analyses recovered congruent topologies using MP, ML, and Bayesian criteria. In Bayesian analyses, multiple runs produced almost identical topologies and posterior probabilities. Given that no strongly supported conflicts were observed when comparing individual gene trees, we proceeded to combine the datasets. The resulting mitochondrial and nuclear topologies are shown in Figs. 2 and 3 (note that incongruences arise between these two topologies; see below). Multiple runs of the complete dataset produced different likelihood values (Fig. 4), from which the topology with the best score was chosen (Fig. 5).

When analyzed separately, most of the genes have limited ability to recover ancient relationships. Three genes (*16S*, *ND1*, and *c-myc*) are unable to resolve relationships among families. The remaining genes (*12S*, *RAG1*, *POMC*) show support, although without reaching statistical significance, for an evolutionary affinity between Centrolenidae and *Allophryne ruthveni*, a relationship that becomes significant in the combined analyses (Fig. 2). The affinities of other anurans to the Centrolenidae + *Allophryne* clade are poorly

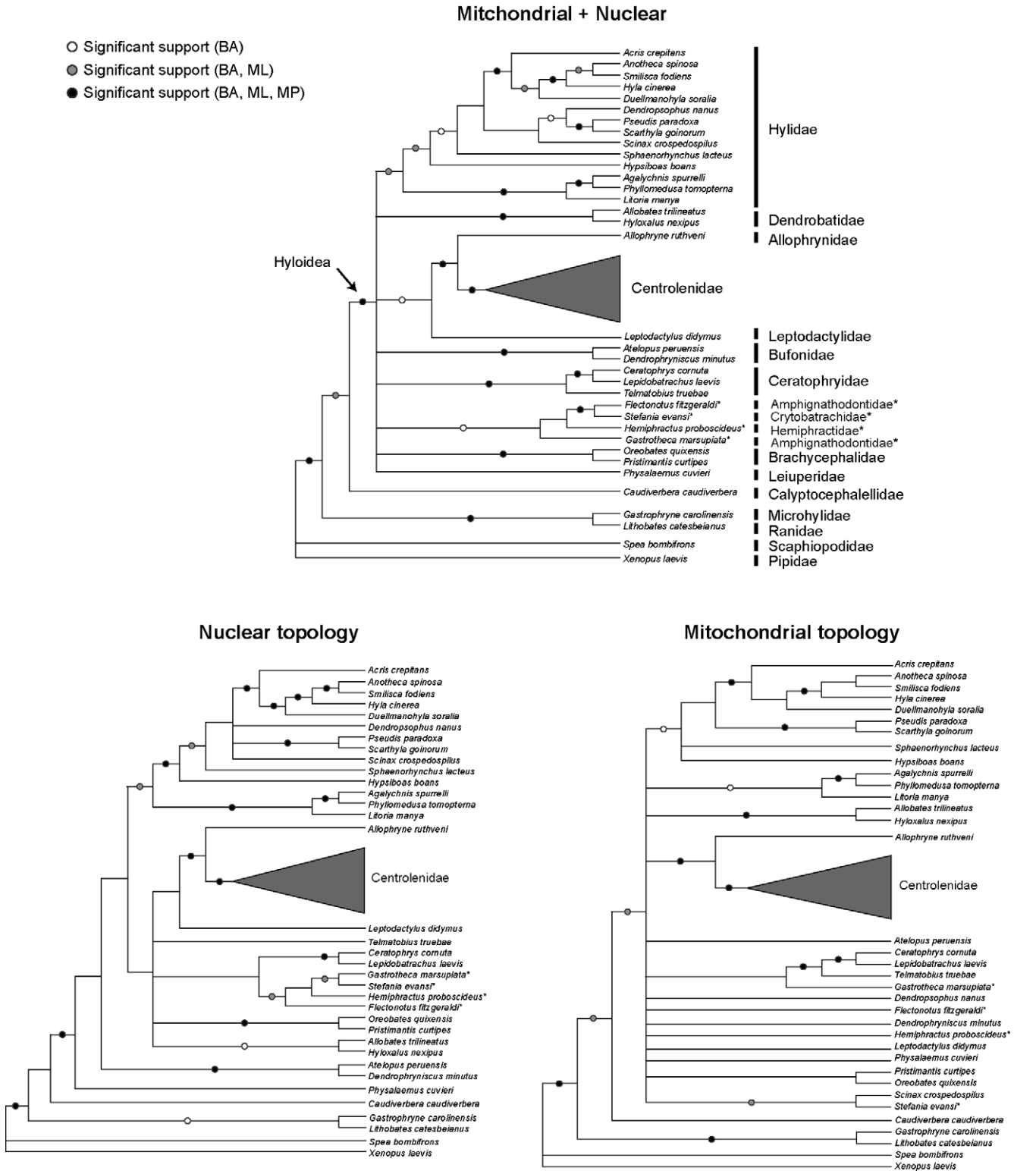

Fig. 2. Schematic trees summarizing relationships between Centrolenidae and other anurans recovered in this study. Note that the *Allophryne* + Centrolenidae clade is recovered consistently. Also, note that the topologies inferred from the nuclear and complete datasets support the monophyly of marsupial frogs (contra Frost et al., 2006; see text); marsupial frogs are indicated with an asterisk (*). Circles indicate significant support values for clades recovered by Bayesian (BA, posterior probability $\geqslant 0.95$), maximum likelihood (ML, bootstrap $\geqslant 70\%$), and maximum parsimony (MP, bootstrap $\geqslant 70\%$) analyses.

resolved, though the topology inferred from the complete dataset places *Leptodactylus didymus* (family Leptodactylidae) as a close relative (Fig. 2).

Other interesting results include the recovery of a clade consisting of all marsupial frogs sampled. Frost et al. (2006) recently split marsupial frogs into three families (Amphignathodontidae, Crytobatrachidae, Hemiphractidae) based on a parsimony analysis of nuclear and mitochondrial genes that found this group to be polyphyletic. Marsupial frog relationships are controversial (reviewed in Frost et al., 2007; Frost, 2007, comments under

