Molecular Phylogenetics and Evolution 62 (2012) 826–838

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

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

Molecular phylogeny and morphometric analyses reveal deep divergence between Amazonia and Atlantic Forest species of *Dendrophryniscus*

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ARTICLE INFO

Article history:
Received 25 March 2011
Revised 8 November 2011
Accepted 24 November 2011
Available online 8 December 2011

Keywords:
Bufonids
Dendrophryniscus
Amazonia
Atlantic Forest
Phylogeny
Morphometry

ABSTRACT

Dendrophryniscus is an early diverging clade of bufonids represented by few small-bodied species distributed in Amazonia and the Atlantic Forest. We used mitochondrial (414 bp of *12S*, 575 bp of *16S* genes) and nuclear DNA (785 bp of *RAG-1*) to investigate phylogenetic relationships and the timing of diversification within the genus. These molecular data were gathered from 23 specimens from 19 populations, including eight out of the 10 nominal species of the genus as well as *Rhinella boulengeri*. Analyses also included sequences of representatives of 18 other bufonid genera that were publically available. We also examined morphological characters to analyze differences within *Dendrophryniscus*. We found deep genetic divergence between an Amazonian and an Atlantic Forest clade, dating back to Eocene. Morphological data corroborate this distinction. We thus propose to assign the Amazonian species to a new genus, *Amazonella*. The species currently named *R. boulengeri*, which has been previously assigned to the genus *Rhamphophryne*, is shown to be closely related to *Dendrophryniscus* species. Our findings illustrate cryptic trends in bufonid morphological evolution, and point to a deep history of persistence and diversification within the Amazonian and Atlantic rainforests. We discuss our results in light of available paleoecological data and the biogeographic patterns observed in other similarly distributed groups.

1. Introduction

Bufonidae is one of the most species-rich families of anurans, with more than 550 species in ca. 50 recognized genera (Frost, 2011). In the last decade, the group has received much attention from biologists. As an example, dramatic extinctions have been documented within the genus *Atelopus* (la Marca et al., 2005; Stuart et al., 2008), while *Rhinella marina* has become a plague in many countries across the globe (Lever, 2003). Recent studies also shed light on the phylogenetic relationships (Frost et al., 2006; Pramuk, 2006), the timing of diversification, and the biogeography of most bufonid lineages (Pauly et al., 2004; Pramuk et al., 2008; Roelants et al., 2007; van Bocxlaer et al., 2009, 2010). Because of these efforts, we now have a better understanding of the diversity and history of Bufonidae relative to most other anuran families (van Bocxlaer et al., 2010).

Recent breakthroughs into the evolutionary history of Bufonidae focused mostly on its "crown" group, i.e. the former genus *Bufo* and its relatives. By contrast, early diverging genera of bufonids, all Neotropical in their distribution, have received comparatively little attention. This is the case for the genus *Dendrophryniscus* (Jiménez de la Espada, 1870), an assemblage of 10 nominal species of small, secretive, forest-dwelling, dull-colored toads. *Dendrophryniscus minutus* was the sole representative of the genus sampled in recent family- and genus-level phylogenetic investigations. These studies indicate that the genus diverged from its putative sister group about 50–70 Ma ago (~52 Ma 95% CI 38–71 Ma, van Bocxlaer et al., 2010; ~65 Ma, Pramuk et al., 2008; >50 Ma as per Heinicke et al., 2009).

Although *Dendrophryniscus* can be diagnosed from other Neotropical bufonids by a set of external and internal morphological characters (Cannatella, 1986; Graybeal and Cannatella, 1995; McDiarmid, 1971), its monophyly has never been formally tested with either extensive taxonomical coverage or molecular data. Moreover, some of its putative diagnostic characters are polymorphic, and the unique skin texture is the single synapomorphy of the genus (Cannatella, 1986).

The biogeographic patterns observed within *Dendrophryniscus* are also intriguing: two nominal species occur in Amazonia

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1055-7903
doi:10.1016/j.ympev.2011.11.023

(*Dendrophryniscus bokermanni and Dendrophryniscus minutus*), whereas eight species (*Dendrophryniscus brevipollicatus; Dendrophryniscus berthalutzae; Dendrophryniscus carvalhoi; Dendrophryniscus krausae; Dendrophryniscus leucomystax; Dendrophryniscus oreites; Dendrophryniscus organensis; Dendrophryniscus stawiarskyi*) (Table 1) occur para-allopatrically, from central to southern Atlantic Forests of Brazil (Cruz and Fusinatto, 2008; Izecksohn, 1968; Izecksohn, 1993a,b). The Atlantic Forest is separated from Amazonia by a northeast–southwest belt of open or dry formations (Prado and Gibbs, 1993; Silva et al., 2004), which acts as a barrier to biotic exchanges between these two forest blocks (Costa, 2003; Mori et al., 1981). Many ancient clades are endemic to each region, having very few species in common.

Although no phylogenetic analysis has been performed within *Dendrophryniscus* so far, Izecksohn (1993b) grouped its species based on reproductive mode. According to him, *D. minutus*, *D. bokermanni* and *D. leucomystax* breed in temporary water bodies, while *D. brevipollicatus*, *D. carvalhoi*, *D. berthalutzae* and *D. stawiarskyi* are known or supposed to breed in bromeliads. Carvalho e Silva et al. (2010) and Cruz and Fusinatto (2008) followed this ecological arrangement, and called the phytotelma-breeding species the "*brevipollicatus* group". However, Recoder et al. (2010) outlined some morphological characters that diagnose Amazonian species from Atlantic Forest species, thus proposing a geographical, rather than ecological, arrangement of the genus. Cassimiro et al. (2010) also noted behavioral differences between Amazonian and Atlantic Forest species, regardless of their breeding sites. Assuming that *Dendrophryniscus* is indeed monophyletic, the previously proposed hypotheses that the genus comprises two clades representing (i) an Amazonian vs. an Atlantic Forest group or (ii) a group of terrestrial pond-breeders vs. phytotelma-breeders with the former distributed across Amazonia and the Atlantic Forest, should be confronted.

Another question relates to the placement of *Rhinella boulengeri*, an Atlantic Forest species originally described as *Phryniscus proboscideus* (Boulenger, 1882) and successively transferred to *Atelopus*, *Dendrophryniscus* and *Rhamphophryne*. The latter was a genus proposed by Trueb (1971) to denote a group of species from the northern Andes. In 1976, Izecksohn extended the range of this genus to include *Dendrophryniscus proboscideus*. Following Pramuk (2006), Chaparro et al. (2007a) considered *Rhamphophryne* to be a junior synonym of *Rhinella*, rendering *Bufo proboscideus* Spix, 1924 (= *Rhinella proboscidea*) and *P. proboscideus* (Boulenger, 1882) (= *Rhamphophryne proboscidea*) secondary homonyms. To resolve this secondary homonymy, Chaparro et al. (2007b,c) proposed the replacement name *R. boulengeri* for *P. proboscideus*. This species, previously assigned to *Dendrophryniscus* has never been included in any phylogenetic analysis. Moreover, Lantyer Silva et al. (2009) reported telocentric chromosomes in *R. boulengeri*, a characteristic supposedly absent in *Rhinella*. Finally, the assignment of this species under *Rhamphophryne* – and later *Rhinella* – is somewhat at odds with its distribution given that *R. boulengeri* is restricted to the Atlantic Forest and thus represents the only non-Andean species of the former genus *Rhamphophryne*. This biogeographical oddity is further emphasized by the apparently relatively young age of *Rhamphophryne* (Pramuk et al., 2008) i.e. a very old divergence would better match such a disjoint distribution.

To shed light on these issues, we generated DNA sequences from mitochondrial and nuclear loci for eight out of the 10 nominal *Dendrophryniscus* species and *R. boulengeri*, and combined them with published data for the main bufonid taxa. Here, these phylogenetic data are used to investigate the phylogenetic position of *R. boulengeri*, to test the monophyly of *Dendrophryniscus*, and to evaluate support for the alternative geographical and reproductive-mode hypotheses of diversification within the genus. The molecular data are further used to estimate the timing of diversification of the group. Intra-generic differences are also evaluated through morphological analyses of the target species.

