SALAMANDRA **53**(1) | 99–113 | 15 February 2017 | ISSN 0036–3375

Integrative taxonomy supports the existence of two distinct species within *Hypsiboas crepitans* (Anura: Hylidae)

Victor G. D. Orrico[1], Ivan Nunes[2,9], Camila Mattedi[2], Antoine Fouquet[3], Arthur W. Lemos[2], Maurício Rivera-Correa[4], Mariana L. Lyra[5], Daniel Loebmann[6], Bruno V. S. Pimenta[7], Ulisses Caramaschi[2], Miguel T. Rodrigues[8] & Célio F. B. Haddad[5]

[1)] Universidade Estadual de Santa Cruz, Departamento de Ciências Biológicas, Rodovia Jorge Amado, Km 16. CEP 45662-900 Ilhéus, BA, Brazil

[2)] Universidade Federal do Rio de Janeiro, Museu Nacional, Departamento de Vertebrados. Quinta da Boa Vista, São Cristóvão. CEP 20940-040, Rio de Janeiro, RJ, Brazil

[3)] CNRS-Guyane, USR 3456 LEEISA – Laboratoire Ecologie, Evolution Interactions des Systèmes Amazoniens, Centre de recherche de Montabo 275 route de Montabo, BP 70620. 97334, Cayenne cedex, French Guiana

[4)] Pontifícia Universidade Católica do Rio Grande do Sul, Faculdade de Biociências, Laboratório de Sistemática de Vertebrados. Av. Ipiranga 6681. CEP 90619-900, Porto Alegre, RS, Brazil

[5)] Universidade Estadual Paulista (UNESP), Instituto de Biociências, Departamento de Zoologia and Centro de Aquicultura da UNESP – CAUNESP, campus Rio Claro, Av. 24A, N 1515. CEP 13506-900, Rio Claro, SP, Brazil

[6)] Universidade Federal do Rio Grande, Instituto de Ciências Biológicas, Laboratório de Vertebrados, Av. Itália Km 8, Carreiros. CEP 96.203-900, Rio Grande, RS, Brazil

[7)] Bicho do Mato Meio Ambiente Ltda. / Bicho do Mato Instituto de Pesquisa. Rua Perdigão Malheiros 222. CEP 30380-234, Belo Horizonte, MG, Brazil

[8)] Universidade de São Paulo, Instituto de Biociências, Departamento de Zoologia, Caixa Postal 11.461. CEP 05508-090, São Paulo, SP, Brazil

[9)] Present address: Universidade Estadual Paulista, Campus do Litoral Paulista, Instituto de Biociências, Laboratório de Herpetologia. Praça Infante Dom Henrique, s/n, Parque Bitarú. CEP 11.330-900, São Vicente, SP, Brazil

Corresponding author: Antoine Fouquet, e-mail: fouquet.antoine@gmail.com

Manuscript received: 10 February 2016

Accepted on 13 September 2016 by Jörn Köhler

Abstract. The Neotropical treefrog *Hypsiboas crepitans* (Wied, 1824) has an intriguing disjunct geographic distribution encompassing two large patches: the Atlantic Forest in southeastern South America and from the Guiana Shield to Central America in the north, that are separated by more than 1500 km. This distribution pattern led us to review the available material and re-examine, under an integrative approach, the taxonomic status of these populations. We assessed data using three lines of evidence: morphology, morphometry, and mitochondrial DNA. All of them suggest that the populations from the two geographical ranges are not conspecific. Given that the type material of *H. crepitans* is from the State of Bahia, Brazil, and that specimens from this area cluster with the southeastern group, we resurrect *Hypsiboas xerophyllus* (Duméril & Bibron, 1841) for the populations of the northwestern group. *Hyla levaillantii* Duméril & Bibron, 1841, *Hyla doumercii* Duméril & Bibron, 1841, *Hyla fuentei* Goin & Goin, 1968, and *Hypsiboas indris* Cope, 1867 are synonymized with *H. xerophyllus*.

Key words. Amphibia, Amazonia, Atlantic Forest, *Hypsiboas faber* species group, revalidation, South America, synonymy.

Introduction

The *Hypsiboas faber* species group was proposed by Faivovich et al. (2005) to accommodate a cluster of eight species: *H. albomarginatus* (Spix, 1824), *H. crepitans* (Wied, 1824), *H. exastis* (Caramaschi & Rodrigues, 2003), *H. faber* (Wied, 1821), *H. lundii* (Burmeister, 1856), *H. pardalis* (Spix, 1824), *H. pugnax* (Schmidt, 1857) and *H. rosenbergi* (Boulenger, 1898). With the exception of *H. albomarginatus* (green, middle sized), all species are large, territorial tree frogs with a lichenous colour pattern, and rugose dorsal skin texture (Faivovich et al. 2005, Kluge 1979, Lynch & Suarez-Mayorga 2001).

One of the species of this group, *Hypsiboas crepitans*, exhibits an extensive and intriguing disjunct geographical distribution (Fig. 1). A southeastern group (hereafter SG)

of populations occurs along the Atlantic Forest and adjacent areas, including the State of Bahia in Brazil, from where the species was originally described (Wied 1824). The northwestern group (hereafter NG) of populations is distributed over the Guiana Shield, Caribbean (Tobago), Llanos, Andes, and Middle America (Panama), from sea level up to 2450 m a.s.l. (Duellman 1997, Frost 2014, Kluge 1979, Lehtinen 2014, Lynch & Suarez-Mayorga 2001). There is, thus, a > 1,500 km gap between the SG and the NG. This unusual disjunct distribution has raised doubts about the conspecificity of these groups of populations (Casal & Juncá 2008, Duellman 1997, Kluge 1979, Lehtinen 2014, Lynch & Suarez-Mayorga 2001). Fouquet et al. (2007) found that the pairwise distance among 16S sequences of specimens from Alagoas, Brazil, and French Guiana was high enough to suggest the presence of two distinct species. Lehtinen (2014) retrieved the same result comparing 16S sequences of specimens from Tobago and Brazil and suggested that the population studied by Casal & Juncá (2008) from the State of Bahia "may represent a different, but currently undescribed, species", not realizing that it could be the other way around given that this particular population is close to the type locality of *H. crepitans* (see below).

However, previous works on tadpoles and calls in both the NG and the SG provide only weak evidence supporting the distinction. Tadpoles of the NG present a spiracle opening directed dorso-posteriorly, and antero-dorsal nostrils (see Lynch 2006, figure 28), while tadpoles from the SG present a spiracle directed backwards, and dorsal nostrils (Casal & Juncá 2008, figure 2). However, given the high plasticity of anuran larvae (e.g., Warketin 1999) we remain skeptical that these slight differences will be maintained in larger samples. The basic structure of the calls of both groups is a periodic pulse train divided in one or some notes. The number of notes from the SG (2–5 notes: Casal & Juncá 2008, Martins et al. 2009) overlaps with the range of number of notes of NG from Panama (2–5 notes; Fouquette 1966, Duellman 1970, Kime et al. 2000) and from Colombia (1–2 notes; Bernal et al. 2004) (Table 1). The range of pulses per second from the SG (68–96; Casal & Juncá 2008, Martins et al. 2009) is slightly different from the value of NG recordings from Panamá (≈110; Duellman 1970). The dominant frequency range of SG recordings (0.53–1.3 kHz; Casal & Juncá 2008, Martins et al. 2009) do not differ from the values recovered for the NG, although being individually lower than values found for NG specimens from Panama (0.96–2.55 kHz;