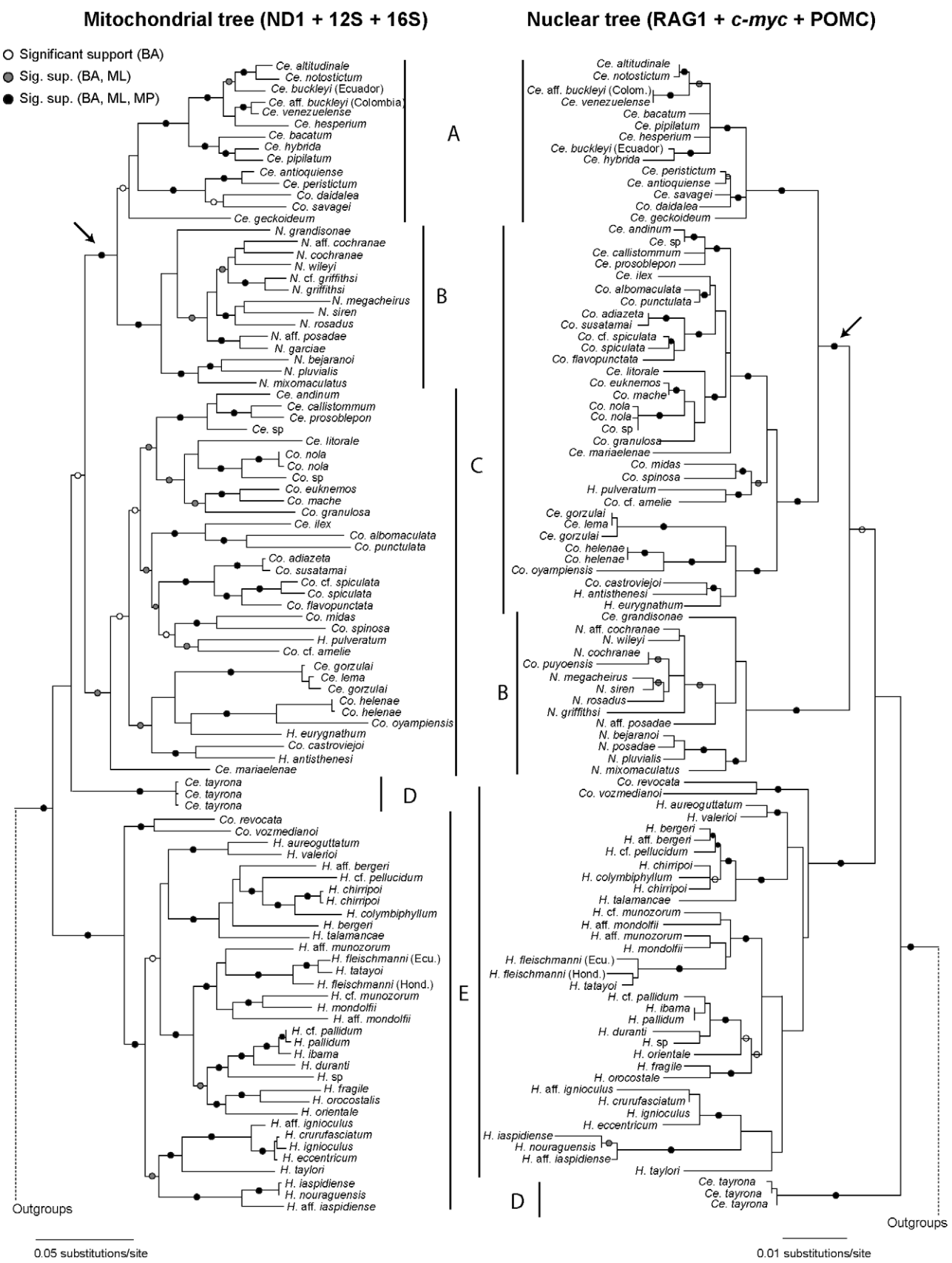

Fig. 3. Maximum likelihood phylogeny of glassfrogs inferred from mitochondrial genes (*12S*, *16S*, *ND1*; ln*L* = −74,810.404) and nuclear genes (*c-myc exon 2*, *RAG1*, *POMC*; ln *L* = −17,244.283) using RAxML. Circles indicate significant support values for clades recovered by Bayesian (BA, posterior probability ⩾ 0.95), Likelihood (ML, bootstrap - 70%), and Parsimony (MP, bootstrap ⩾ 70%) analyses. *Ce.* = *Centrolene*; *Co.* = *Cochranella*; *H.* = *Hyalinobatrachium*; *N.* = *Nymphargus*.

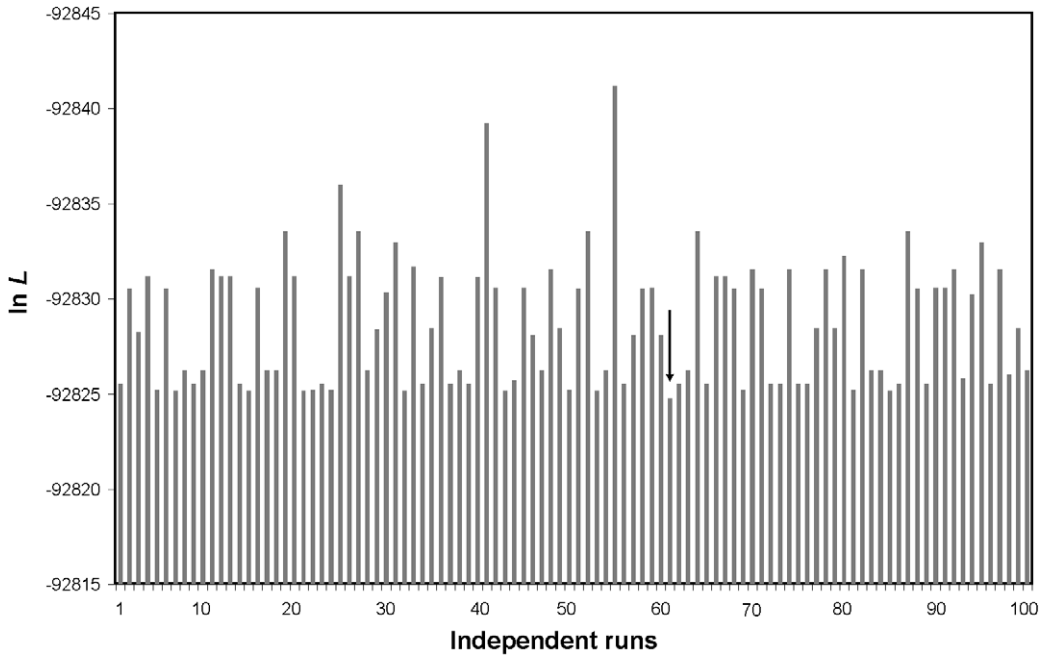

Fig. 4. Distribution of likelihood values inferred from the complete dataset using the program RAxML. The arrow indicates the run with the best likelihood (−92,824.772).

Amphignathodontidae). Topologies only based on maximum parsimony or mitochondrial genes show marsupial frogs as non-monophyletic (Darst and Cannatella, 2004; Faivovich et al., 2005; Wiens et al., 2006; Frost et al., 2006) or monophyletic with weak support (Wiens et al., 2005). In contrast, topologies inferred from data sets including nuclear genes under a Bayesian approach yielded a highly supported clade of marsupial frogs (Wiens et al., 2005, 2007). Frost (2007, comments under Amphignathodontidae) considered that Wiens et al. (2007) work did not correctly evaluate marsupial frogs monophyly because "assumed the monophyly of marsupial frogs and used outgroups that would be considered ingroups in the paradigm suggested by Frost et al. (2006)". Our sampling of marsupial frogs contains four (*Flectonotus*, *Gastrotheca*, *Hemiphractus*, and *Stefania*) of the five genera currently recognized (Frost, 2007). In our analyses, a clade with all marsupial frogs was recovered with significant support in the nuclear (Bayesian and maximum likelihood criteria) and complete (Bayesian criterion) phylogenies, but not in the mitochondrial topology (Fig. 2). It has been shown that analyses of fast-evolving genes (e.g., mitochondrial genes) produce phylogenies with relatively poor resolution among old lineages when compared to those inferred from nuclear genes, probably because the high mutation rate of the mitochondrial DNA leads to homoplasy, obscuring phylogenetic signal (Brown et al., 1979; Swofford et al., 1996, and references therein). Additionally, when topologies are in the 'Felsenstein zone', MP approaches are affected by long-branch attraction (Felsenstein, 1978). We attribute the findings of Darst and Cannatella (2004), Faivovich et al. (2005), Wiens et al. (2006), Frost et al. (2006), and Wiens et al. (2005) to the use of relatively fast-evolving mitochondrial markers and the effect of long-branch attraction (Felsenstein, 1978). Finally, in our analyses, we consistently recovered a clade that is that compatible with Hyloidea as defined by Darst and Cannatella (2004).

Relationships recovered within Centrolenidae are congruent among individual genes (not shown). When combining the genes, five well-defined clades are inferred (A–E, Figs. 3 and 5). However, a noticeable incongruence emerges when comparing nuclear and mitochondrial topologies. The mitochondrial phylogeny suggests a Clade A + B, whereas the nuclear tree places Clade A as the sister taxon to Clade C (Fig. 3). In the gene-by-gene analyses, only *POMC* was found to support the clade A + C. The other nuclear genes either weakly supported A + B (*c-myc*), or did not resolve the relationships between these clades (*RAG1*). Each of the mitochondrial genes inferred an A + B clade with non-significant support.

The only conspicuous uncertainty is the phylogenetic position of *Centrolene tayrona* (Clade D). None of the genes places this species within any larger clade with strong support. For example, two nuclear genes (*POMC*, *RAG1*) suggest an affinity between *C. tayrona* and species in Clade B (not shown). In contrast, the other nuclear gene (*c-myc*) shows some support for *C. tayrona* as the sister species of all other glassfrogs. The mitochondrial genes are ambiguous for the placement of the species as well. The combined datasets place *C. tayrona* as an early divergent species, but fail to resolve its relationship with other clades (Figs. 3 and 5).

3.3. Statistical testing of alternative phylogenies

The non-parametric SH test rejects all previous hypotheses of centrolenid relationships (Fig. 1) when compared to our best tree ($P < 0.001$). In addition, none of the previous hypotheses is represented in the ".trprobs" file generated during the MCMC analyses of the complete dataset, implying that their Bayesian posterior probability is close to zero. Also, the SH test rejects ($P < 0.001$) constrained trees hypothesizing a single origin of humeral spines, a white-bulbous liver, and reduced hand webbing (Fig. 1E).