2. Material and methods

2.1. Molecular methods

Newly collected tissues from *Rhinella boulengeri*, *Dendrophryniscus berthalutzae, Dendrophryniscus bokermanni*, *Dendrophryniscus brevipollicatus*, *Dendrophryniscus carvalhoi*, *Dendrophryniscus krausae*, *Dendrophryniscus leucomystax*, *Dendrophryniscus oreites*, and *Dendrophryniscus* sp. from French Guiana and Amapá [named *D. minutus* in previous studies; Fouquet et al. (2007)] were taken from thigh muscle or liver and preserved in 95% ethanol (Suppl. Material 1). Sequences of *D. minutus* were also retrieved from GenBank (accession number for 12S and 16S DQ158420, for RAG-1 DQ158346) and tentatively associated with the nominal species given that the sampling locality (Ecuador) was the closest to the type locality (Taracúa, Rio Uaupés, Amazonas, Brazil).

Genomic DNA was extracted using Promega DNA extraction kit. Two fragments of the mitochondrial (mtDNA) 12S and 16S genes and one fragment of the nuclear gene (nuDNA) Recombination Activating Gene 1 (RAG-1) were amplified by standard PCR techniques (Salducci et al., 2005). These loci have already been sequenced in most bufonids, are substantially informative at the species- and genus-taxonomic level, and easily amplified with universal primers. Amplifications were conducted with primers by Salducci et al. (2005) for *12S* and *16S*, and by Hoegg et al. (2004) for *RAG-1* (Suppl. Material 2). Sequencing was performed using ABI Big Dye V3.1 (ABI, Foster City, USA) and resolved on an automated sequencer at IQUSP (São Paulo, Brazil). Sequences were edited and aligned with CodonCode Aligner v.3.5.2. Novel sequences were deposited in GenBank (Suppl. Material 1).

Table 1
Dendrophryniscus species list including reproductive mode ("sup." stands for "supposedly").

Genus old	Species	Description	Breeding site
Dendrophryniscus	*minutus*	Melin, 1941	Pond developing
Dendrophryniscus	*bokermanni*	Izecksohn, 1993a	Pond developing
Dendrophryniscus	sp.		Pond developing
Dendrophryniscus	*berthalutzae*	Izecksohn, 1993b	Sup. Phytotelmous
Dendrophryniscus	*brevipollicatus*	Jiménez de la Espada, 1870	Phytotelmous
Dendrophryniscus	*carvalhoi*	Izecksohn, 1993b	Phytotelmous
Dendrophryniscus	*leucomystax*	Izecksohn, 1968	Pond developing
Dendrophryniscus	*stawiarskyi*	Izecksohn, 1993b	Sup. Phytotelmous
Dendrophryniscus	*proboscideus*	Boulenger, 1882	Sup. Phytotelmous
Dendrophryniscus	*krausae*	Cruz and Fusinatto, 2008	Sup. Phytotelmous
Dendrophryniscus	*organensis*	Carvalho e Silva et al., 2010	Sup. Phytotelmous
Dendrophryniscus	*oreites*	Recoder et al., 2010	Sup. Phytotelmous

Sequences from other Bufonidae genera were retrieved from GenBank to represent the major lineages documented so far (van Bocxlaer et al., 2010). Given that relationships among families of Hyloidea (Nobleobatrachia) remain largely unknown (Heinicke et al., 2009), we arbitrarily used two members of the Leiuperidae family, *Physalaemus* and *Pseudopaludicola*, as outgroups. The resulting dataset consisted of 60 terminals.

2.2. Molecular analyses

Preliminary alignment of the sequences was performed with ClustalX (Thompson et al., 1997), under default settings. This led to a 414 bp alignment for the 12S gene, 575 bp for the 16S gene, and 785 bp of the RAG-1 gene. We used Gblocks 0.91b (Castresana, 2000) to eliminate poorly aligned positions of the mitochondrial sequences, where homology was ambiguous (48 bp of the *12S* and 87 bp of the *16S*). The final alignment comprised 1639 bp.

2.3. Phylogenetic analyses

We used the software Modeltest version 3.6 (Posada and Crandall, 1998) to select the substitution model that best fit each mtDNA locus and each *RAG-1* codon position under Akaike's Information Criterion (Akaike, 1981). The five resulting models were employed in a Bayesian analysis (Table 2) with MrBayes 3.1 (Huelsenbeck and Ronquist, 2001). The Bayesian analysis consisted of two independent runs of 1.0×10^7 generations each, starting with random trees and 10 Markov chains (one cold), sampled every 1000 generations. Adequate burn-in was determined by examining likelihood scores of the heated chains for convergence on stationarity, as well as the effective sample size of values in Tracer 1.4 (Rambaut and Drummond, 2003). We also examined convergence on stationarity on bivariate plots of the split frequencies, cumulative split frequency for all splits for the two runs of the simulation, and symmetric tree-difference score within and between runs using AWTY (Wilgenbusch et al., 2004).

We also employed maximum parsimony (MP) with PAUP 4.0b10 (Swofford, 2002) to investigate phylogenetic relationships. Support for proposed clades was assessed via 2000 nonparametric bootstrap pseudoreplicates (Efron, 1979; Felsenstein, 1985) with the heuristic search option, tree bisection–reconnection (TBR) branch swapping and 10 random taxon addition replicates per pseudoreplicate.

We considered relationships strongly supported when posterior probabilities of the Bayesian analysis were equal to or higher than 0.95, and MP bootstrap percentages equaled to or exceeded 70% (Hillis and Bull, 1993).

Independent MP analyses of mtDNA or nuDNA revealed similar topologies among *Dendrophryniscus* species, with only one exception (the position of *R. boulengeri*) that is discussed below. This overall congruence justified the use of a total evidence approach.

2.4. Relaxed Bayesian molecular clock

Previous studies used large datasets and fossil calibrations to infer timing of diversification within Bufonidae (Pramuk et al., 2008; van Bocxlaer et al., 2009, 2010). These estimates provide calibration points for our study. We estimated the time of divergence for basal splits within the genus using a relaxed Bayesian molecular clock with uncorrelated lognormal rates (Beast 1.5.4; Drummond and Rambaut, 2007), with seven divergence estimates from van Bocxlaer et al. (2010) set as normal distributions (Table 3). Heinicke et al. (2009) provided a younger age for Bufonidae (48.7; 95% CI: 67.1–34.0) than did van Bocxlaer et al. (2010) (67.9; 95% CI: 92.7–52.7) and Marjanovic and Laurin (2007) (~62 Ma). Nevertheless, the 95% CIs overlap widely, and we preferred to use more consistent calibration points available from Pramuk et al. (2008) rather than the two estimates of Heinicke et al. (2009). Given the reduced amount of characters used here relative to Pramuk et al.'s (2008) study, we used a subset of the Bufonidae lineages to obtain a fully resolved topology and thus stabilize the chosen calibration points. For the root of the tree, we used a uniform distribution bounded between 110 and 60 Ma, which corresponds to the basal split within Hyloidea as per molecular dating and fossil records so far (Heinicke et al., 2009; Igawa et al., 2008; Marjanovic and Laurin, 2007; Pramuk et al., 2008; Roelants et al., 2007; San Mauro et al., 2005; Wiens et al., 2005). We used a GTR + I + G model, a Birth and Death Process tree prior, and a UPGMA starting tree. Other priors with large distributions were used in a preliminary run of 5.10^6 generations and sampled every 1000 generations, using the auto-optimization option for operators. We subsequently ran 1.10^7 generations and sampled every 1000 generations, using well-bounded prior distributions and optimized operators. We examined convergence on stationarity as explained above. All parameters effective sample size values (ESS) were >340 and generally >1000.

2.5. Morphological analyses

External morphological characters were examined under a stereoscopic microscope and compared amongst specimens. The following morphological measurements were obtained with a digital calliper, rounded to the nearest 0.01 mm: snout vent length (SVL), head length (HL), head width (HW), internarial distance (IND), eye-snout distance (ESD), eye to nostril distance (END), horizontal eye diameter (ED), interorbital distance (IOD), upper eyelid width (UEW), thigh length (THL), tibia length (TL), tarsal length (TAL), foot length (FL), upper arm length (UAL), forearm length, and hand length (HAL), as per Duellman (1970) and Kok and Kalamandeen (2008). External morphological nomenclature follows Izecksohn (1993a,b) and Kok and Kalamandeen (2008); finger nomenclature follows Coloma et al. (2010) and Fabrezzi and Alberch (1996). Eighty-nine specimens belonging to seven of the ten currently recognized species of *Dendrophryniscus* (two Amazonian and five Atlantic species; only *D. krausae*, *D. stawiarskyi* and *D. organensis* were not included), plus *R. boulengeri*, were examined (Suppl. Material 3). This material included 32 specimens from Western Amazonia, including the type locality of *D. minutus*. The examined material is deposited in the herpetological collections of the Museu de Zoologia da Universidade de São Paulo (MZUSP) and Museu de Zoologia da Universidade Estadual de Campinas "Adão José Cardoso" (ZUEC).