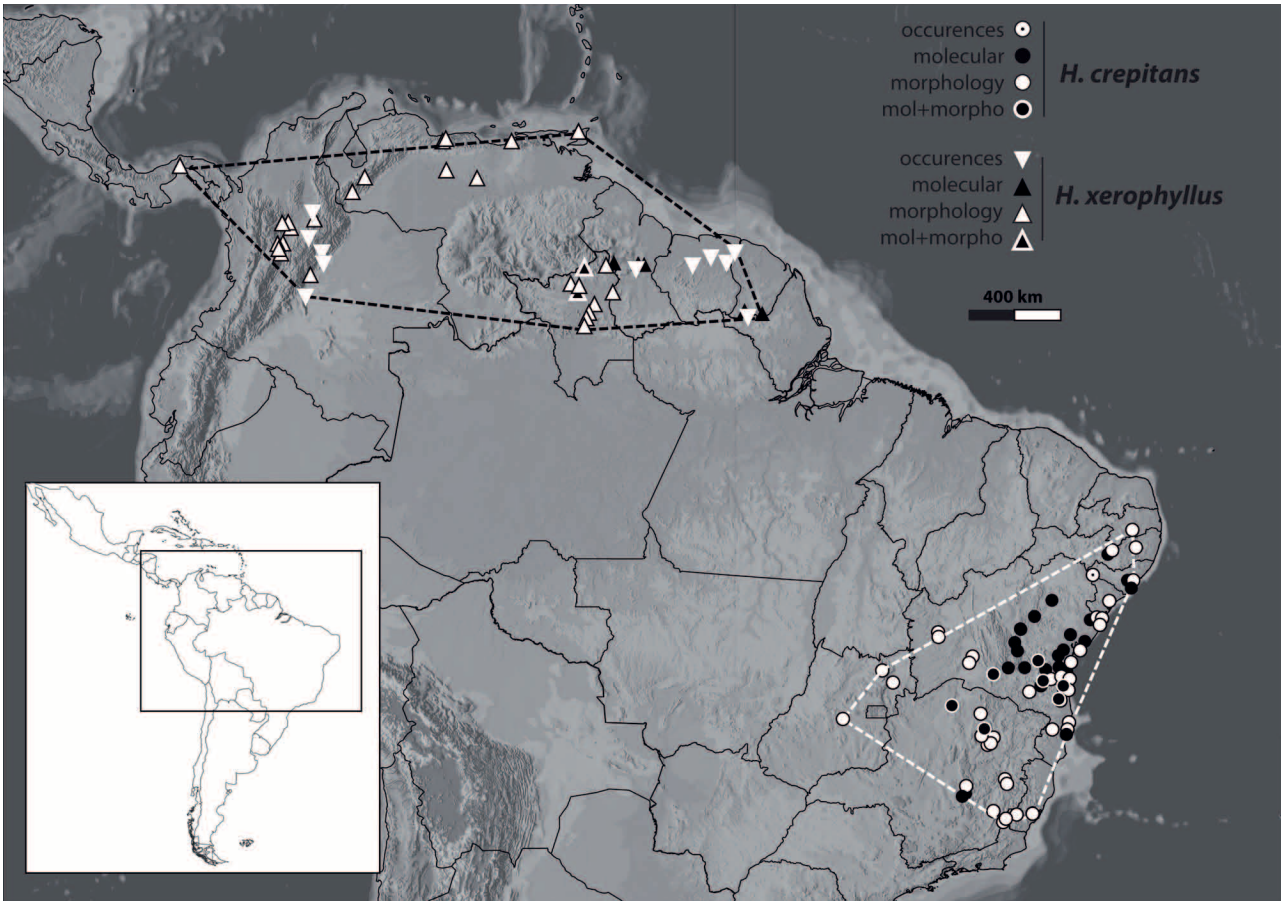

Figure 1. Map showing sampling localities of *Hypsiboas crepitans* (circles) and *H. xerophyllus* (triangles) specimens used in this study.

Table 1. Advertisement call data for *Hypsiboas crepitans* distributional patches. See text for abbreviations.

OTU	Call duration (s)	Intercall interval (s)	Number of notes per call	Number of pulses/ second	Note interval (s)	Number of pulses/note	Pulse duration (s)	Dominant frequency (kHz)	Reference
NG	–	–	2–5	–	–	–	–	2.55	Fouquette (1966)
NG	2.5–5	–	3.3–4.4	110	–	3–5	0.009	0.965–1.288	Duellman (1970)
NG	–	–	2–5	–	–	–	–	2.15	Kime et al. (2000)
NG	0.21–0.31	–	1–2	–	–	–	–	0.35	Bernal et al. (2004)
NG Summary	**0.21–5**	**?**	**1–5**	**110**	**?**	**3–5**	**0.009**	**0.965–2.55**	–
SG	0.51	0.87	1–5	72–96	0.04	3–33	0.010–0.014	0.8	Casal & Juncá (2008)
SG	0.46	0.52	2	68–77	0.049	4–27	0.014–0.013	0.53–1.30	Martins et al. (2009)
SG Summary	**0.46–0.51**	**0.52–0.87**	**1–5**	**68–96**	**0.04–0.049**	**3–27**	**0.01–0.014**	**0.53–1.30**	–

Fouquette 1966, Duellman 1970, Kime et al. 2000), and higher than those found for Colombia (0.35 kHz; Bernal et al. 2004). Therefore, differences between the advertisement calls of specimens from the distinct groups are meager. However, Casal & Juncá (2008) used advertisement call data (dominant frequency mainly) to corroborate the hypothesis that more than one species is hidden under the name *Hypsiboas crepitans*. Therefore, although there is a suspicion that the two groups may represent distinct species, published data on advertisement calls and tadpoles are inconclusive in this respect.

The two groups of populations are separated by the Caatinga, Cerrado and Chaco biomes which are part of the "Dry Diagonal" of the Neotropics (Schmidt & Inger 1951, Werneck 2011), a group of "dry environments" presently separating Atlantic and Amazonian Forests and to which some cladogenic events have been attributed (Costa 2003). Successive fluctuations over time of these contrasting habitats help to explain relationships and faunal exchanges between Amazonian and Atlantic Forests (see discussions in Fouquet et al. 2012, 2014). Most of the documented dispersals of terrestrial anurans between these two biomes are rather ancient (Fouquet et al. 2012) and only a few examples of Atlantic Forest species like *Rhinella hoogmoedi* and *Hypsiboas semilineatus* are nested with relatively low genetic distances (1–3% on 16S) within otherwise Amazonian species (dos Santos et al. 2015, Fouquet et al. 2016). However, no species of anuran restricted to forest habitats is known to be distributed in both biomes while it is the case in only a few open habitat species like *Adenomera hylaedactyla* (Fouquet et al. 2014).

Nevertheless, although *Hypsiboas crepitans* is predominantly associated with forest and mesic habitats, many populations are found in dry or xeric environments. Lescure & Marty (2000) and Ouboter & Jairam (2012) reported that populations from French Guiana and Suriname, respectively, live in open environments such as in savannas and inselbergs surrounded by forest. In fact, the type locality of *H. crepitans* (Tamboril, Municipality of Condeúbas, State of Bahia, Brazil, see Bokermann 1966) is situated in a transitional area between the rocky meadows of Serra do Espinhaço and the Caatinga where both open and forest vegetation are present. Therefore, the influence of the dry diagonal as a barrier for this species is not straightforward. Given these rather wide habitat requirements, a dispersal between Amazonia and the Atlantic Forest, recent enough to support a conspecific status of the two groups, cannot be excluded.

The goals of the present contribution are to review specimens assigned to *Hypsiboas crepitans* from both distribution areas and perform analyses using an integrative approach combining molecular (mtDNA), morphology and morphometry in order to evaluate the specific status of these populations.

If the existence of more than one species is supported, a number of synonyms are available for the NG and should therefore be examined. Additionally, Ouboter & Jairam (2012) also reported *H. fuentei* (Goin & Goin 1968), a species known only from three localities in northeast-central Suriname (Frost 2016), that are nested within the range of the NG. They also stated that they would "not be surprised, if *H. fuentei* proves to be a junior synonym of *H. crepitans*". However, Hoogmoed (1979) suggested that because of the green colours of live specimens, *H. fuentei* seemed somehow related to *H. punctatus* or *H. cinerascens* – both today assigned to the *H. punctatus* species group. Neither Hoogmoed (1979) nor Ouboter & Jairam (2012) compared their specimens with the holotype of *H. fuentei*, solely with the description of Goin & Goin (1968). Although Faivovich et al. (2005) did not assign *H. fuentei* to any of their species groups, they state "the angulate dentigerous process of the vomer suggests that this species could be associated with certain Gladiator Frogs... [that] have this character state. A study of the holotype … should clarify the matter". Given the confusion surrounding *H. fuentei* and the possible relation with *H. crepitans*, we include herein our own observations of the *H. fuentei* holotype and evaluate its taxonomic status.

Materials and methods

General procedures

We examined specimens in zoological collections trying to assemble material from as many populations reported as *H. crepitans* as possible. Institutional abbreviations can be found in Sabaj Pérez (2010), with the addition of AL-MN (Adolpho Lutz Collection, housed at Museu Nacional, Rio de Janeiro, Brazil) and MTR (Miguel T. Rodrigues field numbers). Institutional abbreviations apply to both morphological and molecular (tissue) material. A number of specimens, published sequences, and tissues available were selected and gathered (see below). This material was either considered as NG or SG, allowing us to test the above-cited hypothesis that the two distributional groups of populations would represent two taxa with allopatric distribution (Casal & Juncá 2008, Duellman 1997, Kluge 1979, Lynch & Suarez-Mayorga 2001). Specimens from NG are from Roraima state Brazil, Colombia, French Guiana and Venezuela, while specimens from SG are from several Brazilian localities (Supplementary document 1). Additional species for comparisons were chosen based on their availability according to the present taxonomy i.e., all the other species of the *H. faber* group, as defined by Faivovich et al. (2005). To discuss the taxonomic status of *H. fuentei*, we examined photos of the holotype and types of relevant material (*H. crepitans* lectotype and photos of the holotypes of taxa currently considered synonyms).

Qualitative phenotypic data

We conducted the comparisons of adult specimens based on observations of collection material (Supplementary document 1) and on literature information (see below). Terminology of external morphology follows Duellman (1970). Standards for dorsal outline and profile of the snout follow Heyer et al. (1990). Fingers nomenclature follows Fabrezi & Alberch (1996). Webbing formula notation follows Savage & Heyer (1967), as modified by Myers & Duellman (1982). Types of vocal sac follow Liu (1935). Sex was determined by the presence of vocal sac, vocal slits, and developed projecting prepollex in adult males. Coloration always refers to preserved specimens, except when stated.