4. Discussion

4.1. Relationships among glassfrogs and other anurans

The relationships among centrolenid frogs and other anurans are partially resolved in our analyses. We consistently inferred a monophyletic Centrolenidae + *Allophryne ruthveni* clade with mitochondrial and nuclear genes (Fig. 2). This relationship is not a surprise. It was first proposed by Noble (1931) and since then, has been corroborated by several morphological (Burton, 1998, 2004; da Silva, 1998; Duellman, 2001; Wiens et al., 2005), as well as molecular (Austin et al., 2002; Faivovich et al., 2005; Frost et al.,

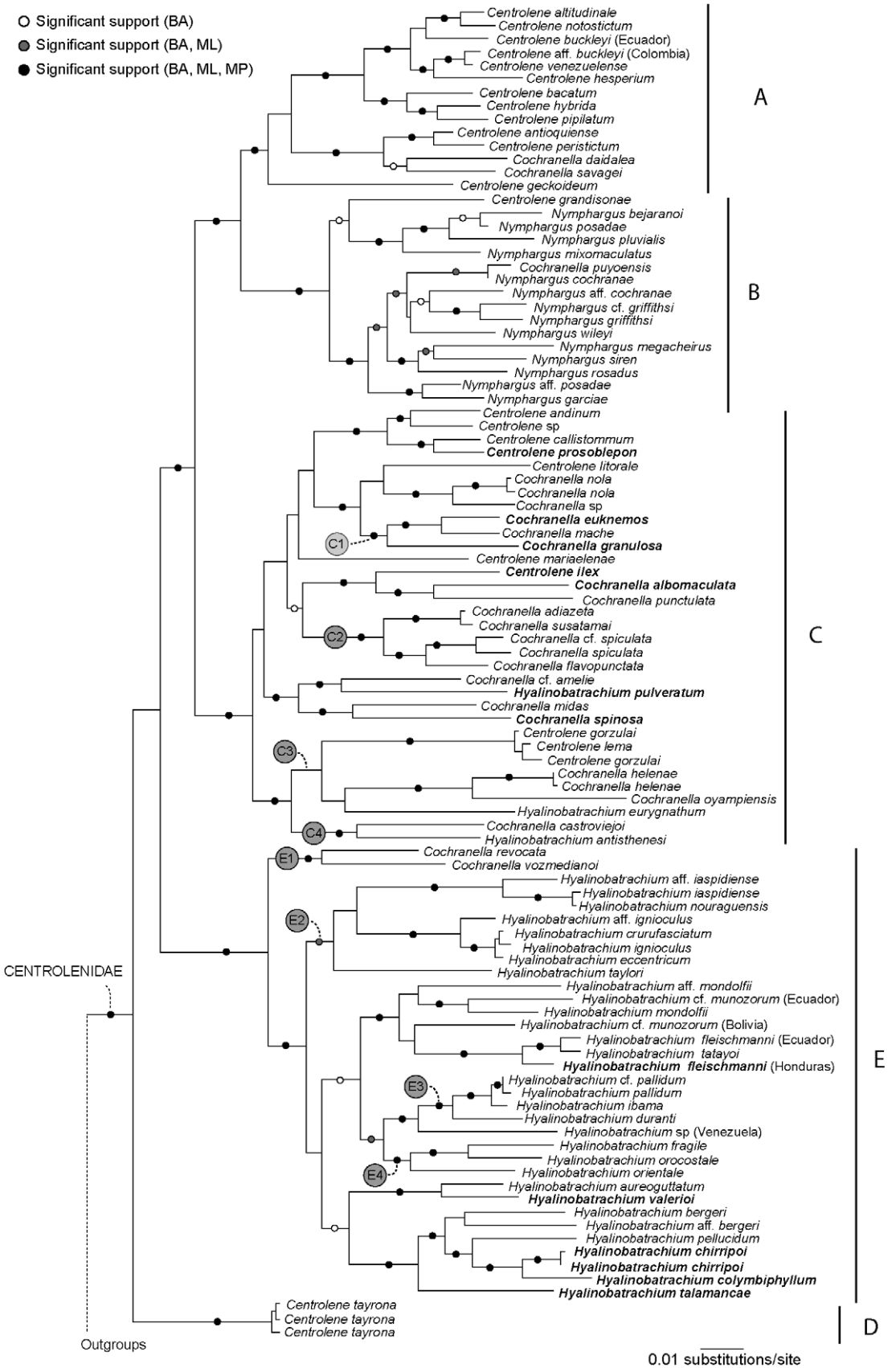

Fig. 5. Maximum likelihood phylogeny of glassfrogs inferred from the complete dataset using RAxML ($\ln L = -92{,}824.772$). Small circles indicate significant support values for clades recovered by Bayesian (BA, posterior probability $\geqslant 0.95$), maximum likelihood (ML, bootstrap $\geqslant 70\%$), and maximum parsimony (MP, bootstrap $\geqslant 70\%$) analyses. Relevant clades for the discussion are labeled with letters and numbers. Species with a geographical distribution that includes Central America are shown in bold; all others are endemic to South America.

2006; Grant et al., 2006; Wiens et al., 2005) characters. Frost et al. (2006) proposed a rearrangement of the Linnaean ranks to formalize the *Allophryne* + Centrolenidae clade. They recognized the family Centrolenidae with two subfamilies (i.e., Allophryninae and Centroleninae). This proposal has the virtue of naming a natural group. On the other hand, if it is accepted, it has an obvious drawback: it will disassociate decades of literature and the names of the merged families. Alternate solutions to avoid taxonomic instability include the use of unranked names (Cantino and de Queiroz, 2006) or using other family-group ranks (e.g., subsuperfamily; ICZN, 1999). A forthcoming work on the taxonomy of glassfrogs will address the pending issue of the name for the *Allophryne*-Centrolenidae clade. At deeper nodes, the mitochondrial topology fails to resolve relationships, a result that is expected given the rapid evolution of mtDNA (Brown et al., 1979). The nuclear genes and complete dataset suggest that leptodactylid frogs are close relatives of the *Allophryne* + Centrolenidae clade, in agreement with Frost et al. (2006).

Marsupial frogs are found to be monophyletic (see Section 3.2); given that this clade is supported by molecular (Wiens et al., 2005, 2007 [with the caveats described by Frost, 2007]; this study; contra Frost et al., 2006), morphological and life-history traits (Burton, 2004; Duellman and Maness, 1980; Wassersug and Duellman, 1984; Wiens et al., 2005), we place Amphignathodontidae Boulenger, 1882, and Cryptobatrachidae Frost et al., 2006, in synonymy of Hemiphractidae Peters, 1862.

4.2. Phylogenetic relationships within Centrolenidae

The phylogeny of glassfrogs contains five main clades (Clades A–E) as inferred from the mitochondrial, nuclear, and complete datasets (Figs. 3 and 5). The mitochondrial and nuclear topologies are congruent except for the position of Clade A. The sister-group relationship between A + C that is recovered in the combined nuclear tree (Fig. 3 right) seems to be influenced mostly by the *POMC* dataset. In contrast, all mitochondrial genes consistently inferred an A + B clade, which is also supported by the complete dataset (Fig. 5). Although the data at hand do not allow us to reach a definite conclusion about which of the arrangements is correct, we favor the hypothesis of a Clade A + B. The observed incongruence among nuclear genes could be caused by stochastic lineage sorting (Tajima, 1983; Neigel and Avise, 1986; see McCracken and Sorenson, 2005). If a rapid radiation originated Clades A, B, and C, stochastic lineage sorting may produce incongruence of individual gene trees with the history of speciation. In such cases, because coalescence time is directly related to effective population size (N_e), and the mitochondrial N_e is about one-quarter that of any nuclear locus, the probability of coalescence is greater for the mitochondrial genome than it is for a nuclear gene; in other words, mitochondrial genes are more likely to recover the species tree (Moore, 1995). Therefore, we base the discussion presented below on the topology shown in Fig. 3 (left) and Fig. 5.

4.3. Taxonomic implications of glassfrog phylogeny

Our phylogeny (Fig. 5) is highly incongruent with all previous hypotheses of centrolenid relationships, most of which were based on phenetics (e.g., Taylor, 1949; Savage, 1967, 2002; Cisneros-Heredia and McDiarmid, 2007a) and not on the principles of phylogenetic systematics and homology (Hennig, 1966; Patterson, 1982; Wiley, 1981). We found that none of the genera historically proposed for the family (*Centrolene*, *Centrolenella*, *Cochranella*, *Hyalinobatrachium*, *Nymphargus*) is monophyletic. All genera proposed in the cladistic studies of Ruiz-Carranza and Lynch (1991, 1998); *Centrolene*, *Cochranella*, *Hyalinobatrachium*) are shown to be polyphyletic. Ruiz-Carranza and Lynch (1991, 1998) based their proposals on a limited number of characters; therefore, the presence of homoplasy has a great impact on the validity of their hypotheses of relationships. A phylogenetic analysis of phenotypic traits already suggested that two of these genera (*Centrolene* and *Cochranella*) were not natural (Guayasamin et al., 2006); however, Ruiz-Carranza and Lynch (1991) already considered the genus *Cochranella* as an unnatural group. *Hyalinobatrachium* (sensu Ruiz-Carranza and Lynch, 1998) is also polyphyletic, contradicting the findings of Guayasamin et al. (2006). However, we infer a clade (Clade E in Fig. 5, not including *Cochranella revocata* and *C. vozmedianoi*) that perfectly agrees with the species content of the infrageneric group *Hyalinobatrachium fleischmanni*. The monophyly of the *fleischmanni* species group, first suggested by Starrett and Savage (1973), also is supported by morphological and behavioral traits (Ruiz-Carranza and Lynch, 1991, 1998; Guayasamin et al., 2006).