Table 2
Models of evolution estimated for each mtDNA locus and each position of the RAG-1 locus with Modeltest.

Gene	Model	Gamma	Substitution matrix	P invar	Base composition
12S	GTR + I + G	0.5952	6.8526, 20.9991, 14.4212, 0.001, 70.4550	0.3090	0.3415,0.2184,0.1932,0.2298
16S	GTR + I + G	0.5290	2.4731, 7.1183, 4.5999, 0.2250, 21.7312	0.4002	0.3503,0.2182,0.1576,0.2739
RAG1 2 position	HKY + I	equal	tr ratio 1.0426	0.8187	0.3097, 0.2150, 0.1922, 0.2831
RAG1 3 position	HKY + G	2.9673	tr ratio 2.1969	0	0.2614,0.2095,0.1935,0.5943
RAG1 1 position	GTR + G	0.3026	2.8170, 2.3156, 1.7531, 0.5389, 6.3593	0	0.3081,0.1615,0.3209,0.2095

Table 3
Calibration points used for molecular dating including the genera included in each clade, the number of the clade in van Bocxlaer et al. (2010); the corresponding number in Fig. 2, the prior distribution of each calibration point. All the calibration points were set as normal distribution except clade 1 which was set with min and max bounds.

Clade	van Bocxlaer et al. (2010)	Fig. 2	Mean	95% inf	95% sup	SD
Hyloidea		1	60–110			
Bufonidae	2	2	67.9	52.7	92.7	12
Atelopus + *Oreophrynella* vs. other Bufonidae	3	3	60.7	45.7	82.3	11
Dendrophryniscus vs. other crown *Bufonidae*	5	4	52.1	38.4	70.9	9
Atelopus vs. *Oreophrynella*	439	5	47.5	28.8	68.4	11
Nannophryne vs. other crown Bufonidae	6	6	47.0	34.8	63.9	8
Rhaebo vs. other crown Bufonidae	7	7	40.8	30.3	55.0	7
Rhinella granulosa vs. *R. marina*	244	8	24.2	12.7	35.5	7

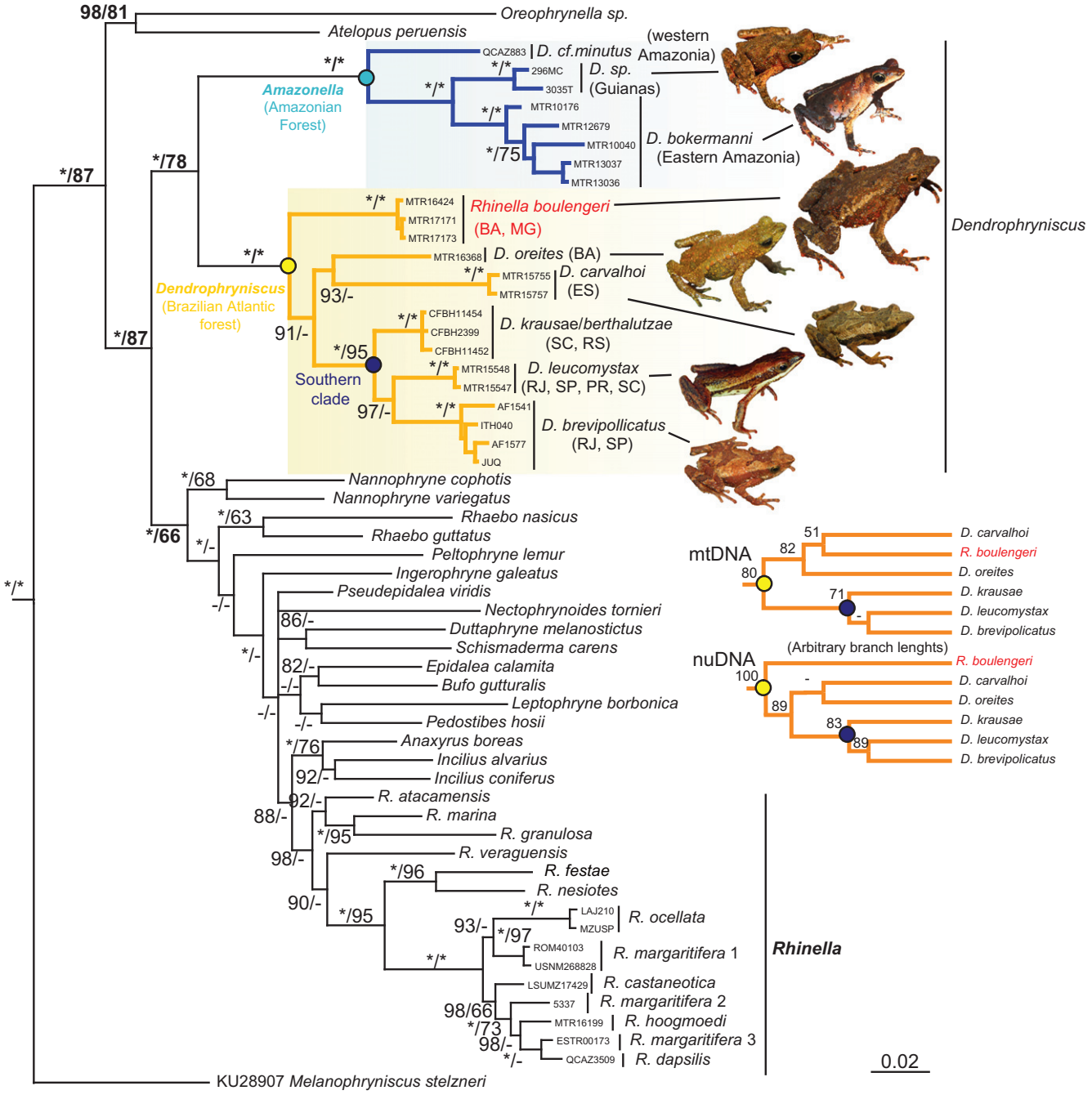

Fig. 1. Phylogenetic relationships among Bufonidae hypothesized from Bayesian analysis using 1639 bp of combined mtDNA. Node support is indicated with values of posterior probability (pp) *100 and MP bootstrap support. Asterisks (*) denote nodes with pp = 1 or 0.99, and bootstrap values = 100 or 99%; hyphens (-) indicate nodes with pp < 0.8 and bootstrap support < 60%. Sub-trees based on MP independent analyses of mtDNA-only and nuDNA-only datasets are also illustrated for the Atlantic Forest clade. We show only the subtrees for the Atlantic Forest *Dendrophryniscus* clade because the topology is the same for the remainder of the tree.

Prior to analysis, all morphometric measures were $\log_{10}$-transformed to conform to requirements of normality (Zar, 2009). A Principal Component Analysis (PCA) was performed on the covariance matrix of morphometric variables to extract dimensions of maximum variation in the data and to visualize overall species grouping in morphological space (Manly, 2004). To remove effects of scale among species, subsequent analyses were performed on a size-corrected dataset obtained by linear-regressing the original morphometric measures of each character with the first principal component of the PCA, a multivariate size estimate (Strauss, 1985). The distinction between Amazonian and Atlantic groups was tested with a Multivariate Analysis of Variance (MANOVA) using "group" and "sex" as factors. A Discriminant Function Analysis (DFA) was performed to test classification of individuals to predicted groups (Manly, 2004).

3. Results

3.1. Molecular phylogenetics

Rhinella boulengeri is unambiguously nested within *Dendrophryniscus* as currently defined (Fig. 1). This result is also supported when the mitochondrial and nuclear markers are analyzed in isolation (Fig. 1, inset). Both methods of phylogenetic reconstruction unambiguously support the monophyly of *Dendrophryniscus*, inclusive of *R. boulengeri* (Fig. 1). However, we recovered a previously undocumented deep divergence within the genus that segregates Amazonian species from Atlantic Forest species. These two groups are reciprocally monophyletic (Fig. 1).