Quantitative phenotypic data

Sixteen morphometric variables were used and are given in millimeters throughout the text. Nine measurements follow Duellman (1970): SVL (snout–vent length), HL (head length), HW (head width), ED (eye diameter), UEW (upper eyelid width), IOD (interorbital distance), IND (internarial distance), TD (tympanum diameter), and TL (tibia length). One measurement follows Heyer et al. (1990): THL (thigh length). Five measurements follow Napoli (2005): END (eye–nostril distance), NSD (nostril-tip of snout distance), FL (foot length, including tarsus), 4FD (fourth finger disk diameter; using nomenclature of Fabrezi & Alberch (1996)), and 4TD (fourth toe disk diameter). We included an additional measurement: FHL (forearm+hand length: straight line distance from elbow to the tip of the third finger). SVL, HL, HW, FHL, THL, TL, and FL were measured with a digital caliper to the nearest 0.05 mm. All other measurements were taken with an ocular micrometer on a Zeiss stereomicroscope.

According to Bernal & Clavijo (2009), specimens preserved at different times can produce an artificial segregation among each time class when measured for morphometric analyses. The reason is the gradual modification of specimens along the years in preservative (see a brief historic account in Deichmann et al. 2009). Given that the measured specimens were collected in several different locations and times (see Supplementary document 1), the large amount of analyzed specimens and, the normal distribution of each measurement, we expect to minimize the possible problems regarding artefacts of preservation.

A total of 276 adult specimens were measured (NG: n = 98 males and 13 females; SG: n = 95 males and 70 females). Prior to analysis, all morphometric measures were log-transformed to conform to requirements of normality and homocedasticity (Zar 2009).

Principal Component Analysis (PCA) was used to explore morphometric differences between groups. Eigenvectors and associated eigenvalues were obtained from a variance-covariance matrix. Scores of individuals were then projected in the reduced space of the main components of larger contributions (Humphries et al. 1981). The first component (hereafter PC1) captures the largest possible variation of the original data; the second component (hereafter PC2) is orthogonal to the first (independent) and provides the remaining of maximum variation (Peres-Neto & Bizerril 1994).

Molecular data procedures

Our survey of GenBank sequences (performed on the 27th of July 2012) showed that, with small additions from our own dataset, it was possible to assemble a nearly complete matrix using sequences of the 12S mitochondrial gene (hereafter 12S; 907 bp) and of the mitochondrial Cytochrome c oxidase subunit 1 (hereafter COI; 671 bp) for all species in the *Hypsiboas faber* group and for some closely related species according to Faivovich et al. (2005) to serve as outgroups. This includes both geographical groups of *H. crepitans* (see Supplementary document 2), however we were not able to gather sequences of both targeted gene fragments for all terminals. In order to reduce missing entries for outgroups, we used five chimerical sequences (see results). Chimerical sequences are solely of distinct individuals assigned to a same species, and no chimerical sequence was produced for ingroup terminals (Supplementary document 2).

To amplify the 12S mtDNA, we used primers MVZ59 (5'–ATAGCACTGAAAAYGCTDAGATG–3'; Graybeal

1997) and 12S F-H (5'–CTTGGCTCGTAGTTCCCTGGCG–3'; Goebel et al. 1999) following the procedures of Faivovich et al. (2005). COI was amplified using primers dgHCO-2198 (5'-TAAACTTCAGGGTGACCAAARAAYCA-3') and dgLCO-1490 (5'-GGTCAACAAATCATAAAGAYATYGG-3') described in Meyer (2003) following his procedures. Fragments were sequenced in both directions and sequencing was performed by Macrogen Inc. (Seoul, South Korea). Data from complementary strands were compared to generate a consensus sequence for each DNA fragment using Sequencher 4.1 (Gene Code Corp, Ann Arbor, USA).

Alignments were conducted in the online version of MAFFT v6 (Katoh et al. 2002), aligning each fragment separately; both genes were aligned using MAFFT alignment strategy L-INS-i. The final matrix comprises 68 terminals. The missing data are two 12S sequences of *H. faber* and 15 COI sequences of various representatives (two *H. crepitans*) of the *H. faber* species group. We collated 46 newly generated sequences of 12S and 45 of COI with 20 for 12S and eight for COI from GenBank. The final matrix can be found at BOLDSYSTEMS.

Genetic (uncorrected pairwise) distances were calculated with MEGA 5.0 (Tamura et al. 2011) considering transitions and transversions. Pairwise distances were computed for a total of 907 bp for 12S and 671 bp for COI. As a first approximation, we considered genetic distances high (i.e., possibly not conspecific) when ≥ 3% for the 12S and ≥ 10% for COI. A threshold of 3% for 12S is common between distinct amphibian species for which genetic distances were studied (e.g., Castroviejo-Fisher et al. 2012, Köhler et al. 2008, Ron et al. 2012). On the other hand, divergence values for COI have been studied for diverse animal groups and although thresholds vary among clades (e.g., Hebert et al. 2003), a minimum value of 10% is commonly found between amphibian species (e.g., Vences et al. 2005).

We used JModelTest 2.1.7 (Guindon & Gascuel 2003, Posada 2008) to select the best nucleotide substitution models according to the Akaike Information Criterion (AIC) for each of the four partitions we considered (12S and each codon position for COI). We used default settings (11 substitution schemes, ML optimization, NNI search). Selected models were GTR+I+G for 12S and TrNef+I, F81, and TrN+G for the first, second and third positions of the COI respectively.

The resulting models were employed in a Bayesian analysis (BA) with MrBayes 3.2 (Ronquist et al. 2012). Instead of TrNef+I and TrN+G we used the closest models (GTR+I and GTR+G respectively) for BA. Gaps were treated as nucleotides of unknown origin in Bayesian inference analyses due to program constrains. The BA consisted of a 20×10^6 generations run and four Markov chains (one cold) sampled every 1,000 generations. A conservative burn-in (25%) was determined by examining stationarity of the likelihood scores (all parameters ESS were > 1,000) and convergence between the two runs using the deviation of split frequencies and mixing between chains. We considered relationships to be strongly supported when posterior probabilities were equal to or higher than 0.95. Outgroup (root) was set as *H. cinerascens* + *H. punctatus* according to Faivovich et al. (2005).

Results

Morphology

Specimens from the two groups can be distinguished based on adults colour patterns and general morphology. However, the two groups share the following characteristics: head in lateral view truncated, rounded, or acuminate; head in dorsal view rounded, truncated, or sub-elliptical; feet webbing formulae I2[1] – 2[1]II1 – 2III1 – 2IV2 – 1V. The usual coloration of the body encompasses several patterns of marbled background with or without small spots (melanophores) mostly resembling a bark-like pattern. The pattern on the posterior surface of thighs is different between both groups; specimens without blotches are found only in the NG, although some specimens may present some dark lines (Fig. 2C; Duellman 1970). Furthermore, the presence of a middorsal longitudinal stripe and the colour of the gular region differ between groups (see the comparisons section).

Morphometric analyses

PCA results are congruent with the hypothesis that the NG and the SG represent different species. Regarding the males (n = 193), PCA shows two major axes (PC1 57.3% of the total variation and PC2 22.4%). The standardized coefficients and factor loadings of the Principal Component axes are presented in Supplementary document 3. The two axes show that the NG is completely separated from the SG, and the separation of these two groups is mostly due to both PC1 and PC2 (Fig. 3). When we look at the axes individually, PC1 is mostly influenced by FL, HW, THL, SVL, and IOD, in this order, and PC2 is mostly influenced by NSD, TD, END, 4FD, and 4TD, in this order (Supplementary document 3).

Regarding the females (n = 83), PCA also display two major axes (PC1 accounted for 63.0% of the total variation and PC2 for 18.8%). The standardized coefficients and factor loadings of the Principal Component axes are presented in Supplementary document 3. The two axes show that the NG is completely separated from the SG, and the separation of these two is mostly due to PC2 (Fig. 3). When we look to the axes individually, PC1 is mostly influenced by SVL, HW, HL, 4FD, and THL, in this order, and PC2 is mostly influenced by IOD, NSD, FL, 4FD, and UEW, in this order (Supplementary document 3).

Molecular analyses

The two groups of *H. crepitans* are reciprocally monophyletic and retrieved as strongly supported sister clades (Fig. 4). Genetic distances between them are high: 4% in

12S and 13% in COI (Table 2). A phylogeographical structure is also apparent within each of these two clades. The NG clade comprises two main lineages segregating eastern populations in French Guiana and Guyana (east of Essequibo river) from western populations in Guyana (west of Essequibo river) and Roraima State (Fig. 4). However, genetic distance between them is low as well as support for the western lineage (< 0.5). The SG also comprises two main lineages segregating the northern part of the Atlantic Forest (AL, PE, BA) from the central part of the Atlantic Forest (BA, MG). Similarly, genetic distance between them is low (Table 2) as well as support for the central Atlantic Forest lineage (< 0.77).