We discover that the recently described *Nymphargus* is paraphyletic (Clade B). Although Cisneros-Heredia and McDiarmid (2007a) proposed the genus for species lacking hand webbing, these authors, arbitrarily, excluded species having this character state (*Cochranella ocellata*, *C. puyoensis*), thereby rendering *Nymphargus* paraphyletic. The species group *Cochranella ocellata* (Ruiz-Carranza and Lynch, 1995), also diagnosed by reduced hand webbing, matches the species composition of Clade B. Only one species, *Cochranella vozmedianoi*, agrees with the diagnosis of the *ocellata* group, but it does not belong to Clade B (Fig. 6).

The molecular phylogeny provides a dramatically new inference of the relationships of glassfrogs based on an extensive dataset, and shows the error of previous hypotheses. The obvious next step is to undertake a major taxonomic revision that will reflect the evolutionary history of the group; however, such a project is beyond the scope of this study and will be addressed elsewhere.

4.4. Phenotypic homoplasy in glassfrogs

At present, the most commonly accepted interpretation of character evolution in glassfrogs is that of Ruiz-Carranza and Lynch (1991, 1995), who proposed an arrangement built on two putative synapomorphies (humeral spine in males of the genus *Centrolene*; white, bulbous liver in species of *Hyalinobatrachium*; Fig. 1E). Also, they proposed the absence of hand webbing as derived in the *Cochranella ocellata* Group (Ruiz-Carranza and Lynch, 1995). The merit of these proposals is that they emphasize the use of synapomorphies and provide a testable, objective topology that relies on the unambiguous evolution of these character states. The SH test rejects a single origin of these putative synapomorphies (Fig. 1C and D), revealing a more complex scenario in which similarity, in some cases, is a product of convergence and/or parallelism (Fig. 6), thereby contradicting the hypotheses of Ruiz-Carranza and Lynch (1991, 1995). Our work on glassfrogs corroborates the idea that morphological homoplasy is not a rare phenomenon (Bossuyt and Milinkovitch, 2000; Manzano et al., 2007; Parra-Olea and Wake, 2001; Wiens et al., 2003, 2006), and that hypotheses of relationships based on few traits are likely to misrepresent the true phylogeny. This point does not imply that morphology is phylogenetically uninformative; it only means that derived characters may often have had more than one origin. For example, humeral spines are prevalent in Clade A; a white-bulbous liver is present in all but two species (*Cochranella vozmedianoi* and *C. revocata*) of Clade E, and reduced hand webbing is present in all species of Clade B. However, these traits are not exclusive in the aforementioned clades (Fig. 6), highlighting the importance of congruence as the mechanism to test hypotheses of homology (de Pinna, 1991; Patterson, 1982).

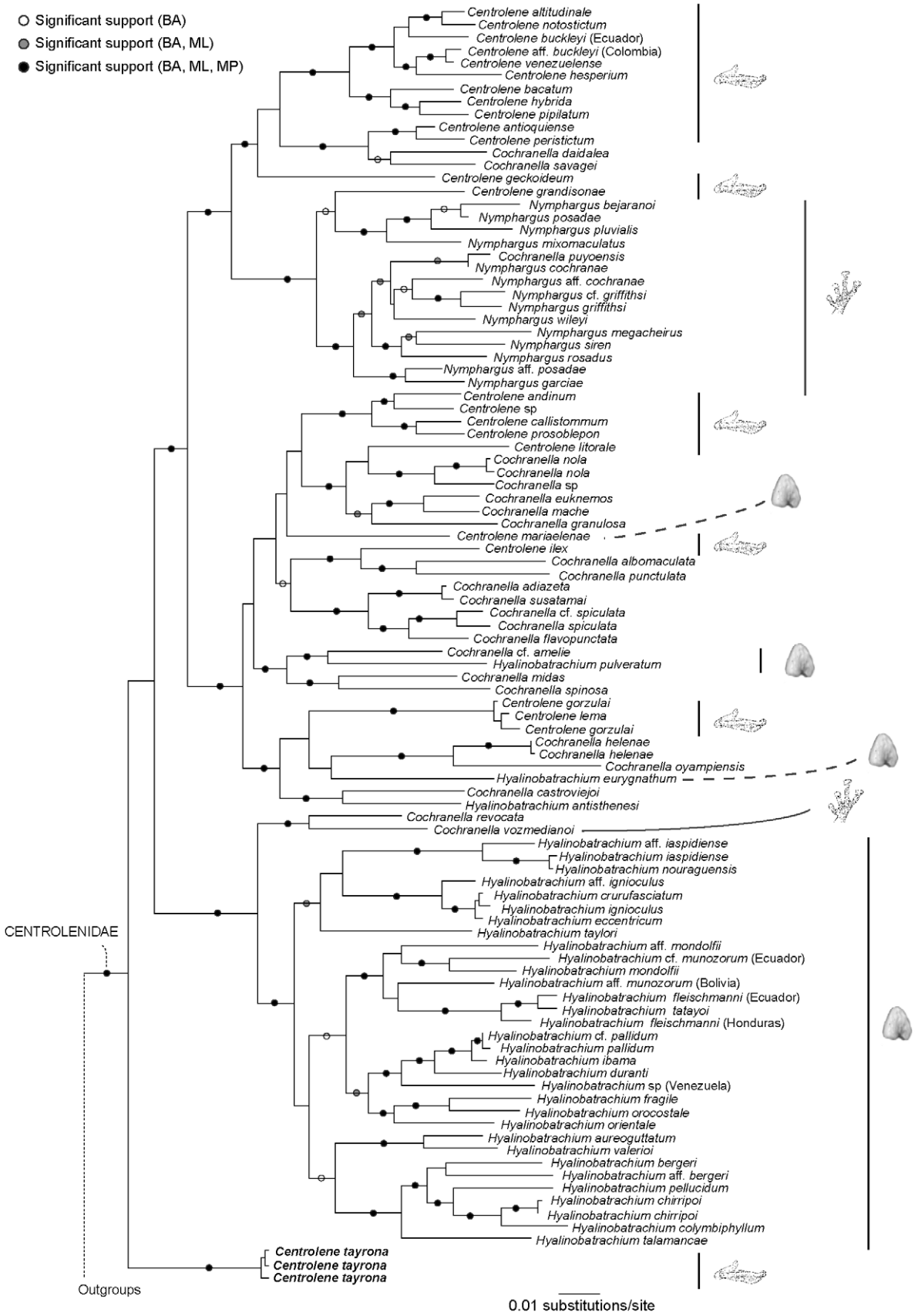

Fig. 6. Glassfrogs' phylogeny (same as in Fig. 5) showing the evolution of three derived traits: humeral spines, reduced hand webbing, and white-bulbous liver. One species, *Centrolene mariaelenae*, has humeral spines and a white-bulbous liver. Note that all traits have some homoplasy, but are informative at different levels of the phylogeny.

4.5. Biogeographic implications

Previous hypotheses of centrolenid relationships implied that closely related species were distantly distributed geographically. For example, species in the genus *Centrolene* (sensu Ruiz-Carranza and Lynch, 1991) are found in the Andes, Guiana Shield, Chocó, and Central America. Similarly, Cisneros-Heredia and McDiarmid (2006a, 2007b) hypothesized a biogeographic connection between Andean and Guianan faunas based on the morphological similarities between Andean (*Centrolene mariaelenae*) and Guianan centrolenids (*Centrolene gorzulai*, *Centrolene lema*). Our results (Figs. 3 and 5) reject (SH test and Bayesian posterior probabilities) their hypotheses. Moreover, the topology shown in Fig. 5 indicates that most clades ultimately have radiated within specific geographical regions and that closely related species usually are distributed within the same ecoregion. For instance, species of Clades A, B, C2, and E3 are exclusively from Andean highlands (>800 m above sea level), Clades C3 and E2 are restricted to the eastern Pre-Cambrian Shields (Guiana Shield and Brazilian Atlantic forest), Clades C4, E1, and E4 to the Cordillera de la Costa, and C1 to the Chocó and Central America (Fig. 5).