Within the Atlantic Forest clade, a strongly supported subclade includes all species from the southern part of the range: *D. brevipollicatus*, *D. leucomystax*, *D. berthalutzae* and *D. krausae* (with the last two being either very closely related, synonyms or our material was actually misidentified for one or the other). The most basal relationships within the Atlantic Forest clade (*R. boulengeri*, *D. carvalhoi*, and *D. oreites*) are poorly resolved (Fig. 1). When analyzed separately using MP (with the same methodology described for the entire dataset), the mtDNA and the nuDNA data recovered a similar topology among *Dendrophryniscus* species, except for the placement of *R. boulengeri*. Using mtDNA alone, this species forms a strongly supported clade with *D. carvalhoi* and *D. oreites*, two species also from the northern half of the distribution within the Atlantic Forest. However, the nuDNA data place *R. boulengeri* as the sister taxon to all other Atlantic Forest species, the latter forming a strongly supported clade.

Within the Amazonian group, the undescribed species from the eastern Guiana shield is the sister taxon to *D. bokermanni*, which together form a sister clade to *D. minutus*. This grouping is well-supported in both mtDNA-only and nuDNA-only analyses.

3.2. Molecular dating

The recovered age of the basal split among *Dendrophryniscus* species is mid-Eocene (~44 Ma 95%CI: 36.3–51.7, Fig. 2). As a matter of comparison, none of the other bufonid genera is recovered to be that old; this age corresponds to the basal node of all other crown bufonids. However, the age estimates for both the Amazonian clade (~24 Ma) and the Atlantic Forest clade (~26 Ma) are comparable to those of other bufonid genera, including *Rhinella* or *Rhaebo* (Pramuk et al., 2008; van Bocxlaer et al., 2010).

3.3. Morphological analyses

The first two principal components extracted by the PCA account for 95.3% of all variation found in the dataset (Table 4). The coefficients of the first principal component, which alone ac-

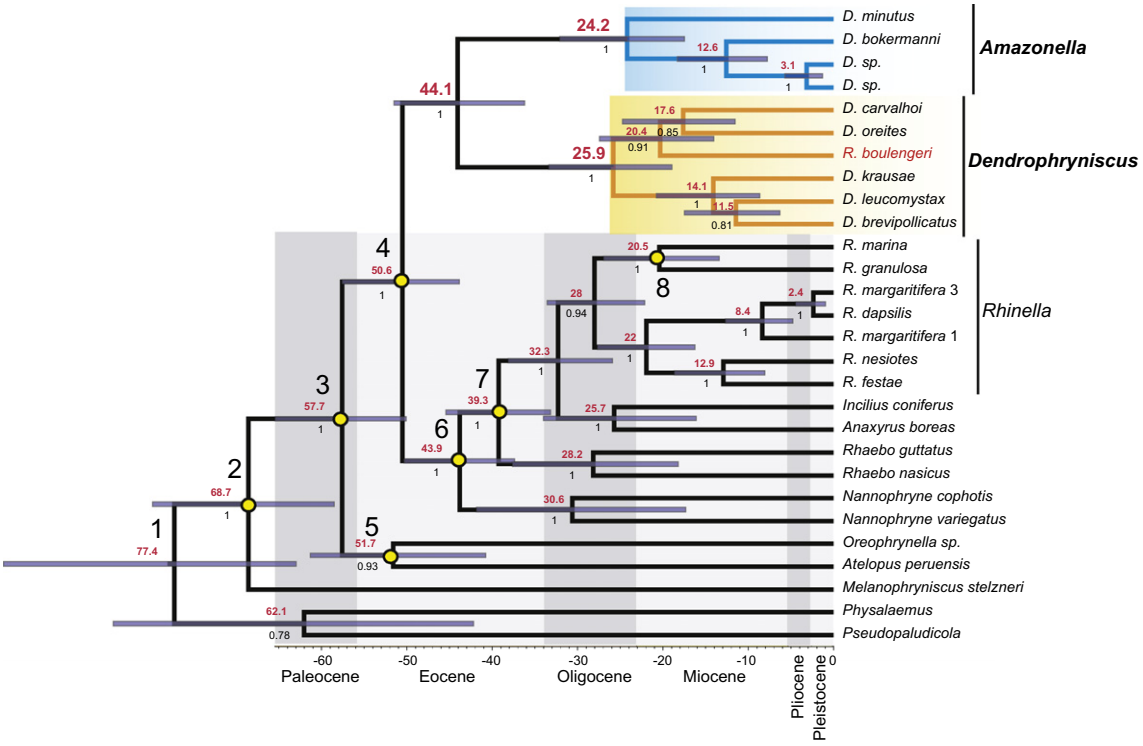

Fig. 2. Bayesian time-calibrated, maximum clade-credibility tree using relaxed clock with the same dataset but selected terminals. Calibration points and corresponding numbers (see Table 3) are indicated with yellow circles. Posterior probabilities are indicated on the lower left of the nodes, while the modes of the posterior distributions of the age of the nodes are indicated on the upper left in red. 95% credibility intervals are indicated with blue bars centered on the nodes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
The first three principal components extracted by the PCA on log-tranformed morphometric variables. The coefficients with significant correlation with the component are in bold. In the bottom are presented the individual and cumulative contributions of each component to total variation. Measure abbreviations are in Section 2.

Measures	PC1	PC2	PC3
SVL	**0.985**	−0.120	−0.020
HL	**0.985**	−0.059	0.100
HW	**0.977**	0.030	0.167
IND	**0.935**	−0.087	**0.285**
ESD	**0.980**	−0.100	0.075
END	**0.958**	**−0.223**	−0.019
ED	**0.916**	**0.233**	**0.243**
IOD	**0.952**	−0.017	0.0162
UEW	**0.940**	0.0394	0.167
THL	**0.940**	**0.318**	−0.064
TL	**0.969**	**0.212**	−0.097
TAL	**0.974**	0.100	−0.143
FL	**0.984**	0.049	−0.103
FAL	**0.952**	−0.178	−0.116
UAL	**0.979**	0.076	−0.053
HAL	**0.973**	−0.111	−0.163
% of Variation	93.196	2.124	1.780
Cumulative %	93.196	95.320	97.100

Table 5
Classificatory matrix from size-free DFA analysis, showing the number of individuals correctly allocated to each group based on original and cross-validated grouped cases.

		Predicted group membership		Total
		Atlantic Forest	*Amazonia*	
Original				
Count	Atlantic Forest	28	1	29
	Amazonia	0	13	13
%	Atlantic Forest	96.5	3.5	100
	Amazonia	0	100	100
Cross-validated				
Count	Atlantic Forest	25	4	29
	Amazonia	1	12	13
%	Atlantic Forest	86.2	13.8	100
	Amazonia	7.7	92.3	100

counts for 93.2% of all variation, are all positive and highly correlated (values >0.9). This can hence be interpreted as a vector of overall size (Strauss, 1985). In the first principal component axis, *R. boulengeri* is distinguished from all other species due to its larger size (Fig. 3). The second component explains 2.1% of the variation; it has significant positive loadings for THL, ED and TL, and negative for END (Table 4). Along this axis, which represents a shape variation vector, the Atlantic Forest species are well distinguished from Amazonian species, except for *R. boulengeri* (Fig. 3).