Most of the nodes of the tree are strongly supported. However, the *Hypsiboas faber* species group was not retrieved as monophyletic with the *H. albopunctatus* species group (*Hypsiboas lanciformis* and *H. multifasciatus*) being nested within it with uncertain relationship (Fig. 4). The basal divergence within the clade *H. faber* group + *H. albopunctatus* group separates *H. albomarginatus* from the rest. However, this relationship is also poorly supported. In fact, four major groups can be recognized in this clade, the *H. albopunctatus* group (widespread in South America) and three strongly supported clades formed by the species of the *Hypsiboas faber* group. The first holds the two samples of *H. albomarginatus* (Atlantic Forest), the second samples of *H. exastis*, *H. faber*, *H. lundii*, and *H. pardalis* (hereafter the *H. faber* clade – Atlantic Forest, Cerrado), and the third *H. crepitans*, *H. pugnax*, and *H. rosenbergi* (hereafter the *H. crepitans* clade – from the Guiana Shield to Central America and the Atlantic Forest).

Observations on the holotype of *Hyla fuentei*

The holotype of *Hyla fuentei* (CM 44218; Fig. 5D) is a female in good condition of preservation. The abdomen has a sagittal opening, possibly made for sex determination. The specimen presents two dorsal cuts in the skin: one over the frontoparietal fontanel and one from the right shoulder blade to the left hip. Colours have faded and no clear pattern can be seen.

Some specimens of the NG agree with the holotype of *H. fuentei*. Many specimens of the NG are dark green in

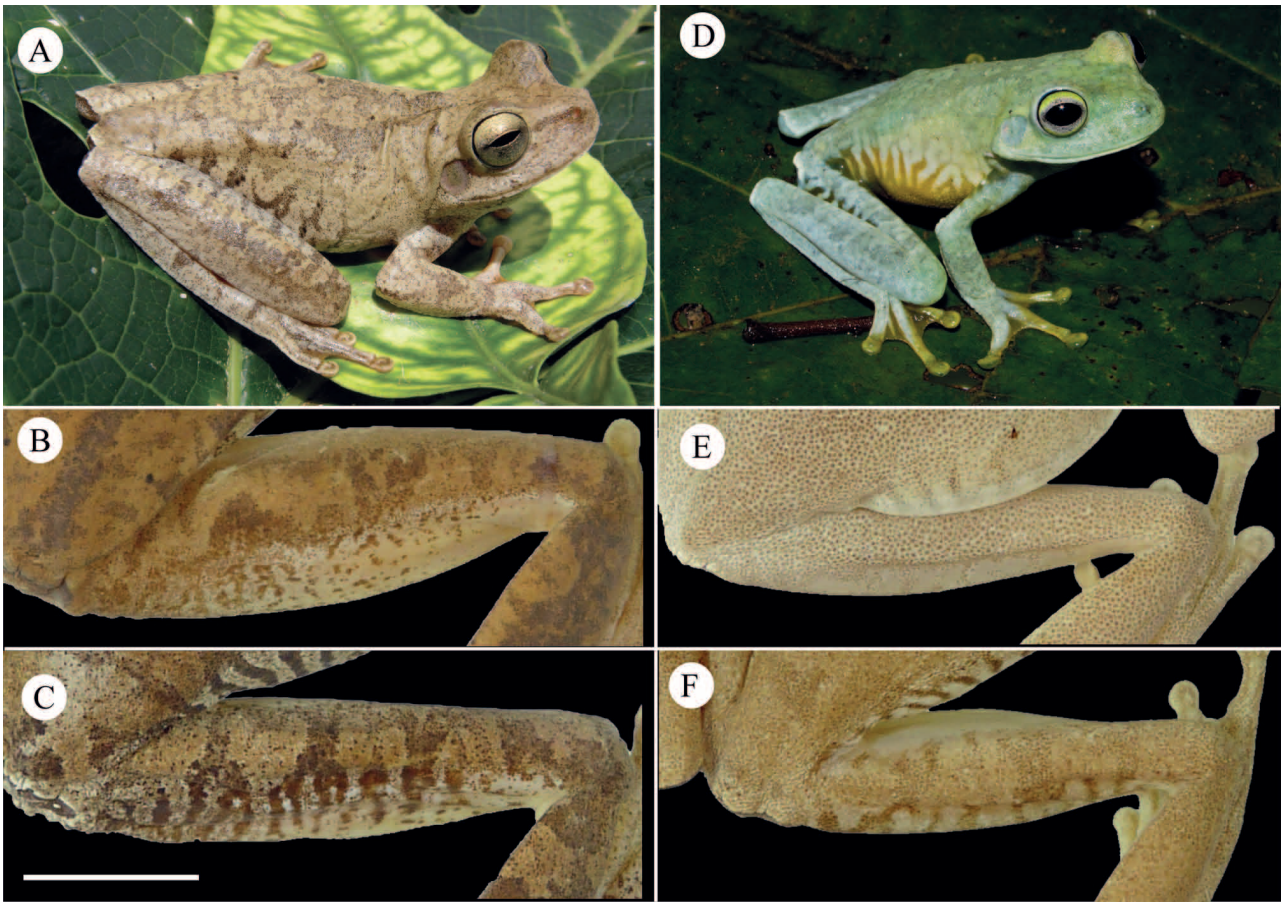

Figure 2. (A) Living specimen of *Hypsiboas crepitans* in diurnal coloration (photo by M. Solé) from the UESC campus in Ilhéus, Bahia, Brazil (specimen not collected). (B, C) Thigh patterns of two specimens of *Hypsiboas crepitans* (B = MNRJ32440 and C = MNRJ64374). (D) Living specimen of *Hypsiboas xerophyllus* in diurnal coloration (photo by A. FOUQUET) from Pacaraima, Roraima, Brazil. (E, F) Thigh patterns of two specimens of *H. xerophyllus* (E = MZUSP65854 and F = MZUSP68669). Scale bar = 1 cm.

Table 2. Mean intra (first column) and inter-specific uncorrected pairwise distances among species of the *Hypsiboas faber* group. Above the diagonal 12S (907 bp); below, COI (671 bp).

		[COI/12S]	1	2	3	4	5	6	7	8	9
1	*H. crepitans*	[0.01/0]	–	0.04	0.07	0.06	0.07	0.09	0.07	0.08	0.09
2	*H. xerophyllus*	[0.01/0]	0.13	–	0.06	0.05	0.07	0.08	0.07	0.07	0.08
3	*H. rosenbergi*	[–/0.03]	–	–	–	0.07	0.08	0.09	0.09	0.09	0.1
4	*H. pugnax*	[–/0]	–	–	–	–	0.07	0.07	0.07	0.07	0.07
5	*H. faber*	[0.06/0.02]	0.18	0.17	–	–	–	0.06	0.05	0.05	0.08
6	*H. exastis*	[–/–]	0.17	0.2	–	–	0.16	–	0.03	0.05	0.08
7	*H. pardalis*	[0/0]	0.19	0.2	–	–	0.15	0.11	–	0.04	0.08
8	*H. lundii*	[0.01/0]	0.18	0.18	–	–	0.16	0.15	0.15	–	0.08
9	*H. albomarginatus*	[0.11/0.02]	0.17	0.17	–	–	0.18	0.17	0.18	0.18	–

life also, but specimens from a given population can exhibit the dark green coloration while other present the pale coloration. Moreover, many specimens of the NG present small melanophores on the dorsal surface, like the holotype of *H. fuentei*. The nostril shape, the reduced webbing, and the absence of dermal ridges of the holotype conform to what is observed in specimens of the NG.

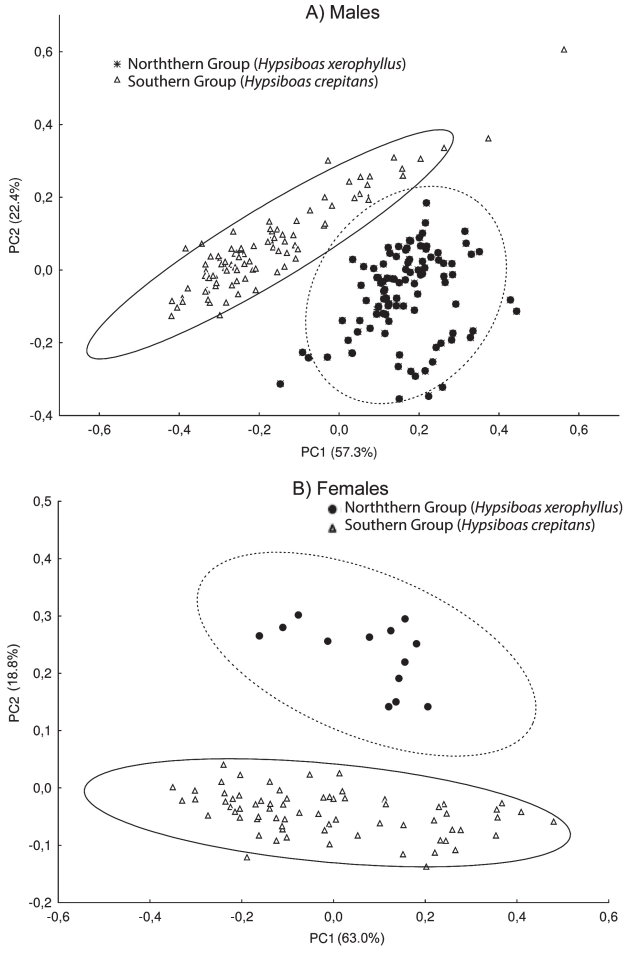

Figure 3. Plots of individual scores resulted from Principal Component Analysis (PCA) of morphometric data from two groups of adult males (A) and adult females (B) of *Hypsiboas crepitans* (SG) and *H. xerophyllus* (NG) in the space of the first with the second canonical axes. Confidence ellipses (95%) for the scores of each group are shown.