There are few examples of sister species that involve two ecological regions, and these are informative about major geologic events that have contributed in shaping Neotropical biodiversity. For example, the Venezuelan Cordillera de la Costa, isolated from the Guiana Shield by the Orinoco River, harbors *Cochranella castroviejoi* and *Hyalinobatrachium antisthenesi* (Clade C4) that are the sister-group of Clade C3, restricted to the eastern Pre-Cambrian Shields. However, the Orinoco River has occupied its current course only since the late Miocene (~11–5 million years ago); earlier, it drained into the Caribbean (Albert et al., 2006; Hoorn, 1995). The history of this river agrees with the biogeographic connection between the Cordillera de la Costa and the Guiana Shield. Similarly, the uplift of the Eastern Andean Cordillera explains why Amazonian species have their closest relatives in the Chocó and Central America (i.e., *Co.* cf. *amelie/H. pulveratum* and *Co. spinosa/Co. midas*). The Eastern Cordillera formed a continuous range between 12.9 and 11.8 Ma (Hoorn, 1995); however, it probably became an important vicariant barrier to lowland species during the early Pliocene (5.3–3.6 Ma; Hooghiemstra et al., 2006).

Our results are concordant with a recent exchange of species between South America and Central America. Considering that anurans usually have limited dispersal abilities and are not tolerant to salt water (Duellman and Trueb, 1994), the most likely scenario is that glassfrogs colonized Central America from South America after the closure of the Isthmus of Panama (ca. 3 Mya; Coates and Obando, 1996). The argument is based on the observation that all Central American species are well embedded in South American clades (Fig. 5), fitting the expectations of a scenario of South American origin and subsequent dispersal to Central America.

By comparing the extant ranges of sister species and phylogeny, we can have a first approximation to their relative importance of different speciation modes (i.e., allopatric, parapatric, and sympatric; Lynch, 1989). In spite of the limitations of this method (assumes complete sampling and no dispersal or range contraction since the time of divergence), all but three of the sister species compared occur in allopatry; therefore, speciation in glassfrogs is better explained model of vicariance. This supports results of Barraclough and Vogler (2000), Fitzpatrick and Turelli (2006), Kozak and Wiens (2006), Lynch (1989), and Ribera et al. (2001) contra those of Graham et al. (2004), Hall (2005), Ogden and Thorpe (2002), and Schneider et al. (1999). Given that different studies have reached a variety of conclusions concerning speciation in the Neotropics, it is reasonable to conclude that dissimilar mechanisms are important in the cladogenesis of distinctive groups. This variation is likely to be associated with the dispersal ability, reproductive mode, and niche breath of organisms.

In summary, the novel hypothesis of centrolenid relationships presented herein opens numerous avenues of research that invite future studies. The interpretation of character evolution in glassfrogs should be reevaluated carefully, especially with respect to the origin of similar morphologies in distantly related species. Similarly, the estimation of ancestral areas, dispersal-vicariance episodes, divergence times, and correlation of diversification rates with phenotypic traits can inform us about the tempo and mode of the origins of Neotropical biodiversity.

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Appendix A

Ingroup sampling listing species, voucher numbers, localities, and Genbank accession numbers of the sequences analyzed in this study

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12S (~949 bp)	*16S* (~882 bp)	ND1 (961 bp)	POMC (616 bp)	*c-myc* ex 2 (~406 bp)	*Rag1* (456 bp)
Centrolene andinum	JMG 366	Venezuela: Estado de Mérida: Quebrada Azul, on the road between La Azulita and El Hato (08°41′13″ N, 71°29′55″ W)	EU663335	EU662976	EU663072	EU663166	EU663250	EU663435
Centrolene altitudinale	MHNLS 17194	Venezuela: Estado Mérida: Quebrada Albarregas (08°37′ N, 71°09′ W; 2100 m)	EU663333	EU662974	EU663070	EU663165	EU663249	–
Centrolene altitudinale	MHNLS 17225	Venezuela: Estado Mérida: Quebrada Albarregas (08°37′ N, 71°09′ W; 2100 m)	EU663334	EU662975	EU663071	–	–	EU663433
Centrolene antioquiense	NRPS 014	Colombia: Departamento Antioquia: Municipio Anori: Vereda El Roble, bosque de la Forzosa, 2127 m	EU663336	EU662977	EU663073	EU663167	EU663251	EU663436
Centrolene bacatum	QCAZ 22728	Ecuador: Provincia Napo: Yanayacu Biological Station (00°41′ S, 77°53′ W; 2100 m).	EU663337	EU662978	EU663074	EU663168	EU663252	EU663437
Centrolene callistommum	QCAZ 28555	Ecuador: Provincia Esmeraldas: Stream affluent of Río Bogotá, nearby San Francisco de Bogotá (0105′13.8″ N, 7841′25.8″ W; 83 m)	EU663340	EU662981	EU663076	EU663171	EU663255	EU663439
Centrolene buckleyi	KU 178031	Ecuador: Provincia Imbabura: Near Lago Cuicocha (00°18′09″ N, 78°36′67″ W; 3010 m)	EU663338	EU662979	EU663075	EU663169	EU663253	–
Centrolene aff. *buckleyi*	MAR 371	Colombia: Departamento Cundinamarca: Municipio Fomeque: Sitio Monte Redondo: Parque Nacional Chingaza, 3035 m	EU663339	EU662980	EU663069	EU663170	EU663254	EU663438
Centrolene geckoideum	KU 178015	Ecuador: Provincia Pichincha: 1 km SW San Ignacio (00°26′55″ S, 78°44′52″ W; 1920 m)	EU663341	EU662982	EU663077	–	–	EU663440
Centrolene gorzulai	BPN 1193	Guyana: Cuyuni-Mazaru Distrit: Upper Partang River (05°48′20.9″ N, 60°12′57.1″ W)	EU663342	EU662983	EU663078	EU663172	–	EU663441
Centrolene gorzulai	MHNLS 16036	Venezuela: Estado Bolívar: Parque Nacional Canaima, Cuenca alta del río Cucurital, Atapare, (05°42′ N, 62°33′ W)	EU663343	EU662984	EU663079	EU663173	EU663256	EU663442
Centrolene grandisonae	QCAZ 22310	Ecuador: Provincia Pichincha: Mindo Biology Station (00°04′40.8″ S, 78°43′55″ W; 1600 m)	EU663344	EU662985	EU663080	EU663174	EU663257	EU663443
Centrolene hesperium	MHNSM 25802	Peru: Departamento Cajamarca: Provincia Santa Cruz: Quebrada Chorro Blanco (06°50′49″S, 79°05′13.3 W, 1795 m), 3.1 Km NE Monte Seco (air distance)	EU663345	EU662986	EU663081	–	EU663258	EU663444
Centrolene hybrida	MAR 347	Colombia: Departamento Boyacá: Municipio Garagoa: Vereda Ciénega Balvanera: Sitio Reserva Natural El Secreto: Quebrada Las Palmitas, 2000 m	EU663346	EU662987	EU663082	EU663175	EU663259	EU663445
Centrolene ilex	UCR 16861	Costa Rica: Provincia de Limón: Finca owned by Brian Kubicki	EU663347	EU662988	EU663083	EU663176	EU663260	EU663446

(*continued on next page*)

Appendix A (*continued*)