The Amazonian and Atlantic groups are significantly distinct in shape (MANOVA, $F_{1,85} = 12.03$, $P < 0.001$). Significant differences ($P < 0.001$) occur in HW, END, ED, UEW, THL, TL, TAL, FL, and UAL. Sexual differences are also present ($F_{1,85} = 5.63$, $P < 0.001$), but shape differences between the groups maintain significance for females ($F_{1,48} = 8.88$, $P < 0.001$) and males ($F_{1,37} = 18.74$, $P < 0.001$) separately. A discriminant function analysis found good membership prediction between the two genera, with 94.4% cases correctly classified in the original grouping, and 87.6% in the cross-

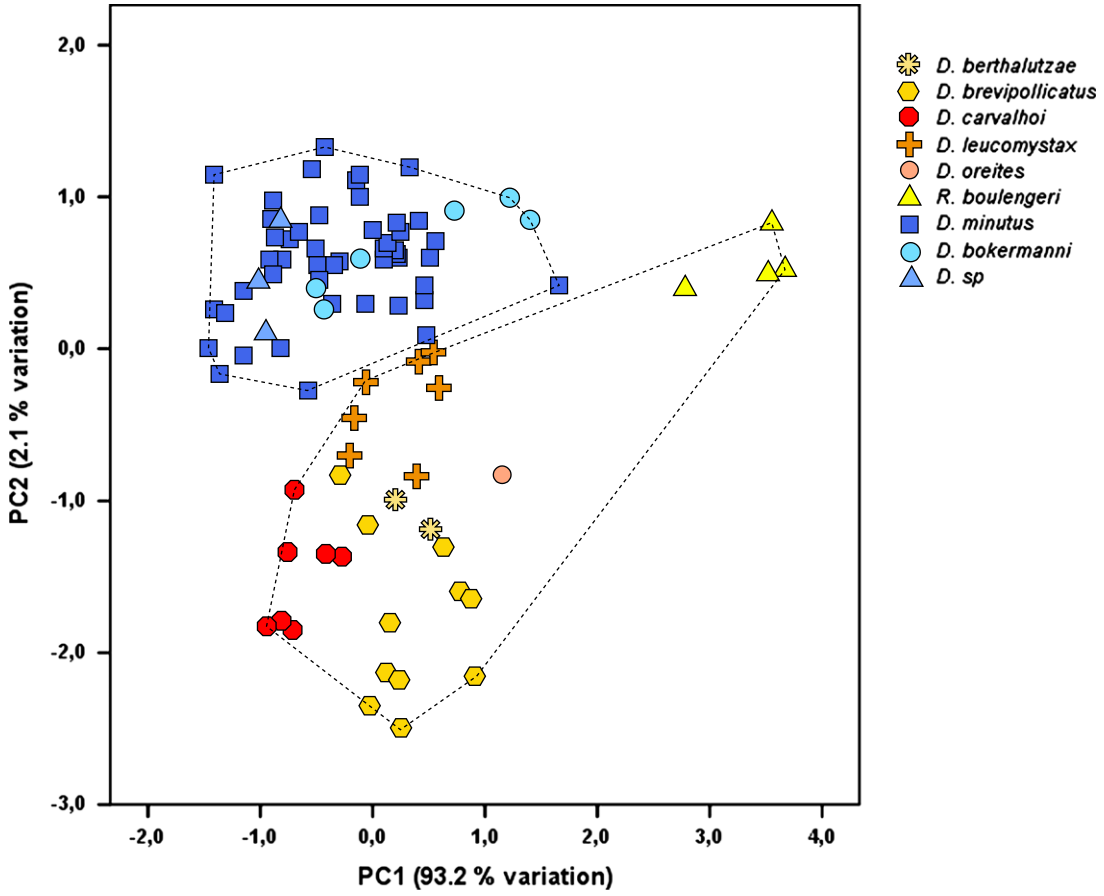

Fig. 3. Results of the PCA on log-transformed morphometric variables. Symbols represent specimens on the first two principal components. The contribution of each axis for total variation is indicated in parenthesis. The two groups, Amazonia and Atlantic Forest, are delimited with dashed lines.

Table 6
Discriminant variables ordered by absolute size of correlation with the canonical discriminant function. Measure abbreviations are in Section 2.

Measures	Discriminant function
THL	0.691
TL	0.561
ED	0.543
UAL	0.494
HW	0.385
TAL	0.314
FL	0.265
END	−0.224
HL	0.196
UEW	0.183
FAL	−0.166
IOD	0.154
HAL	−0.084
ESD	0.067
IND	0.066

validated cases (Table 5). In the discriminant function analysis, THL, TL, ED and UAL coefficients had the most significant and positive loadings (Table 6).

4. Discussion

4.1. Taxonomic changes

4.1.1. A new genus of Bufonidae

There are no strict criteria about how to delimit a genus, and the identification of supraspecific taxonomic levels always holds some degree of arbitrariness (Cain, 1956; Hennig, 1966; Simpson, 1963). Taxonomists nonetheless have the responsibility to describe higher-level taxa that are not only monophyletic, but also practical. Gill et al. (2005) outlined the following guidelines for genus recognition: (1) monophyly; (2) reasonable compactness (a genus should not be expanded needlessly); and (3) distinctiveness in evolutionarily relevant criteria, i.e. ecology, morphology, or biogeography. In the case of *Dendrophryniscus*, these three criteria converge and point to the need to reconsider generic inclusiveness: (1) the genus consists of two major subclades, (2) each subclade holds multiple species, (3) the subclades occupy different positions in the morphometric space (i.e., can be differentiated morphologically), and (4) the subclades are allopatrically distributed in morphoclimatic domains that have been historically isolated and that differ environmentally and biologically. An additional point is that the split between the Amazonian and the Atlantic Forest *Dendrophryniscus* is very ancient (Eocene), older than any other bufonid genera dated so far. It has been proposed that supraspecific groups with similar ages be given the same Linnaean rank, which would allow for appropriate comparisons (Hennig, 1966; Avise and Liu, 2011). Although this controversial topic lies out of the scope of this work, considering the two major subclades of *Dendrophryniscus* as different genera also improves the consistency of crown-group ages relative to other Bufonidae genera.

Despite using a reduced dataset outside of *Dendrophryniscus*, our molecular dating results are in complete agreement with previous studies that were based on extensive taxonomic and genomic sampling within Bufonidae (Pramuk et al., 2008; van Bocxlaer et al., 2009, 2010). We thus argue that allocating Amazonian and Atlantic Forest clades to different genera constitutes a logical improvement of the current taxonomy. Because the type species of the genus is *D. brevipollicatus* (Jiménez de la Espada, 1870), from Rio de Janeiro, the generic name *Dendrophryniscus* becomes restricted to the Atlantic Forest species. We here propose a new generic name for the Amazonian clade.

4.1.1.1. *Amazonella gen. nov. Type species*: *Atelopus minutus* Melin, 1941.

Etymology: The generic epithet *Amazonella* is a composition of the words: Amazon, and the Latin suffix "-ella", a feminine diminutive particle. It means "small Amazonian", alluding to the small body size of the species and their Amazonian distribution.

Definition: A genus of small-sized bufonid species (12.0–26.5 mm), hind limbs well developed (THL + TL/SVL > 0.90), snout protruding in profile, parotoid glands absent, cranial crests indistinct, tympanum absent, vocal slits absent, foot basally webbed, skin uniformly and finely granulose, dorsal color cryptic, ventral surfaces with pattern of blotches, presence of longitudinally elliptical subarticular tubercles on hands, reproduction in ponds and Amazonian distribution. Phylogenetically, the new genus is defined as a clade comprising the most recent common ancestor of *Amazonella minuta* and *Amazonella bokermanni*, and all of its descendants.

We have not investigated the internal morphology of the species of *Amazonella* and no data on the osteology or musculature of *A. bokermanni* are available in the literature. As such, we are unable to identify internal morphological synapomorphies at this time. Nevertheless, variable amounts of osteological and/or myological data on *Amazonella* and/or *Dendropsophus* were provided notably by McDiarmid (1971) which provides some characters that could be tentatively considered as defining the genus: the depressor mandibulae muscle arises on the anterior projection as well as the posterior arm of the squamosal and on the lateral portion of the prootic; the frontoparietal and prootic are fused; the broader lateral edge of the septomaxilla is reduced; the palatines are greatly reduced or absent; vomers are crescent-shaped and smaller than in *Dendrophryniscus*; the dorsal arm of the squamosal barely overlays the prootic; the quadratojugal is reduced to only an ossified tip of the quadrate; the fourth and fifth trunk vertebrae point posteriorly, while the sixth and seventh are directed laterally (it is not clear here whether only *A. minuta* or both *A. minuta* and *D. brevipollicatus* possess fusion of the vertebrae); the coccyx is fused; rectangular shaped girdle; columella absent; well-developed opercularis muscle as well as typical middle ear structure but absence of ostia pharyngea; Bidder's organs are present. From Graybeal and Cannatella (1995) we can also add that *Amazonella* possess a posterolateral process of the hyoid and that it does not present inguinal fat bodies (da Silva and Mendelson, 1999).