Discussion

Taxonomic conclusions

Although previous authors have used larval morphology and advertisement call data (Casal & Juncá 2008) to suggest that populations of *Hypsiboas crepitans* from the two geographical groups could represent two distinct species, published data on this subject remained ambiguous (see Introduction). Numerical parameters of advertisement call overlap (or nearly so, see Table 1), and call descriptions, especially for the populations of the NG, are too succinct and do not address intraspecific variation and other factors known to influence amphibian calls (e.g., the social context; see Wells & Greer 1981). Larval morphology of *Hypsiboas* species presents high levels of intraspecific variation and many closely related species have nearly indistinguishable larvae (see Kolenc et al. 2008, Orrico et al. 2007). However, since important character states are sometimes overlooked in tadpole descriptions (see Kolenc et al. 2008), we cannot exclude that the larvae of the two distributional groups are actually distinct. Additional studies are needed for a comprehensive comparison of larval morphology and bioacoustics of the two groups.

However, the molecular and morphological data presented herein are concordant with the recognition of the populations from the two distributional groups as distinct species. The SG is restricted to eastern Brazil, from the State of Paraíba to the northern State of Rio de Janeiro, including the State of Minas Gerais. The type locality of *H. crepitans* was originally designated as "Tamburil, Jiboya, Arrayal da Conquista", State of Bahia, Brazil (Frost 2014). Bokermann (1966) restricted it to "Tamburil, [Municipality of] Condeúbas, Bahia, Brazil". Based on the aforementioned characteristics, type series data, and type locality, the SG is considered to represent *H. crepitans* (Fig. 6).

The NG occurrence area comprises a region where five taxon names are available. Three names were proposed

by Duméril & Bibron (1841): *Hyla doumercii* Duméril & Bibron, 1841, from Suriname (holotype MNHN 766 [Fig. 5A], SVL 47.2 mm, adult male, vocal slits present, supplementary bone on Finger II =prepollical spine; A. Ohler, pers. comm.); *Hyla levaillantii* Duméril & Bibron, 1841, from Suriname (holotype MNHN 764 [Fig. 5B], SVL 48.1 mm, adult male, vocal slits present, prepollical spine on Finger II); *Hyla xerophylla* Duméril & Bibron, 1841, from "Cayenne", French Guiana (holotype MNHN 752 [Fig. 5C], SVL 44.9 mm, probably an adult female, no male sexual secondary character, flat tubercle instead of prepollical spine). Article 24 of the International Code of Zoological Nomenclature (ICZN 1999) argues about the "Precedence between simultaneously published names, spellings or acts". Following the rules of the Code, we cannot apply the Section 24.2 about the "Determination by First Reviser" because this was already done (see Duellman 1970, Kluge 1979).

Although we cannot establish precedence, Article 24 of the Code still allows us to choose which of the names of Duméril & Bibron (1841) we will revalidate if applicable to a natural population. Thus, any of the three names described by Duméril & Bibron (1841) are available and can be applied to the NG. Herein, we decided to consider the

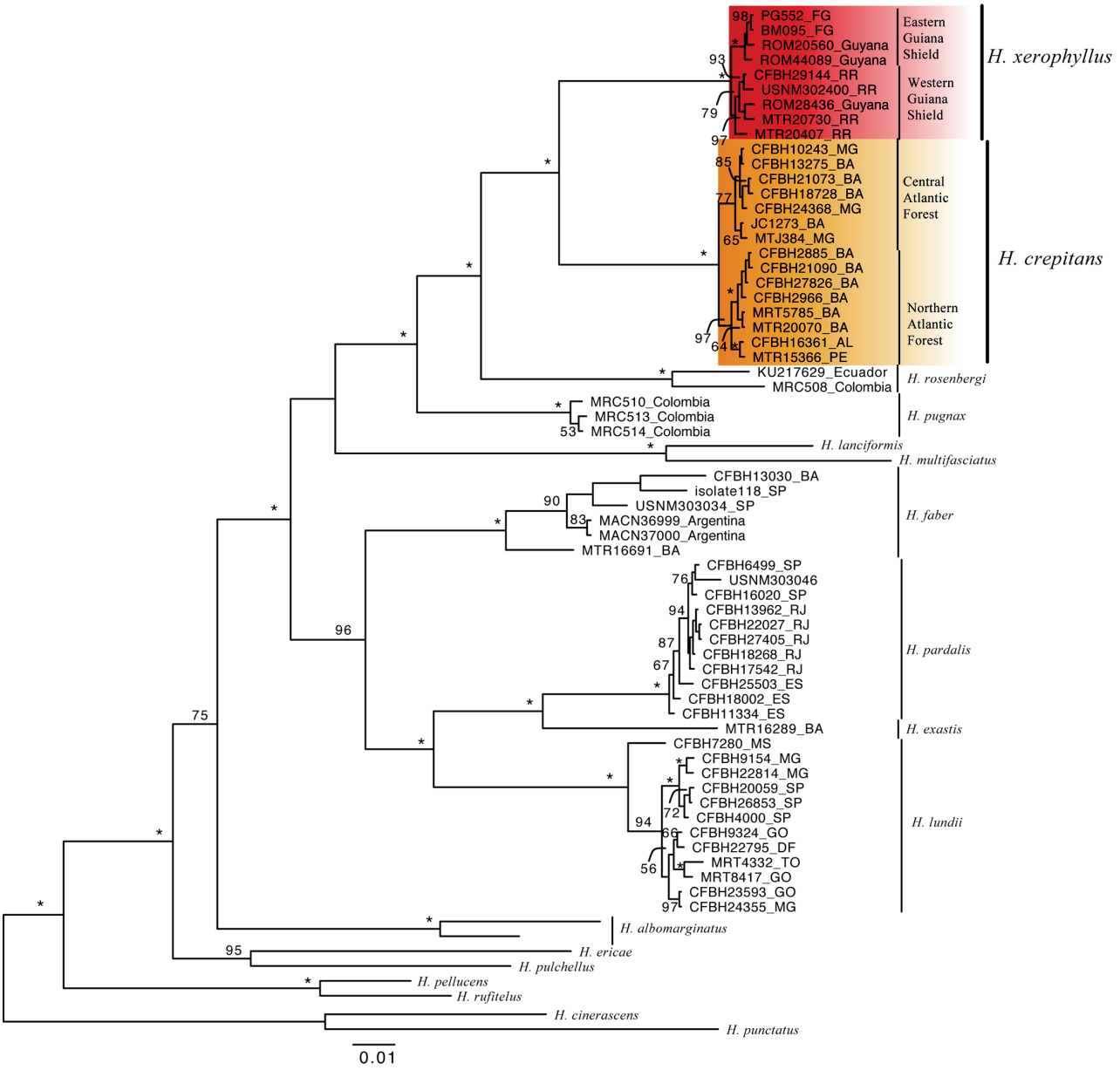

Figure 4. Phylogram (50% majority rule consensus with frequencies of all observed bipartitions) hypothesized from Bayesian analysis using 1578 bp of 12S and COI. Numbers above nodes are posterior probabilities (* indicates pp ≥ 0.99; pp < 0.5 are not indicated). Samples are labeled according to collection numbers followed by origin (country in full or Brazilian state official acronym if from Brazil).

Figure 5. Dorsal and ventral views of the holotypes of (A) *Hyla doumercii* [SVL = 47.2 mm, male], (B) *H. levaillantii* [SVL = 48.1 mm, male], (C) *H. xerophyllus* [SVL = 44.9 mm, likely a female], and (D) *H. fuentei* [SVL = 57.0 mm, female]. Figures are not to scale.

NG as *Hypsiboas xerophyllus*, resurrecting the name *Hyla xerophylla* Duméril & Bibron, 1841 from the synonymy of *Hypsiboas crepitans* (Wied, 1824) and transferring the species to the genus *Hypsiboas* based on phylogenetic relationships.

The two remaining names, *Hypsiboas indris* Cope, 1867 and *Hyla fuentei* Goin & Goin, 1968, are more recent and based on material from Suriname. *Hyla fuentei* holotype from "Suriname, Suriname District Powakka" (CM 44218 [Fig. 5D], SVL 57.0 mm, adult female; Goin & Goin 1968) presents a colour pattern in accordance with what was stated by Goin & Goin (1968). The sagittal opening at the abdomen seems to be made for information on ovaries and oocytes (see Goin & Goin 1968). In fact, all our observations about the holotype agree well with Goin & Goin (1968). In addition, The holotype agreed with the NG populations and they exhibit a dark green coloration, while other present the pale coloration, a phenomenon probably not uncommon in *Hypsiboas*, as also revealed in Andean species of the *H. pulchellus* species group (Köhler et al. 2010). After the examination of the holotype of *Hyla fuentei* we considered this species as belonging to the NG and, thus, to be a junior synonym of the name applicable to this group (*H. xerophyllus*). We have not examined the holotype of *Hypsiboas indris* Cope, 1867. However, the distribution of this taxa completely overlaps with the more inclusive distribution of the NG. In the light of the present results – where the genetic diversity within each group is low (Table 3) – it seems very unlikely that it represents a different species from *H. crepitans*. Therefore, we provisionally transfer it from the synonymy of *Hypsiboas crepitans* to the synonymy of *Hypsiboas xerophyllus*.