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12S (~949 bp)	*16S* (~882 bp)	ND1 (961 bp)	POMC (616 bp)	*c-myc* ex 2 (~406 bp)	*Rag1* (456 bp)
Centrolene lema	KU 181128	Venezuela: Estado Bolívar: km 127 on the El Dorado-Santa Elena de Uairén road, 860 m	EU663348	EU662989	EU663084	EU663177	EU663261	EU663447
Centrolene litorale	QCAZ 27693	Ecuador: Provincia Esmeraldas: Stream near Durango (01°02′49″ N, 78°37′05″ W; 220 m)	EU663349	EU662990	EU663085	EU663178	EU663262	EU663448
Centrolene mariaelenae	QCAZ 31729	Ecuador: Provincia Tungurahua: Stream on the Río Negro–Río Verde road (01°24′24″ S, 78°15′19″ W; 1423 m)	EU663350	EU662991	EU663086	EU663179	EU663263	EU663449
Centrolene notostictum	MAR 510	Colombia: Departamento Norte de Santander: Municipio La Playa de Belem: Vereda Piritama: Quebrada Piritama, 1800 m	EU663351	EU662992	EU663087	EU663180	EU663264	EU663450
Centrolene peristictum	QCAZ 22312	Ecuador: Provincia Pichincha: Mindo Biology Station (00°04′40.8″ S, 78°43′55″ W; 1600 m)	EU663352	EU662993	EU663088	EU663181	EU663266	EU663451
Centrolene pipilatum	KU 178154	Ecuador: Provincia Napo: Río Salado, 1 km upstream from Río Coca (00°11′30″ S, 77°41′59″ W; 1420 m)	EU663353	EU662994	EU663089	–	–	EU663452
Centrolene prosoblepon	MVZ 149741	Costa Rica: Provincia Puntarenas: Monteverde (10.3000 N, 84.8167 S)	–	–	**AY819466**	**AY819085**	**AY819170**	–
Centrolene prosoblepon	UCR 17102	Costa Rica: Provincia Cartago: Cantón Paraíso: Distrito Cachí: Bajos de Cachí (0950′2.4″ N; 8348′22.32″ W; 1010 m)	EU663354	EU662995	–	–	–	EU663453
Centrolene sp.	MHUA 4099	Colombia: Departamento Antioquia: Municipio Anorí: Vereda El Retiro: finca El Chaquiral (06°58′ N, 757′50″ W, 1730 m)	EU663355	EU662996	EU663090	EU663182	–	EU663454
Centrolene tayrona	MAR 544	Colombia: Departamento Magdalena, Sierra Nevada de Santa Marta: road to San Lorenzo, 1800 m	EU663356	EU662997	EU663091	EU663183	EU663330	EU663455
Centrolene tayrona	MAR 545	Colombia: Departamento Magdalena, Sierra Nevada de Santa Marta: road to San Lorenzo, 1800 m.	EU663357	EU662998	EU663092	EU663184	EU663331	EU663456
Centrolene tayrona	MAR 546	Colombia: Departamento Magdalena, Sierra Nevada de Santa Marta: road to San Lorenzo, 1800 m	EU663358	EU662999	EU663093	EU663185	EU663332	EU663457
Centrolene venezuelense	MHNLS 16497	Venezuela: Estado Mérida: Cordillera de Mérida.	EU663360	EU663001	EU663095	–	EU663267	EU663459
Centrolene venezuelense	EBRG 5244; MHNLS/ADN 17340	Venezuela: Estado Mérida: Páramo de Maraisa (08°50′31″ N, 70°43′52″ W; 2450 m)	EU663359	EU663000	EU663094	EU663186	–	EU663458
Cochranella adiazeta	MAR 483	Colombia: Departamento Santander: Municipio Charala: Correjimiento de Virolín: Vereda El Reloj	EU663361	EU663002	EU663096	EU663187	EU663268	EU663460
Cochranella albomaculata	USNM 534151	Honduras: Departamento Gracias a Dios: Quebrada Machin (15°19′10″ N, 85°17′30″ W; 540 m)	EU663362	EU663003	EU663097	EU663188	EU663270	EU663461
Cochranella cf. *amelie*	MHNC 5646/ADN 20619	Peru: Departamento Cusco: Provincia Ouspicanchis: Stream 10 km from Quincemil towards Puerto Maldonado (13°12′03.6″ S; 70°40′28.9″ W; 572 m)	EU663365	EU663005	EU663099	EU663190	EU663327	EU663463

Cochranella castroviejoi	MHNLS 16446	Venezuela: Estado Sucre: Península de Paria, 2.5 km W and 3.2 km N of Macuro (10°41′32″ N, 61°57′44″ W; 580 m)	EU663363	EU663004	EU663098	EU663189	EU663271	EU663462
Cochranella daidalea	MHUA 3271	Colombia: Departamento Cesar: Municipio González: Vereda San Cayetano (0825′30.1″ N, 7324′3.4″ W; 1600 m)	EU663366	EU663007	EU663101	EU663192	EU663272	EU663465
Cochranella euknemos	CH 5109	Panama: Provincia Coclé: Cerro Escaliche, Quebrada Escaliche.	EU663367	EU663008	EU663102	EU663193	–	EU663466
Cochranella flavopunctata	QCAZ 32265	Ecuador: Provincia Morona Santiago: 7.6 W of 9 de Octubre (02°13′30.5″ S, 78°17′25.6″ W; 1715 m), on the 9 de Octubre–Guamote road	EU663368	EU663009	EU663103	EU663194	EU663273	EU663467
Cochranella granulosa	CH 5121	Panama: Provincia Coclé: Quebrada Guabalito, Palmarazo, Parque Nacional Omar Torrijos	EU663369	EU663010	–	–	–	EU663468
Cochranella granulosa	USNM 559082	Honduras: Departamento Gracias a Dios: Rus Rus (14°43′ N, 82°27′ W; 60 m)	EU663370	–	EU663104	EU663195	EU663274	EU663469
Cochranella helenae	MHNLS 17128	Venezuela: Estado Bolivar: San Ignacio de Yuraní, Quebrada de Jaspe (04°55′ N, 61°05′ W; 800–1000 m)	EU663371	EU663011	EU663105	EU663196	EU663275	EU663470
Cochranella helenae	MHNLS 17139	Venezuela: Estado Bolivar: Salto Karuay (05°41′27″ N, 61°51′40″ W; 900 m)	EU663372	EU663012	EU663106	EU663197	EU663276	EU663471
Cochranella mache	QCAZ 27747	Ecuador: Provincia Esmeraldas: Río Balthazar (00°58′28″ N, 78°37′0.3″ W; 645 m)	EU663373	EU663013	EU663107	EU663198	EU663277	EU663472
Cochranella midas	KHJ	Ecuador: Provincia Napo: Jatun Sacha, 450 m.	EU663374	EU663014	EU663108	EU663199	EU663278	EU663473
Cochranella nola	CBG 1094	Bolivia: Departamento Cochabamba: Villa Fatima, 700 m	EU663375	EU663015	EU663109	EU663200	–	EU663474
Cochranella nola	CBG 814	Bolivia: Departamento La Paz: Boquerón (15°36′63″ S, 67°20′60″ W; 1000 m)	EU663376	EU663016	EU663110	EU663201	EU663279	EU663475
Cochranella oyampiensis	MB 165	French Guiana: Terrain Comté	–	EU663017	–	–	–	–
Cochranella oyampiensis	MB 292	French Guiana: Cayenne: Aya, Trinité	EU663377	–	EU663111	EU663202	EU663326	EU663476
Cochranella punctulata	MHUA 4071	Colombia: Departamento Antioquia: Municipio de Maceo: Vereda Las Brisas, Hacienda Santa Bárbara (06°32′49″ N, 74°38′37″ W; 520 m)	EU663378	EU663018	EU663112	EU663203	EU663280	EU663477
Cochranella puyoensis	DFCH-USFQ D285	Ecuador: Provincia Napo: 45 E of Narupa, on the Hollín–Loreto road, 800 m	–	–	–	–	–	EU663478
Cochranella revocata	MHNLS 17319	Venezuela: Estado Aragua: Colonia Tovar (10°24′16″ N, 67°17′06″ W; 1800 m)	EU663379	EU663019	EU663113	EU663204	EU663281	EU663479
Cochranella sp	CBG 1096	Bolivia: Departamento Cochabamba: Repechón (500 m)	EU663381	EU663021	EU663115	EU663206	EU663283	EU663481
Cochranella cf. *spiculata*	CBG 806	Bolivia: Departamento La Paz: Boquerón (15°36′63″ S, 67°20′60″ W; 1000 m)	EU663364	EU663006	EU663100	EU663191	EU663269	EU663464
Cochranella spiculata	MHNSM 24867	Peru: Departamento Junin: Provincia Satipo: Distrito Llaylla: Vista Alegre (11°40′95″ S, 74°64′92″ W; 1340 m)	EU663382	EU663022	EU663116	EU663207	EU663284	EU663482
Cochranella spinosa	USNM 538863	Honduras: Departamento Olancho: Quebrada El Guasimo (14°35′ N, 85°18′ W; 140 m)	EU663383	EU663023	EU663117	EU663208	EU663285	EU663483

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Appendix A (*continued*)