Diagnosis: *Amazonella* is readily diagnosed from Neotropical genera formerly included in *Bufo* (i.e.: *Bufo*, *Incilius*, *Nannophryne*, *Rhaebo* and *Rhinella*) and other Neotropical bufonids (*Andinophryne*, *Metaphryniscus*, *Peltophryne*, *Truebella*) by (comparative character in parentheses): absence of parotoid glands (present), small size, with maximum SVL of 26.5 mm (mean SVL higher than 27 mm), absence of external tympanum (present), and cranial crests not evident (evident). *Amazonella* is distinguished from *Atelopus* by the presence of uniformly granular skin (smooth skin or with presence of irregularly distributed small warts); from *Truebella* by its basally webbed foot (foot not webbed) and hind limb long, TL 0.43–0.55SVL (short hind limbs, TL 0.28–0.37SVL); from *Crepidophryne* by cranial crests indistinct (distinct), absence of parotoid glands (present); from *Frostius* by absence of external tympanum (present), and absence of vocal slits (present); from *Melanophryniscus* by granular skin (rugose), absence of vocal slits (present), and atelopodiform habitus with snout pointed in profile (bufoniform habitus with snout rounded in profile); from *Oreophrynella and Osornophryne* by hands and foot basally webbed (hands and foot webbed or palmate). From *Dendrophryniscus*, *Amazonella* is distinguished by the presence of longitudinally elliptical subarticular tubercles (transversely elliptical subarticular tubercles), ventral region with pattern of blotches or spots (ventral surfaces pale colored, or with marginally distributed colored blotches), and skin finely granular with small scattered tubercles (skin granular to

warty). Also, *Amazonella* has proportionately larger limbs (upper arm length, thigh length and tibia length), larger eyes (eye diameter), and shorter snout (eye to nostril distance).

Content: *Amazonella* contains two nominal species: *A. minuta* (Melin, 1941) com. nov. and *A. bokermanni* (Izecksohn, 1993) com. nov. The genus probably contains additional undescribed species, including one from the lowlands of eastern Guiana Shield (Fouquet et al., 2007).

Distribution and natural history: *Amazonella* is known to occur throughout Amazonia, at low to moderate altitudes in Brazil, Bolivia, Peru, Ecuador, Colombia, Venezuela, Guyana and French Guyana (Fig. 4). *Amazonella minuta* has been documented to be diurnal (e.g. Hödl, 1990) but to preferably breed by night (Aichinger, 1985) in ponds near water courses in the forest floor, laying egg strings of 175–420 small eggs (Duellman and Lynch, 1969). *Dendrophryniscus bokermanni* supposedly has the same reproductive mode (Izecksohn, 1993a). However, the delineation of species and their ranges in *Amazonella* is problematic as many putative species have been lumped under the name *A. minuta* (Fouquet et al., 2007; Frost, 2011). Extensive analyses, beyond the scope of the present study, are needed to determine species' boundaries within this genus. Nonetheless, given our geographically broad sampling of this taxon in Amazonia (Figs. 3 and 4; $n = 57$ specimens), we are confident that we recovered a relatively good representation of the morphological variation within *Amazonella* and that the observed cohesion is not an artefact of limited sampling.

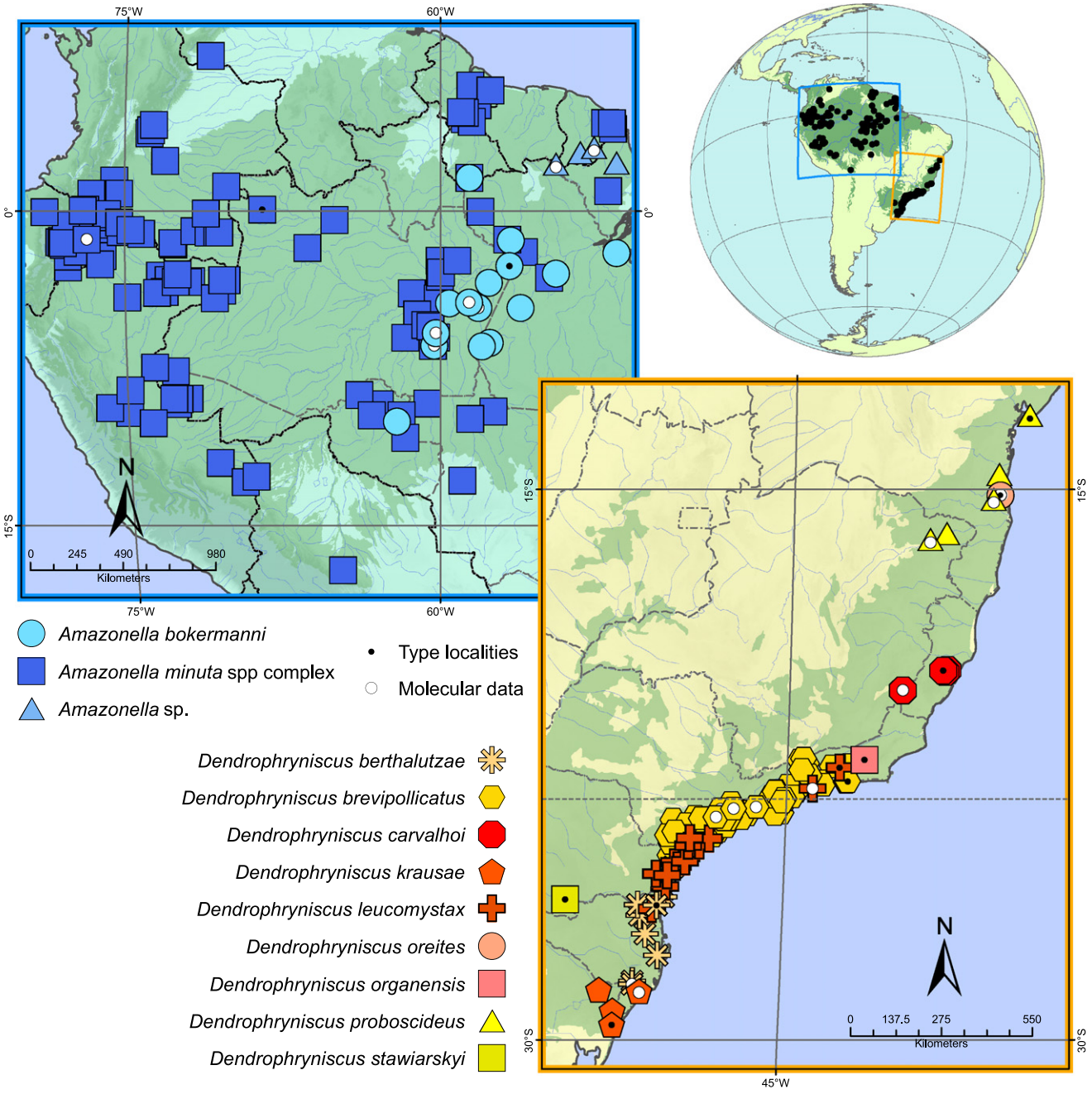

Fig. 4. Occurrence records of *Dendrophryniscus* spp. and *Amazonella* gen. nov., over respectively the Atlantic Forest and Amazonia. Type localities are indicated with black dots and localities from which we obtained molecular data used in our analysis with white dots. Molecular data from Ecuador is presented in a general locality, as no further information is available for this location.

4.1.2. Redefinition of Dendrophryniscus

Given our results, we place the Atlantic Forest species currently referred to as *R. boulengeri*, and originally described as *P. proboscideus*, in the genus *Dendrophryniscus*.

4.1.2.1. Dendrophryniscus Jiménez de la Espada, 1870. Type species: *Dendrophryniscus brevipollicatus* Jiménez de la Espada, 1870.

Definition: A genus of small to medium-sized bufonid (15.0–46.8 mm), habitus atelopodiform, hind limbs well developed (THL + TL/SVL > 0.75), snout protruding in profile, parotoid glands absent, cranial crests indistinct in most species, external tympanum absent, foot basally webbed, skin uniformly granulose to warty, cryptic dorsal color, ventral surfaces pale colored or with marginally distributed colored blotches on the belly and limbs, presence of longitudinally elliptical subarticular tubercles on hands, reproduction in ponds or phytotelmata, Atlantic Rainforest distribution.