Species account

Hypsiboas xerophyllus (Duméril & Bibron, 1841).
New combination, revalidation

Hyla levaillantii Duméril & Bibron, 1841 – Erp. Gen. 8:550. New synonymy.

Hyla doumercii Duméril & Bibron, 1841 – Erp. Gen. 8:551. New synonymy.

Hyla fuentei Goin & Goin, 1968 – Copeia 1968:581. New synonymy.

Hypsiboas indris Cope 1867 – J. Acad. Nat. Sci. Philadelphia, Ser. 2, 6: 201. New synonymy.

Holotype: MNHN 652, SVL 44.9 mm, probably female (no male secondary characters), "Cayenne" [= French Guiana] (Fig. 5C).

Diagnosis: *Hypsiboas xerophyllus* is a member of the *H. faber* species group (*sensu* Faivovich et al. 2005), characterized by: SVL 42.9–63.8 mm in adult males, 40.9–71.8 mm in adult females; in dorsal view, head nearly rounded with rounded, truncated, or sub-elliptical snout; single, well developed projecting prepollex in adult males; tympanum

Table 3. Mean pairwise distances within species of the *Hypsiboas faber* group and within other groups (gr.) of *Hypsiboas*. 12S sequences have 907 bp; COI, 671 bp.

	12S	COI
H. albomarginatus	0.02	0.11
H. albopunctatus gr.	0.04	0.13
H. crepitans	0.00	0.01
H. exastis	–	–
H. faber	0.02	0.06
H. lundii	0.00	0.01
H. pardalis	0.00	0.00
H. pellucens gr.	0.01	0.11
H. pugnax	0.00	–
H. pulchellus gr.	0.08	–
H. punctatus gr.	0.09	–
H. rosenbergi	0.03	–
H. xerophyllus	0.00	0.01

and tympanic annulus visible externally and in contact with the posterior margin of eye; well-developed supratympanic fold covering the upper edge of the tympanum; lower edge of the tympanum close to the mouth (at jaw articulation level); males with vocal slits under the tongue and parallel to the lower lip, vocal sac median, subgular (sensu Liu 1935); presence of two groups of vomerine teeth between choanae; dermal ridges (fimbriae) absent or rudimentary along arms and feet; skin smooth dorsally and granular ventrally; colour of gular region similar to belly colour in preserved males and females (cream); cloacal region composed by a subcloacal fold, white tubercles around the cloacal opening, and a well-developed flap (horizontal, above vent); flanks usually show parallel transverse thin bars; well-developed tubercles on the ventral surface of thighs; dorsal surface of thighs usually with parallel bars, if present, spaced and wider dorsally, thinner and lighter posteriorly; colour on dorsum ranging from pale gray to brown in preservative, with a X-shaped mark sometimes over the suprascapula.

Comparisons with other species: *Hypsiboas xerophyllus* is easily differentiated from *H. crepitans*, its sister taxon and morphologically most similar species, by the absence of a mid-dorsal longitudinal dark brown stripe (present, of different widths, sometimes incomplete – i.e., not connecting snout and vent – in *H. crepitans*); immaculate cream gular region in both sexes (brown in males of *H. crepitans*); absence of, or barely visible, bifurcated vertical dark brown bars on ventroposterior surfaces of thighs (present in *H. crepitans*; Fig. 2).

From other species of the *H. faber* group, *H. xerophyllus* differs by having a smooth texture of skin on dorsal surfaces, and low development of ulnar and outer tarsal dermal ridges (skin texture lumpy, and well developed dermal ridges in *H. pardalis*, *H. exastis*, *H. rosenbergi*, and *H. lundii*). The absence of well-developed calcars distinguishes *H. xerophyllus* from *H. pardalis*, and *H. exastis*. The absence of extensive hand webbing distinguishes *H. xerophyllus* from *H. pardalis*, *H. exastis*, and *H. lundii*. The supratympanic fold of *H. albomarginatus* is white (or whitish green in live specimens) while in *H. xerophyllus* it presents the same lichenous colour as the dorsum. The gular region is immaculate cream in *H. xerophyllus* while it has brown flecks in *H. pugnax*. In life, the iris coloration is whitish around the pupil and yellow-green on he outer edge in *H. xerophyllus* while it is golden yellow in *H. pugnax*.

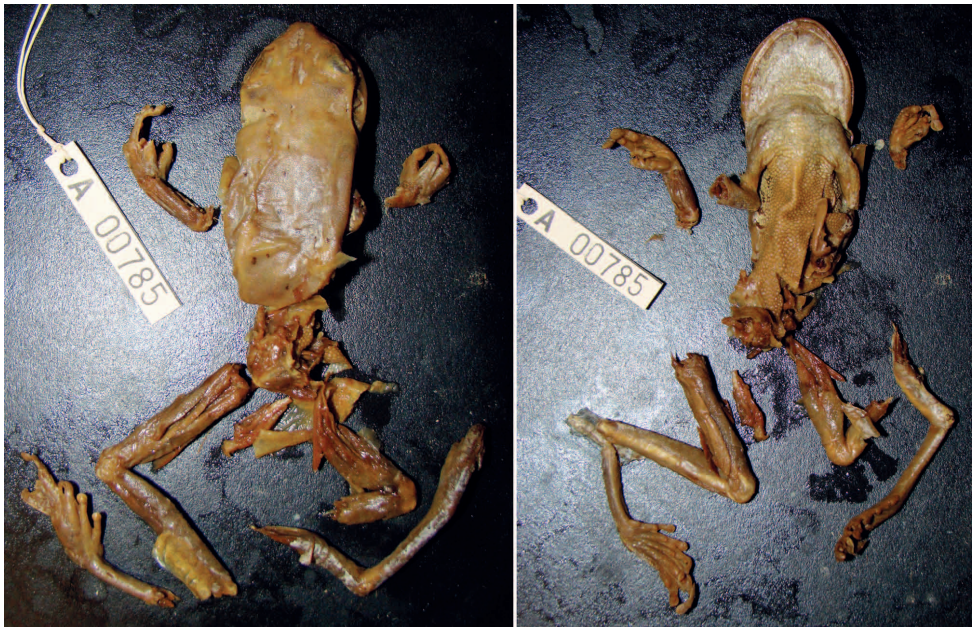

Figure 6. Lectotype of *Hypsiboas crepitans*, AMNH 785, adult female, SVL ca. 69 mm, from Vitória da Conquista, State of Bahia, Brazil.

Table 4. Descriptive statistics (in millimeters) for measurements of *Hypsiboas crepitans* and *H. xerophyllus*. The results are presented as mean ± standard deviation (range).

Measures	*Hypsiboas crepitans*		*H. xerophyllus*	
	Males (n=95)	Females (n=70)	Males (n=98)	Females (n=13)
SVL	58.1±7.6 (33.2–71.8)	55.4±4.59 (51.0–64.7)	50.6±3.86 (42.9–63.8)	57.7±7.91 (40.9–71.8)
HL	19.2±2.2 (11.4–22.8)	18.4±2.23 (14.7–21.6)	16.6±1.5 (13.7–19.9)	19.1±2.21 (13.8–22.8)
HW	20.3±2.5 (12.0–24.1)	18.9±1.32 (17.1–21.3)	17.7±1.25 (15.4–21.7)	20.0±2.51 (14.5–24.1)
ED	6.1±0.7 (3.2–7.3)	2.2±0.15 (1.9–2.5)	5.4±0.54 (4.1–6.9)	3.7±0.43 (2.9–4.7)
END	5.6±0.7 (3.5–6.8)	3.6±0.39 (2.8–4.3)	5.5±0.72 (4.3–9.8)	1.9±0.28 (1.3–2.6)
TD	4.4±0.6 (2.1–5.5)	4.6±0.47 (4.0–5.5)	4.4±0.39 (3.4–5.3)	4.4±0.69 (2.7–6.3)
UEW	4.6±0.7 (2.7–6.3)	5.6±0.47 (4.7–6.5)	4.4±0.63 (2.2–6.0)	6.0±0.67 (4.2–7.3)
IOD	10.8±1.2 (7.0–12.8)	5.7±0.50 (5.0–6.6)	5.4±0.63 (4.0–7.0)	10.7±1.21 (7.7–12.8)
IND	3.8±0.5 (1.7–4.8)	5.6±0.44 (5.0–6.1)	3.3±0.66 (1.8–4.6)	5.5±0.69 (4.2–6.8)
NSD	1.9±0.3 (1.1–2.6)	4.5±0.50 (3.7–5.3)	2.5±0.63 (1.6–3.9)	4.4±0.63 (2.1–5.5)
FAL	27.7±3.6 (15.4–34.4)	25.7±3.46 (16.8–30.3)	23.9±2.1 (12.0–29.6)	27.4±3.55 (19.2–34.4)
4FD	2.6±0.4 (1.5–3.4)	2.7±0.38 (2.1–3.2)	2.4±0.25 (1.8–3.3)	2.6±0.38 (1.8–3.4)
THL	32.4±4.2 (18.3–40.0)	30.6±1.91 (27.7–34.1)	28.3±1.92 (23.1–34.8)	31.9±4.19 (23.2–40.0)
TL	31.7±4.1 (18.0–39.3)	30.4±1.85 (27.8–33.8)	28.0±1.99 (23.0–35.0)	31.5±4.13 (23.3–39.3)
FL	40.8±5.2 (22.5–51.9)	38.6±2.40 (34.5–42.4)	35.5±2.61 (28.8–44.3)	40.4±5.14 (30.2–51.9)
4TD	2.3±0.3 (1.2–3.0)	2.4±0.32 (2.0–3.0)	2.1±0.25 (1.5–2.9)	2.2±0.33 (1.6–2.9)

Colour in preservative: Dorsal background light brown to cream with dark brown blotches; an interocular stripe and a X-shaped mark on the dorsum can be present. Flanks with well-defined dark brown transverse stripes. Chest and gular region beige, edge of lips green. Belly and ventral surfaces green, sometimes orange. Fingers beige, dark green, or orange. Iris grey with green outer border.