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12S (~949 bp)	*16S* (~882 bp)	ND1 (961 bp)	POMC (616 bp)	*c-myc* ex 2 (~406 bp)	*Rag1* (456 bp)
Cochranella susatamai	MAR 337	Colombia: Departamento Tolima: Municipio Ibagué: Vereda El Tutumo: Finca La Magnolia, Quebrada El Coral, 1100 m	EU663384	EU663024	EU663118	EU663209	EU663286	EU663484
Cochranella cf. *savagei*	MHUA 4094	Colombia: Departamento Antioquia: Municipio Anorí: Vereda El Retiro: Finca El Chaquiral (06°58′ N, 757.83′ W, 1732 m)	EU663380	EU663020	EU663114	EU663205	EU663282	EU663480
Cochranella vozmedianoi	MHNLS 17877	Venezuela: Estado Sucre: Península de Paria, Cerro Humo (10°42′ N, 62°37′ W; 800 m)	EU663385	EU663025	EU663163	EU663247	EU663324	EU663531
Hyalinobatrachium aureoguttatum	QCAZ 32105	Ecuador: Provincia Esmeraldas: 2 km E San Francisco, on the road San Francisco–Durango (01°05′09″ N, 78°41′26″ W; 63 m)	EU663391	EU663032	EU663124	EU663214	EU663288	EU663491
Hyalinobatrachium antisthenesi	MHNLS 17909	Venezuela: Estado Aragua: Parque Nacional Henri Pittier, Estación Biológica Rancho Grande, 1000 m	EU663390	EU663031	EU663123	EU663213	EU663287	EU663490
Hyalinobatrachium aff. *bergeri*	MTD 46305	Peru: Departamento Pasco: km 34 on the Oxapampa–Yaupi road (10°44′44.4″ S, 75°30′02.2″ W; 1770 m)	EU663393	EU663026	EU663119	EU663210	EU663290	EU663485
Hyalinobatrachium bergeri	MHNC 5676; MNCN/ADN 5547	Peru: Deptartamento Cusco: Provincia Ouispicanchis: 6.1 km from Puente Fortaleza towards Quince Mil (13°11′09.5″ S, 70°34′50.1″ W; 464 m)	EU663392	EU663033	EU663125	EU663215	EU663289	EU663492
Hyalinobatrachium chirripoi	USNM 538586	Honduras: Departamento Olancho: Quebrada El Guasimo (14°35′ N, 85°18′ W; 140 m)	EU663399	EU663038	EU663130	EU663220	EU663295	EU663497
Hyalinobatrachium chirripoi	UCR 17424	Costa Rica: Provincia Limón: Aguas Zarcas, Cuenca del Río Banano	EU663398	EU663037	EU663129	EU663219	EU663294	EU663496
Hyalinobatrachium colymbiphyllum	UCR 17423	Costa Rica: Provincia Puntarenas: Reserva Monteverde	EU663400	EU663039	EU663131	EU663221	EU663296	EU663498
Hyalinobatrachium crurifasciatum	MHNLS 16475	Venezuela: Estado Bolívar: 13 km S Las Claritas, on the road Las Claritas–Santa Elena de Uairén	EU663401	EU663040	EU663132	EU663222	EU663297	EU663499
Hyalinobatrachium duranti	MHNLS 16493	Venezuela: Estado Mérida: El Chorotal Alto, on the road between Mérida and La Azulita, 2100 m	EU663402	EU663041	EU663133	EU663223	EU663298	EU663500
Hyalinobatrachium eccentricum	MHNLS 17335	Venezuela: Estado Bolívar: Top of Auyan-tepui, 1800 m	EU663403	EU663042	EU663134	–	–	EU663501
Hyalinobatrachium eurygnathum	CFBH 5729	Brazil: Estado Minas Gerais: Itamontes	**AY843595**	**AY843595**	EU663135	–	–	**AY844383**
Hyalinobatrachium fleischmanni	USNM 559092	Honduras: Departamento Gracias a Dios: Rus Rus Biological Reserve (14°43′ N, 82°27′ W; 60 m)	EU663406	EU663045	EU663137	EU663225	EU663300	EU663504
Hyalinobatrachium fleischmanni	QCAZ 22303	Ecuador: Provincia Esmeraldas: La Tola (00°24′16.8″ N, 79°54′41″ W; 31 m)	EU663405	EU663044	EU663136	EU663224	EU663299	EU663503
Hyalinobatrachium fragile	MHNLS 17161	Venezuela: Estado Cojedes: Road Manrique-La Sierra (09°52′52.3″ N, 68°33′03.3″ W; 530 m)	EU663407	**EU447286**	EU663138	EU663226	EU663301	EU663505
Hyalinobatrachium aff. *iaspidiense*	MB 247	French Guiana: Crique Wapou	EU663386	EU663027	–	–	EU663328	EU663486
Hyalinobatrachium iaspidiense	MHNLS 17126	Venezuela: Estado Bolivar: San Ignacio de Yuraní: Quebrada de Jaspe (04°55′ N, 61°05′W; 800–1000 m)	EU663408	EU663047	EU663139	–	EU663302	EU663506

Hyalinobatrachium ibama	MAR 503	Colombia: Departamento de Santander: Municipio Playa de Belén: Vereda Piritama: Quebrada Piritama, 1780 m	EU663409	EU663048	EU663140	EU663227	EU663303	EU663507
Hyalinobatrachium ignioculus	BPN 1315	Guyana: Cuyuni-Mazaru Distrit: Upper Partang River (05°48′20.9″ N, 60°12′ 57.1″ W)	EU663410	EU663049	EU663141	EU663228	EU663304	EU663508
Hyalinobatrachium aff. *ignioculus*	SMNS 12251	Guyana: Upper Demerara–Berbice Distrit: Mabura Hill Forest Reserve, Maiko creek (05° 09′ 19.30″ N, 58° 41′ 58.96″ W; 60 m)	EU663394	EU663028	EU663120	–	–	EU663487
Hyalinobatrachium mondolfii	MHNLS 17119	Venezuela: Delta Amacuro: Slopes of Serranía de Imatáca, first stream of Caño Acoima, tributary of río Grande (08°22′ N, 61°32′ W; 15 m)	EU663411	EU663050	EU663142	EU663229	EU663305	EU663509
Hyalinobatrachium aff. *mondolfii*	MB 254	French Guiana: Cayenne: Rivière de Kaw	EU663387	EU663029	EU663121	–	–	–
Hyalinobatrachium aff. *mondolfii*	MB 260	French Guiana: Crique Gabrielle	–	–	–	EU663211	EU663329	EU663488
Hyalinobatrachium cf. *munozorum*	QCAZ 31056	Ecuador: Provincia Zamora Chinchipe: Destacamento Militar Shaime, 920 m	EU663395	EU663034	EU663126	EU663216	–	EU663493
Hyalinobatrachium aff. *munozorum*	CBG 1099	Bolivia: Departamento Cochabamba: Repechón, 500 m	EU663388	EU663030	EU663122	EU663212	EU663291	EU663489
Hyalinobatrachium nouraguensis	SMNS 12247	Guyana: Upper Demerara–Berbice Distrit: Mabura Hill Forest Reserve, Maiko creek (05° 09′ 19.30″ N, 58° 41′ 58.96″ W; 60 m)	EU663412	EU663051	EU663143	–	–	EU663510
Hyalinobatrachium orocostale	MHNLS 17247	Venezuela: Estado Guárico: Cerro Platillón, southern slope, Hacienda Picachito, main creek (09°51′ 23″ N, 67°30′ 09.1″ W; 1500 m)	EU663414	**EU447284**	EU663145	EU663231	EU663307	EU663512
Hyalinobatrachium orientale	MHNLS 17878	Venezuela: Estado Sucre: Península de Paria, Cerro Humo (10°41′ N, 61°37′ W; 850 m)	EU663413	**EU447289**	EU663144	EU663230	EU663306	EU663511
Hyalinobatrachium cf. *pallidum*	MHNLS 17881	Venezuela: Estado Barinas: San Isidro (08°50′05″ N, 70°34′41″ W; 1500 m)	EU663396	EU663035	EU663127	EU663217	EU663292	EU663494
Hyalinobatrachium pallidum	MHNLS 17238	Venezuela: Estado Táchira: Road from Sabana Grande to La Grita, Quebrada Guacharaquita (08°10′02.8″ N; 71°58′44.2″ W; 1650 m)	EU663415	EU663052	EU663146	–	–	EU663513
Hyalinobatrachium cf. *pellucidum*	QCAZ 29438	Ecuador: Provincia de Morona Santiago: km 6.6 on the Limón–Macas road	EU663397	EU663036	EU663128	EU663218	EU663293	EU663495
Hyalinobatrachium pulveratum	USNM 538588	Honduras: Departamento Olancho: Matamoros (14°40′ N, 85°23′ W; 150 m)	EU663416	EU663053	EU663147	EU663232	EU663308	EU663514
Hyalinobatrachium sp	MIZA 317	Venezuela: Estado Aragua: Parque Nacional Henri Pittier, Estación Biológica Rancho Grande, 1000 m	EU663417	**EU447290**	EU663148	–	EU663309	EU663515
Hyalinobatrachium tatayoi	MHNLS 17174	Venezuela: Estado Zulia: stream near Tokuko (09° 50′ 30.6″ N, 72° 49′ 13.6″ W; 301 m)	EU663419	EU663055	EU663150	EU663234	EU663310	EU663517
Hyalinobatrachium taylori	MHNLS 17141	Venezuela: Estado Bolivar: Salto Karuay (05°41′27″ N, 61°51′40″ W; 900 m)	EU663420	EU663056	EU663151	EU663235	EU663311	EU663518
Hyalinobatrachium valerioi	UCR 17418	Costa Rica: Provincia Puntarenas: Rincón de Osa	EU663421	EU663058	EU663152	EU663236	EU663312	EU663519
Hyalinobatrachium talamancae	CH 5330	Panama: Provincia Coclé: Río Indio	EU663418	EU663054	EU663149	EU663233	EU663313	EU663516

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Appendix A (*continued*)