Diagnosis: *Dendrophryniscus* is readily diagnosed from Neotropical genera formerly included in *Bufo* (i.e. *Bufo*, *Incilius*, *Nannophryne*, *Rhaebo* and *Rhinella*) and other Neotropical bufonids (*Andinophryne*, *Metaphryniscus*, *Peltophryne*) by (comparative character between parentheses): absence of parotoid glands (present), absence of external tympanum (present), and cranial crests usually not evident (evident). From the other bufonids, *Dendrophryniscus* is distinguished from *Atelopus* by presence of uniformly granular or warty skin (smooth skin or with presence of irregularly distributed small warts); from *Truebella* by its basally webbed foot (foot not webbed) and hind limb longer, TL 0.38–0.50 SVL (shorter hind limbs, TL 0.28–0.37 SVL); from *Crepidophryne* by absence of parotoid glands (present); from *Frostius* by absence of external tympanum (present); from *Melanophryniscus* by granular skin (rugose) and atelopodiform habitus with snout pointed in profile (bufoniform habitus with snout rounded in profile); from *Oreophrynella and Osornophryne* by hands and foot basally webbed (hands and foot webbed or palmate). From *Amazonella*, *Dendrophryniscus* is distinguished by the presence of transversely elliptical subarticular tubercles (longitudinally elliptical subarticular tubercles), ventral surfaces pale colored, or with marginally distributed colored blotches (ventral region with pattern of blotches or spots), and skin granular to warty (skin finely granular with small scattered tubercles). Also, *Dendrophryniscus* has proportionately larger snout (eye to nostril distance), smaller limbs (thigh length, tarsal length), and smaller eyes (eye diameter).

Content: *Dendrophryniscus* contains eight nominal species: *D. brevipollicatus* Jiménez de la Espada, 1870; *D. leucomystax* Izecksohn, 1968; *D. carvalhoi* Izecksohn, 1993b; *D. berthalutzae* Izecksohn, 1993b and *D. stawiarskyi* Izecksohn, 1993b; *D. krausae* Cruz and Fusinatto, 2008; *D. oreites* Recoder et al., 2010; *D. organensis* Carvalho e Silva et al., 2010 and *D. proboscideus* Boulenger, 1882.

Distribution and natural history: Species of *Dendrophryniscus* are distributed along the Atlantic Rainforest of eastern Brazil, from southern Bahia to the northern coast of Rio Grande do Sul. The species occur in altitudes ranging from sea-level up to 1050 m a.s.l. (Carvalho e Silva et al., 2010; Izecksohn, 1993b). They inhabit the leaf-litter and low vegetation of well-preserved rainforests, being usually cryptic in habits (Heyer et al., 1990). *D. brevipollicatus* has smaller clutch sizes, depositing between 50 and 65 large eggs (Carvalho, 1949). *D. brevipollicatus* and *D. carvalhoi* breed in phytotelmata. Field observations suggest that *D. berthalutzae*, *D. krausae*, *D. organensis*, *D. oreites*, and *D. stawiarskyi* may have the same reproductive mode, while *D. leucomystax*, the only lowland species of *Dendrophryniscus*, breeds in temporary ponds of the forest floor (Carvalho e Silva et al., 2010; Cruz and Fusinatto, 2008; Izecksohn, 1993b; Recoder et al., 2010). *D. proboscideus* likely breeds in streams (Feio et al., 2003).

4.1.2.2. D. proboscideus (Boulenger, 1882). Phryniscus proboscideus (Boulenger, 1882), p. 150, pl.13, Fig. 1. Syntypes: BM 69.2.22.8, 69.11.3.24, 69.11.3.28 (according to M.S. Hoogmoed in Frost, 1985:76.). Type locality: "Bahia", Brazil ("Probably in vicinity of Salvador, Bahia" according to Feio et al. (2003), p. 108).

Atelopus proboscideus Boulenger, 1894, pp. 374–375.
Dendrophryniscus proboscideus McDiarmid, 1971, p. 40.
Rhamphophryne proboscidea Izecksohn, 1976, p. 342.
Rhinella proboscidea Chaparro et al., 2007, p. 211; a junior homonym of *Bufo proboscideus* Spix, 1824.
Rhinella boulengeri Chaparro et al., 2007, p. 1027.

Diagnosis: *Dendrophryniscus proboscideus* is distinguished from other congeners by: (1) large size for the genus (maximum SVL = 46.8 mm, vs. 18.0–26.5 mm in other species); (2) warty aspect of the body, with numerous enlarged granules presenting *coni apicali*; (3) protruding snout in lateral view; (4) presence of nuptial pads on males; (5) inner finger just slightly reduced; (6) ventral region with pattern of colored blotches on the belly and limbs.

Comparisons: *Dendrophryniscus proboscideus* is diagnosed from all its congeners by: SVL larger than 32 mm (smaller than 27 mm), toe tip of third finger not expanded (tip of third finger expanded), and ventral pattern with presence of colored blotches irregularly distributed on the belly and limbs (pale ventral surfaces without pattern). Furthermore, *D. proboscideus* is distinguished from *D. brevipollicatus*, *D. berthalutzae*, *D. krausae* and *D. stawiarskyi* by presence of enlarged granules on canthal region (smooth canthal region); from *D. oreites* and *D. carvalhoi* by the more ossified head, with cranial crests evident.

Distribution and natural history: *Dendrophryniscus proboscideus* is known to occur in the coastal Atlantic Forest of Bahia (near Salvador), Serra das Lontras (southern Bahia), and northeastern Minas Gerais (Jequitinhonha river valley; Izecksohn, 1976; Feio et al., 2003). All specimens recently obtained were found in well-preserved forest fragments, associated with mountainous areas that reach 500–800 m a.s.l. (Feio et al., 2003; our data). These are terrestrial, leaf-litter toads, often found while active during the day and near small streams (Feio et al., 2003; our data). Reproduction has never been directly observed, but the recurrent observation of adults and juveniles next to small mountain streams suggests that the species may use pools in stream beds to breed (Feio et al., 2003).

Remarks: When Izecksohn (1976) removed *P. proboscideus* from *Dendrophryniscus* and assigned it to *Rhamphophryne*, he examined four old and poorly preserved exemplars. Recently collected individuals reveal that some of the characters that were used to assign the species to *Rhamphophryne*, such as the presence of parotoid glands, are actually absent (pers. obs.). Furthermore, the diagnostic characters proposed by Izecksohn (1976) to distinguish the species from other *Rhamphophryne* (i.e. slender body, warty skin, protruding snout, longer limbs, and occurrence in the Atlantic Forest of eastern South America) actually align it with species of *Dendrophryniscus*. At the time, Izecksohn justified his generic rearrangement on the basis of the overall morphological differences between *D. proboscideus* and *D. brevipollicatus*/*D. leucomystax*, given the larger size, warty aspect of the skin, and slender fingers of *D. proboscideus*. The recent discovery of *D. oreites*, with remarkable warty skin and large male size for the genus (Recoder et al., 2010) fills the morphological gap between *D. probocideus* and the other *Dendrophryniscus* and is congruent with the arrangement based on the molecular data.

4.2. Morphological evolution

Studies of amphibian diversity and evolutionary trends remain a difficult task because morphology can be extremely conserved in

the group (Cherry et al., 1977, 1978; Emerson, 1986), and is often homoplasic (Bossuyt and Milinkovitch, 2000; Parra-Olea and Wake, 2001). Our morphological analysis supports this view: almost all morphometric variation among species in both *Dendrophryniscus* and *Amazonella* can be explained by size differences; shape variation is subtle. The use of molecular tools reinforces the notion that morphological conservatism and convergence mask a great proportion of amphibian diversity that remains to be discovered, both at the species level and in deeply rooted lineages (e.g. Chek et al., 2001; Maxson, 1984; Richards and Moore, 1996; Stuart et al., 2006), leading to a revitalization of amphibian taxonomy (Faivovich et al., 2005; Frost et al., 2006; Grant et al., 2006; Guayasamin et al., 2009; Hedges et al., 2008; Heinicke et al., 2009; Padial et al., 2010; Wiens et al., 2005). The case of *D. proboscideus* is compelling. For more than 30 years, this species has been placed in genera that share a common ancestor with *Dendrophryniscus* going back ~40 Ma ago, and occurring in very distant morphoclimatic domains. This is a striking example of the inherent difficulty of assessing homology of morphological characters in amphibians.