Variation: *Hypsiboas xerophyllus* presents some variation in size, with females usually larger than males (Table 4). Adult males have hypertrophied forearms, enlarged and projecting prepollices, and vocal slits, all characteristics that are absent in females. In dorsal view, shape of head ranges from rounded to oval, and in lateral view from rounded to truncated. Specimens usually lack any colour pattern at inguinal region and posterior surface of thighs. In living specimens, the dorsal pattern can vary in colour (white, green, or brown), often depending on light intensity, and dorsal blotches can be present or absent.

Distribution: Eastern Panama, through northern Colombia, Venezuela, the Guiana Shield, including adjacent northwestern Brazil, below 2,450 m a.s.l. The species seems absent from most French Guiana, only occurring around inselbergs at isolated localities in the south and in disturbed forest along the lower course of the Maroni River (Apatou, Saint Laurent). The species has not been recorded in the states of Amapá (AF pers. obs., Dias-Lima 2005) and Pará, north of the Amazon River (Ávila-Pires et al. 2010). However, it occurs throughout most Guyana (Cole et al. 2013) and northern Suriname (Ouboter & Jairam 2012), although apparently absent from Kaieteur National Park (Kok & Kalamandeen 2008).

Natural history: According to Duellman (1970; as *H. crepitans*), specimens from Panama do not exhibit the habit to build clay nests. However, Lynch & Ramirez (2000) reported this behavior as usual in Colombian populations, and Lehtinen (2014) confirms this behavior for Trinidad populations. One of us (AF) observed building of clay nests in the state of Roraima in Brazil and in Suriname. Therefore, nest building behavior is likely facultative as already observed by Caldwell (1992) in other species of *Hypsiboas*. Males have been observed calling at night in small streams, ponds, and even flasks during the rainy and the dry season. The species seems to inhabit ecotonal forest and is often found in disturbed habitat.

Hypsiboas crepitans (Duméril & Bibron, 1841)

Lectotype: AMNH 785, SVL ca. 69 mm, adult female, from Tamburil, [Municipality of] Condeúba, Bahia, Brazil (Fig. 6).

Diagnosis: *Hypsiboas crepitans* is a member of the *H. faber* species group (*sensu* Faivovich et al. 2005), characterized by: SVL 48.1–72.0 mm in males and 42.3–70.6 mm in females; interdigital membranae poorly developed; fimbrias absent in arms and tibia; Dorsal coloration brown, usually with an X-shape above the scapular region or a fragmented mid-dorsal line; dorsal skin smooth; flank and thigh with conspicuous transversal bars; absence of calcar flap or appendage; presence of subcloacal dermal fold, surrounded by granules; upper cloacal flap poorly developed; absence of lichenous subcloacal plate.

Distribution: Central to eastern Brazil, from the Atlantic coast of the states of Rio de Janeiro to Paraíba (present study).

Phylogenetic relationships and biogeography

The *Hypsiboas faber* species group, as defined by Faivovich et al. (2005), has not been recovered as monophyletic in our results – the weak relationship between *H. albomarginatus* and the remaining species of the group has been already reported by previous authors (Kolenc et al. 2008, Wiens et al. 2006), and the nested position of the *H. albopunctatus* group within the *H. faber* group is strongly supported. However, we refrain to take any formal action regarding this matter as our molecular analyses were simply aimed at exploring genetic divergence within *H. crepitans*.

Faivovich et al. (2005) listed the *H. faber* group as an example of a clade having an Atlantic Forest (or at least an eastern Brazilian) origin with subsequent radiations into neighbouring regions. At that time the authors had only Atlantic Forest dwellers in their dataset (their sample of *H. crepitans* was from State of Alagoas, Brazil) and probably assumed that *H. albomarginatus* would be sister to the remaining species of the group based on its unique colour. Our results suggest a more complex history of successive dispersals between Amazonia, Cerrados and the Atlantic Forest. However, the lack of support at the base of the clade prevents any further biogeographic analyses, and additional data are needed. Nevertheless, the *H. faber* clade is endemic to eastern Brazil while the *H. crepitans* clade is restricted to northern South America and Panama with the exception of *H. crepitans*. Such a pattern suggests that *H. crepitans* originates from a dispersal event from Amazonia to the Atlantic Forest probably via a northern route (Costa 2003). Given the genetic distances between *H. crepitans* and *H. xerophyllus* we hypothesize that this dispersal occurred before the Pleistocene and was probably favored by forest connection due to more humid climate. Such a route is supported by biogeographical analyses of different groups of organisms, as well as by climatic and floristic evidence (Batalha-Filho et al. 2013, Cabanne et al. 2008, Costa 2003, Melo Santos et al. 2007, Wang et al., 2004).

Hypsiboas pardalis and *H. lundii* were long considered synonyms (Caramaschi & Napoli 2004) due to their morphological similarity. Although the relationship of *H. exastis* with *H. pardalis* and *H. lundii* was not unexpected (see Caramaschi & Rodrigues 2003), we – unexpectedly – found that *H. lundii* is instead sister to a clade composed of *H. exastis* + *H. pardalis*. Caramaschi & Rodrigues (2003) related *H. exastis* to *H. pardalis* and *H. lundii* due to their similar skin texture and colour pattern; the lichenous aspect similar to tree bark were evidence that these species were more closely related to each other than to all other members of the *Hyla boans* species group, as defined at that time.

The IUCN distribution for *Hypsiboas crepitans* (La Marca et al. 2010) and *H. xerophyllus* needs to be re-evaluated. Although Frost (2016) stated that the species occurs in the south of the Atlantic Forest, from São Paulo to Santa Catarina states, we were unable to find a specimens from this region (Fig. 1). We propose that the southern part of the IUCN distribution is based on misidentified *H. lundii* or *H. pardalis* specimens and that the distribution of *H. crepitans* (as re-defined here) is restricted to an area north of 22° southern latitude. Despite the fact that the respective ranges are now confined to a smaller portion of the formerly supposed range of *H. crepitans*, both species are still widespread and, given their habitat requirements, are likely relatively tolerant to human disturbance. Therefore, we suggest considering them as Least Concern (LC).

Acknowledgements

We are grateful toward S. Ron, P. J. R. Kok and an anonymous reviewer for valuable comments on a previous version. For their invaluable help and company in the field and for the gift of tissue samples, we thank P. Gaucher, M. Blanc, J. Cassimiro, C. M. Carvalho, F. Curcio, M. Dixo, A. Camacho, R. Damasceno, D. Pavan, R. Recoder, M. Sena, S. M. Souza, M. Teixeira Jr., V. Verdade, H. de Niemeyer, and D. F. de Moraes Jr. Pictures of the holotype of *Hypsiboas fuentei* were kindly provided by S. Rogers and J. M. Padial. VGDO thanks Sao Paulo Research Foundation (FAPESP; grants #2007/57067-9, #2012/1200-5, BIOTA #2013/50297-0) and ANPCyT PICT 2202/2007 for financial support and C. S. Cassini for support and comments on a previous versions of this manuscript. AF benefited from an "Investissement d'Avenir" grant managed by Agence Nationale de la Recherche (CEBA, ref.ANR-10-LABX-25-01), France. IN thanks Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for support and fellowship, respectively. CM thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for fellowship. UC thank CNPq for financial support. MLL thanks FAPESP (#2010/50124-0) and CNPq (#478670/2010-9; #564955/2010-8) for fellowship and financial support. CFBH and MTR thank FAPESP (Grant #2013/50741-7) and CNPq for financial support. This study was co-funded by NSF (DEB 1343578) and NASA. This research was supported by the Núcleo de Computação Científica (NCC/GridUNESP) from the Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP). The Centro de Estudos de Insetos Sociais (CEIS), UNESP, Rio Claro, Brazil, allowed the utilization of the facilities for molecular analyses.