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12S (~949 bp)	*16S* (~882 bp)	ND1 (961 bp)	POMC (616 bp)	*c-myc* ex 2 (~406 bp)	*Rag1* (456 bp)
Nymphargus bejaranoi	CBG 1488	Bolivia: Departamento Cochabamba: Chaquisacha (17°41′ S, 65°25′ W; 1500 m)	EU663422	EU663059	EU663155	EU663239	EU663314	EU663522
Nymphargus cochranae	QCAZ 31113	Ecuador: Provincia Napo: Pacto Sumaco (00°43′ S, 77°34′ W; 1400 m)	EU663425	EU663061	EU663156	EU663240	EU663317	EU663523
Nymphargus aff. *cochranae*	QCAZ 31340	Ecuador: Provincia Zamora Chinchipe: Estación Cientifica San Francisco (03°58′ S, 79°04′ W; 1960 m)	EU663423	EU663060	EU663153	EU663237	EU663315	EU663520
Nymphargus garciae	KU 202796	Ecuador: Provincia Sucumbíos: 18 km E Santa Bárbara, 2550 m	**AY326022**	**AY326022**	–	–	–	–
Nymphargus cf. *griffithsi*	KU 202801	Ecuador: Provincia Carchi: ~5 km W La Gruel, 2340 m	**AY326025**	**AY326025**	–	–	–	–
Nymphargus griffithsi	QCAZ 31768	Ecuador: Provincia Imbabura: Santa Rosa, Reserva Biológica Alto Chocó (00°23′ N, 78°26′ W; 2100 m)	EU663426	EU663062	EU663157	EU663241	EU663318	EU663524
Nymphargus megacheira	KU 143272	Ecuador: Provincia Napo: 16.5 km NNE Santa Rosa (00°13′ S; 77°43′ W; 1700 m)	EU663427	EU663063	EU663158	EU663242	EU663319	EU663525
Nymphargus mixomaculata	MTD 45200	Peru: Departamento Huánuco: Provincia Huánuco: Cordillera Carpish, vicinity of Caserío Carpish de Mayobamba (09°43′50″ S, 76°06′46″ W; 2625 m)	–	EU663064	EU663159	EU663243	EU663320	EU663526
Nymphargus pluvialis	KU 173224	Peru: Departamento Cusco: Pistipata, Rio Umasbamba, 12 km SE Huyro, 1820 m	EU663428	EU663065	EU663160	EU663244	EU663321	EU663527
Nymphargus aff. *posadae*	AAV 119	Colombia: Departamento Santander: Santuario de Fauna y Flora Guanentá–Alto Río Fonce, Río Cercados, 2650 m	EU663424	EU663058	EU663154	EU663238	EU663316	EU663521
Nymphargus posadae	QCAZ 26023	Ecuador: Provincia Napo: Yanayacu Biological Station (00°41′ S, 77°53′ W; 2100 m)	–	–	–	–	–	EU663528
Nymphargus rosada	MHUA 4308	Colombia: Departamento Antioquia: Municipio Anorí: Vereda El Retiro: Finca El Chaquiral (0658′ N, 757.83′ W; 1732 m)	EU663429	EU663066	EU663161	EU663245	EU663322	EU663529
Nymphargus siren	KU 179171	Ecuador: Provincia Napo: 3.2 km NNE Oritoyacu (00°27′ S, 77°52′ W; 1910 m)	EU663430	EU663067	EU663162	EU663246	EU663323	EU663530
Nymphargus wileyi	QCAZ 27435	Ecuador: Provincia Napo: Yanayacu Biological Station (00°41′ S, 77°53′ W; 2100 m)	EU663431	EU663068	EU663164	EU663248	EU663325	EU663532

Sequences that were obtained from Genbank are shown in bold. Underlined sequences of *Centrolene altitudinale*, *C. venezuelense*, and *Cochranella granulosa* were used for phylogenetic analyses. Institutional abbreviations are as in Frost (2007), with the following additions: CBG = Centro de Biodiversidad y Genética, Cochabamba, Bolivia; CH = Círculo Herpetológico, Panama; MHUA = Museo de Herpetología de la Universidad de Antioquia, Colombia; MIZA = Museo del Instituto de Zoología Agrícola Francisco Fernández Yépez, Venezuela; MHNC = Museo de Historia Natural Cusco, Universidad Nacional de San Antonio Abad del Cusco. Abbreviations for field series of individuals are as follow: AAV = Alvaro Andres Velasquez; CFBH = Célio F. B. Haddad; BPN = Brice P. Noonan; DFCH-USFQ = Diego F. Cisneros-Heredia, Universidad San Francisco de Quito, Ecuador; IDLR: Ignacio De la Riva; KHJ = Karl-Heinz Jungfer; MAD = Maureen A. Donnelly; MAR = Marco Rada; NRPS = Nely Rocio Pinto; MB = Michel Blanc.

Appendix B

Outgroups included in this study

Species	Gene region					
	12S	*16S*	*ND1*	*RAG1*	*c-myc ex 2*	*POMC*
Allophrynidae						
Allophryne ruthveni	AY819328	EU662973	AY819458	EU663432	AY819162	AY819077
Amphignathodontidae*						
Flectonotus fitzgeraldi	AY819355	DQ679381	AY819486	DQ679274	AY819189	AY819104
Gastrotheca marsupiata	AY819356	DQ679397	AY819487	DQ679289	AY819190	AY819105
Brachycephalidae						
Oreobates quixensis	–	DQ679380	AY819474	–	AY819178	AY819093
Pristimantis curtipes	AY819343	DQ679379	AY819473	DQ679272	AY819177	AY819092
Bufonidae						
Atelopus peruensis	AY819329	–	AY819459	–	AY819163	AY819078
Dendrophryniscus minutus	AY819332	–	AY819462	DQ503337	AY819166	AY819081
Calyptocephalellidae						
Caudiverbera caudiverbera	AY819341	DQ872913	AY819471	–	AY819175	AY819090
Ceratophryidae						
Ceratophrys cornuta	AY819342	DQ679376	AY819472	DQ679269	AY819176	AY819091
Lepidobatrachus laevis	AY819345	DQ679377	AY819475	DQ679270	AY819179	AY819094
Telmatobius truebae	AY819348	–	AY819478	DQ679271	AY819182	AY819097
Cryptobatrachidae*						
Stefania evansi	AY819359	DQ679416	AY819490	DQ679307	AY819193	AY819108
Dendrobatidae						
Allobates trilineatus	AY819339	DQ502118	AY819469	DQ503290	AY819173	AY819088
Hyloxalus nexipus	AY819340	AY364553	AY819470	DQ503285	AY819174	AY819089
Hemiphractidae*						
Hemiphractus proboscideus	AY819358	DQ679413	AY819489	DQ679304	AY819192	AY819107
Hylidae						
Acris crepitans	AY819360	–	AY819491	–	AY819194	AY819109
Agalychnis spurrelli	AY819401	–	AY819532	–	AY819236	AY819151
Anotheca spinosa	AY819361	DQ830813	AY819492	–	AY819195	AY819110
Dendropsophus nanus	AY819373	–	AY819505	AY844437	AY819208	AY819123
Duellmanohyla soralia	AY819362	–	AY819493	AY844378	AY819196	AY819111
Hyla cinerea	AY819366	–	AY819498	AY323766	AY819201	AY819116
Hypsiboas boans	AY819364	–	AY819496	–	AY819199	AY819114
Litoria manya	AY819397	–	AY819529	–	AY819232	AY819147
Phyllomedusa tomopterna	AY819404	–	AY819535	AY844497	AY819239	AY819153
Pseudis paradoxa	AY819353	–	AY819483	AY323773	AY819187	AY819102
Scarthyla goinorum	AY819389	–	AY819521	AY844514	AY819224	AY819139
Scinax crospedospilus	AY819391	–	AY819523	–	AY819226	AY819141
Smilisca fodiens	AY819387	AY843743	AY819519	–	AY819222	AY819137
Sphaenorhynchus lacteus	AY819394	–	AY819526	AY844527	AY819229	AY819144
Leiuperidae						
Physalaemus cuvieri	AY819347	AY843729	AY819477	AY844499	AY819181	AY819096
Leptodactylidae						
Leptodactylus didymus	AY819346	–	AY819476	–	AY819180	AY819095
Microhylidae						
Gastrophryne carolinensis	AY819349	–	AY819479	–	AY819183	AY819098
Pipidae						
Xenopus laevis	M27605	NC001573	NC001573	L19324	AY819160	AY819075
Ranidae						
Lithobates catesbeianus	AY819354	–	AY819484	–	AY819188	AY819103
Scaphiopodidae						
Spea bombifrons	AY819327	–	AY819457	–	AY819161	AY819076

All sequences were obtained from Genbank, except those in bold. Family names follow Frost et al. (2006). *See text for suggested changes that apply to marsupial frogs.

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