Despite general conservatism and the similar crown ages of the two groups, the Atlantic Forest *Dendrophryniscus* display a wider array of shape and size than do the Amazonian *Amazonella*. This is visible on the PCA, with the morphometric space occupied by *Dendrophryniscus* appears much wider than the space occupied by *Amazonella* (Fig. 3). This is true for the range of body size as shown by the dispersion along PC1, with *D. proboscideus* being much larger. It is also demonstrated by the diversity of shapes along PC2, even when *D. proboscideus* is excluded. This may be related to the sharp altitudinal and latitudinal gradient of the Atlantic Forest relative to Amazonia. However, this observation is difficult to interpret with confidence given the samplings within Amazonia and the Atlantic Forest are very different. Even if we did sample more *Amazonella* (57, with material spanning western Amazonia, the Guianas and south-eastern Amazonia) than *Dendrophryniscus* (37), we included unambiguously the majority of the diversity within *Dendrophryniscus* while the diversity within *Amazonella* remains virtually unknown. Consequently, the relatively reduced morphological variation within *Amazonella* compared to *Dendrophryniscus* is conditional on the data at hand and require further sampling and evaluation.

4.3. Biogeography

The divergence between *Dendrophryniscus* and *Amazonella* coincides with the middle Eocene, a period of southern uplift of the Andes (Hoorn et al., 2010), isolation of Antarctica and the creation of a circumpolar current, dramatic drop of the sea level, and major climatic changes (Ortiz-Jaureguizar and Cladera, 2006). This period also corresponds to the arrival of immigrant taxa in South America (rodents and primates), prevalence of large grazing herbivores, and 'modernization' of other faunal aspects during the mid-Cenozoic, reflecting adaptation to major environmental changes, including increased aridity and cooling (Flynn and Wyss, 1998). The middle Eocene witnessed the spread of open vegetation at the expense of the rainforest that previously dominated the Southern continent, and likely the origination of the dry corridor that isolates Amazonia and the Atlantic Forest (Roig-Juñent et al., 2006; Romero, 1986). Interestingly, the origination of the bufonid "range expansion phenotype", as coined by van Bocxlaer et al. (2010), corresponds to this period of habitat modification. The middle Eocene matches divergence times of major clades in the higher taxon Terrarana that are almost exclusively associated with forest habitat (Heinicke et al., 2009), with some of these clades being endemic to either the Atlantic Forest or Amazonia. There is little doubt that the origin of Atlantic Forest endemic clades was concomitant in other forest-restricted groups, such as Centrolenidae (Guayasamin et al., 2009).

The dry corridor of open vegetation in central South America has been considered an important restraint to species migration between Amazonia and the Atlantic rainforests (Costa, 2003; Mori et al., 1981; Raven and Axelrod, 1974). Several phylogenetic hypotheses place early branching events in the Atlantic Forest biota (Bates et al., 1998; Cracraft and Prum, 1988; Eberhard and Bermingham, 2005; Fiaschi and Pirani, 2009; Santos et al., 2007). As a matter of fact, many genera or even families of amphibians and reptiles are endemic to one or the other region [e.g. for Atlantic Forest morphoclimatic domain: Anurans: *Bokermannohyla*, Brachycephalidae, *Crossodactylodes*, *Cycloramphus*, *Frostius*, Hylodidae, *Itapotihyla*, *Paratelmatobius*, *Phyllodytes*, *Scythrophrys*; *Zachaenus*, and Squamates: *Ecpleopus*, *Placosoma*, etc.]. Only a few anuran species or species groups occur in both forest biomes (Lynch, 1979): those that do are mostly in complexes of ecologically versatile species that may have invaded one of the domains in the late Tertiary or Quaternary (Costa, 2003; Cracraft and Prum, 1988). Maps of present-day vegetation suggest that the ubiquitous gallery forests and series of deciduous and semi-deciduous forest patches that constitute a network of interconnected forests through the otherwise open landscape of the dry corridor may provide opportunities for these recent invasions (Costa, 2003; Oliveira-Filho and Ratter, 1995; Wang et al., 2004). Thus, the Atlantic Forest biota has contributions from both old endemics and recently dispersing lineages (Costa, 2003; Pennington et al., 2006). The fact that groups like *Dendrophryniscus/Amazonella* or Terrarana failed to disperse during Amazonia/Atlantic Forest connection periods likely results from the combined effects of strong physiological and ecological sensitivity and poor dispersal abilities. The dry corridor must have remained unsuitable or too fleeting for such sensitive species.

Crown ages of both *Dendrophryniscus* and *Amazonella* seem concomitant (~25 Ma), as suggested by our molecular dating. Yet, too few data are available for *Amazonella* to recover the full evolutionary history of the group. Although older lineages may have been missed by our analyses, it is possible that initial diversification within the genus coincides with the Oligocene/Miocene transition, a period of cooling and mountain building that matches the diversification of the first modern Andean genera of plants and animals (Hoorn et al., 2010). This mountain build-up had major impacts on Amazonia's hydrological system (Hoorn et al., 2010) and may have driven diversification in terrestrial, forest-associated organisms such as *Amazonella*.

Similarly, the North vs. South Atlantic Forest pattern observed within *Dendrophryniscus* is concordant with several studies of vicariant forms whose limits are more or less coincident with the Rio Doce valley (northern Espírito Santo state; Carnaval et al., 2009; Costa, 2003; Cracraft and Prum, 1988; Pellegrino et al., 2005; Pinto da Rocha et al., 2005; Silva et al., 2004). Several plant taxa are restricted to either one of these areas, producing a strong floristic differentiation between the northern and southern Atlantic Forests (Oliveira-Filho and Fontes, 2000). The occurrence of narrow endemic species of *Dendrophryniscus* in the northern part of the Atlantic Forest, diverging between ~25 and ~17 Ma ago, are the testimony that some of these forest fragments remained relatively stable during the entire Miocene and Quaternary, a much longer time period than that modeled by Carnaval and Moritz (2008).

4.4. Life-history evolution

Assuming that the common ancestor of *Dendrophryniscus* and *Amazonella* was a pond breeder (van Bocxlaer et al., 2010), as are most bufonid lineages, then the most parsimonious hypothesis to explain the evolution of reproductive modes in the group is that

the phytotelmous breeding habit evolved within the Atlantic Forest clade, with a reversion later occurring in *D. leucomystax*, the only species that breeds in ponds and which is associated with lowlands and "restingas" of the Atlantic Forest. Interestingly, *D. leucomystax* also appears to be the closest to *Amazonella* in the morphometric space (Fig. 3). Lentic water bodies are more frequently found in these open environments relative to the steep forests of the mountains adjacent to the coast, where the other species are found. The use of phytotelmata as breeding sites by most *Dendrophryniscus* may have been driven by the abundance of bromeliads and the rarity of lentic-water ponds in the steep Atlantic Rainforest, yet predator or competitor avoidance cannot be rejected as potential underlying mechanisms (Magnusson and Hero, 1991). Evolutionary shifts to bromeliad-breeding occurred recurrently and independently in many lineages of Atlantic Forest frogs (e.g. *Bokermannohyla astartea*, *Crossodactylodes* spp., *Flectonotus fissilis*, *Frostius* spp., *Phyllodytes* spp., *Scinax spp.* gr. *perpusillus*; Haddad and Prado, 2005), supporting the hypothesis that this strategy may be advantageous in the coastal rainforest environment.

Acknowledgments

We are grateful to the many people and institutions that made this study possible. Célio F.B. Haddad, Christian Marty, François Catzeflis and Ross McCulloch authorized tissue loans. Sabrina Baroni, Manoel Antunes Jr. and Maira Concistré (Instituto de Biociências da Universidade de São Paulo) assisting with lab work. Carolina Mello and Hussan Zaher (Museu de Zoologia da Universidade de São Paulo), and Luis Felipe Toledo (Museu de Zoologia "Adão José Cardoso", Universidade de Campinas) granted access to specimens under their care. We also thank Taran Grant as well as an anonymous reviewer and Allan Larson (AE) for their thoughtful suggestions on this manuscript and Germano Woehl Jr. and Pedro Peloso for letting us use their pictures of *D. leucomystax* and *A. bokermanni*. Bird Life and Gabriel Rodrigues dos Santos (Instituto de Estudos Socioambientais do Sul da Bahia, IESB) provided logistical support in the field. Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) granted collection permits and assisted in field. Funding was provided by Conselho Nacional de Desenvolvimento Científico and Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2003/10335-8 and 2007/57067–9), and the National Science Foundation (NSF, DEB-817035, DEB-1035184, DEB-1120487).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.11.023.

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