References

Ávila-Pires, T. C. S., M. S. Hoogmoed & W. A. D. Rocha (2010): Notes on the vertebrates of northern Pará, Brazil: a forgotten part of the Guianan Region, I. Herpetofauna. – Boletim do Museu Paraense Emílio Goeldi de Ciências Naturais, **5**: 13–112.

Batalha-Filho, H., J. Fjeldsa, P.-H. Fabre & C. Y. Miyaki (2013): Connections between the Atlantic and the Amazonian forest avifaunas represent distinct historical events. – Journal of Ornithology, **154**: 41–50.

Bernal, M. H. & J. A. Clavijo (2009): An essay on precision in morphometric measurements in anurans: inter-individual, intra-individual and temporal comparisons. – Zootaxa**, 2246**: 32–44.

Bernal, M. H., D. P. Montealegre & C. A. Páez (2004): Estudio de la vocalización de trece especies de anuros del municipio de Ibagué, Colombia. – Revista de la Academia Colombiana de Ciencias Exactas, Fisicas y Naturales, **28**: 385–390.

Bokermann, W. C. A. (1966): Lista anotada das localidades tipo de anfíbios brasileiros. – Serviço de Documentação, Reitoria da Universidade de São Paulo: São Paulo.

Boulenger, G. A. (1898): An account of the reptiles and batrachians collected by Mr. W. F. H. Rosenberg in western Ecuador. – Proceedings of the Zoological Society of London, **1898**: 107–126.

Burmeister, C. H. C. (1856): Erläuterungen zur Fauna Brasiliens, enthaltend Abbildungen und ausführliche Beschreibungen neuer oder ungenügend bekannter Thier-Arten. – G. Reimer: Berlin.

Cabanne, G. S., F. M. d'Horta, E. H. Sari, F. R. Santos & C. Y. Miyaki (2008): Nuclear and mitochondrial phylogeography of the Atlantic forest endemic *Xiphorhynchus fuscus* (Aves: Dendrocolaptidae): biogeography and systematics implications. – Molecular Phylogenetics and Evolution, **49**: 760–773.

Caldwell, J. (1992): Diversity of reproductive modes in anurans: facultative nest construction on gladiator frogs – pp. 85–97. In: Hamlett, W. C. (ed.): Reproductive biology of South American vertebrates. Springer, New York, USA.

Caramaschi, U. & M. F. Napoli (2004): Nomenclatural status of the synonyms of *Hyla pardalis* Spix, 1824, and taxonomic position of *Hyla biobeba* Bokermann and Sazima, 1974 (Anura: Hylidae). – Journal of Herpetology, **38**: 501–509.

Caramaschi, U. & M. T. Rodrigues (2003): A new large treefrog species, genus *Hyla* Laurenti, 1768, from southern Bahia, Brazil (Amphibia, Anura, Hylidae). – Arquivos do Museu Nacional, **61**: 255–260.

Casal, F. C. & F. A. Juncá (2008): Girino e canto de anúncio de *Hypsiboas crepitans* (Amphibia: Anura: Hylidae) do Estado da Bahia, Brasil, e considerações taxonômicas. – Boletim do Museu Paraense Emílio Goeldi de Ciências Naturais, **3**: 217–224.

Castroviejo-Fisher, S., P. E. Perez-Pena, J. M. Padial & J. M. Guayasamin (2012): A second species of the family Allophrynidae (Amphibia: Anura). – American Museum Novitates, **3739**: 1–17.

Cole, C. J., C. R. Townsend, R. P. Reynolds, R. D. MacCulloch & A. Lathrop (2013): Amphibians and reptiles of Guyana, South America: illustrated keys, annotated species accounts, and a biogeographic synopsis. – Proceedings of the Biological Society of Washington, **125**: 317–578.

Cope, E. D. (1867): On the families of the raniform Anura. – Journal of the Academy of Natural Sciences of Philadelphia, **2**: 189–206.

Costa, L. P. (2003): The historical bridge between the Amazon and the Atlantic Forest of Brazil: a study of molecular phylogeography with small mammals. – Journal of Biogeography, **30**: 71–86.

Deichmann, J. L., J. Boundy & G. B. Williamson (2009): Anuran artifacts on preservation: 27 years later. – Phyllomedusa, **8**: 51–58.

Dias Lima, J. (2008): A herpetofauna do Parque Nacional Montanhas do Tumucumaque, Amapá, Brasil, Expedições I a V – pp. 38–50. in: Bernard, E. (ed.): Inventários Biológicos Rápidos no Parque Nacional Montanhas do Tumucumaque, Amapá, Brasil. – RAP Bulletin of Biological Assessment 48, Conservation International, Arlington, USA.

Duellman, W. E. (1970): Hylid frogs of Middle America. – Monographs of the Museum of Natural History, University of Kansas, **1/2**: 1–753.

Duellman, W. E. (1997): Amphibians of La Escalera Region, southeastern Venezuela: taxonomy, ecology, and biogeography. – Scientific Papers, Natural History Museum University of Kansas, **2**: 1–52.

Duméril, A. M. C. & G. Bibron (1841): Erpétologie générale ou Histoire naturelle complète des reptiles. – Librarie Enclyclopedique de Roret, Paris.

Fabrezi, M. & P. Alberch (1996): The carpal elements of anurans. – Herpetologica, **52**:188–204.

Faivovich, J., C. F. B. Haddad, P. C. A. Garcia, D. R. Frost, J. A. Campbell & W. C. Wheeler (2005): Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. – Bulletin of the American Museum of Natural History, **294**: 1–240.

Fouquet, A., A. Gilles, M. Vences, C. Marty, M. Blanc & N. J. Gemmell (2007): Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. – PLoS ONE, **2**: e1109.

Fouquet, A., D. Loebmann, S. Castroviejo-Fisher, J. M. Padial, V. G. D. Orrico, M. L. Lyra, I. J. Roberto, P. J. R. Kok, C. F. B. Haddad & M. T. Rodrigues (2012): From Amazonia to the Atlantic forest: molecular phylogeny of Phyzelaphryninae frogs reveals unexpected diversity and a striking biogeographic pattern emphasizing conservation challenges. – Molecular Phylogenetics and Evolution, **65**: 547–561.

Fouquet, A., C. S. Cassini, C. F. B. Haddad, N. Pech & M. T. Rodrigues (2014): Species delimitation, patterns of diversification and historical biogeography of the Neotropical frog genus *Adenomera* (Anura, Leptodactylidae). – Journal of Biogeography, **41**: 855–870.

Fouquet, A., Q. Martinez, L. Zeidler, E. A. Courtois, P. Gaucher, M. Blanc, J. Dias Lima, S. Marques Souza, M. T. Rodrigues & P. J. R. Kok (2016): Cryptic diversity in the *Hypsiboas semilineatus* species group (Amphibia, Anura) with the description of a new species from the eastern Guiana Shield. – Zootaxa, **4084**: 079–104.

Fouquette, M. J. (1966): Some hylid frogs of the canal zone with special reference to cell structure. – Caribbean Journal of Science, **6**: 167–172.

Frost, D. R. (2016): Amphibian Species of the World: An Online Reference. Version 6.0 (01 February 2016). Eletronic database accessible at http://research.amnh.org/herpetology/amphibia/index.html. American Museum of Natural History: New York, USA.

Goebel, A. M., J. M. Donnelly & M. E. Atz (1999): PCR primers and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase 1, and cytochrome b in bufonids and other frogs, and an overview of PCR primers which have amplified DNA in amphibians successfully. – Molecular Phylogenetics and Evolution, **11**: 163–199.

Goin, C. J. & O. B. Goin (1968): A new green tree frog from Suriname. – Copeia**, 1968**: 581–583.

Graybeal, A. (1997): Phylogenetic relationships of bufonid frogs and tests of alternate macroevolutionary hypotheses characterizing their radiation. – Zoological Journal of the Linnean Society, **119**: 297–338.

Guindon, S. & O. Gascuel (2003): A simple, fast, and accurate algorithm to estimate large phylogenies by Maximum Likelihood. – Systematic Biology**, 52**: 696–704.

Hebert, P. D. N., A. Cywinska, S. L. Ball & J. R. deWaard (2003): Biological identifications through DNA barcodes. – Proceedings of the Royal Society of London. Series B: Biological Sciences, **270**: 313–321.

Heyer, W. R., A. S. Rand, C. A. G. Cruz, O. L. Peixoto & C. E. Nelson (1990): Frogs of Boracéia. – Arquivos de Zoologia, **31**: 231–410.

Hoogmoed, M. S. (1979): Resurrection of *Hyla ornatissima* Noble (Amphibia, Hylidae) and remarks on related species of green tree frogs from the Guiana area. Notes on the herpetofauna of Surinam VI. – Zoologische Verhandelingen, **172**: 1–46.

Humphries, J. M., F. L. Bookstein, B. Chernoff, G. R. Smith, R. L. Elder & S. G. Poss (1981): Multivariate discrimination by shape in relation to size. – Systematic Zoology, **30**: 291–308.

ICZN – International Commission on Zoological Nomenclature (1999): International Code of Zoological Nomenclature. – International Trust of Zoological Nomenclature: London.

IUCN – Standards and Petitions Subcommittee (2014): Guidelines for using the IUCN red list categories and criteria. Version 11. Prepared by the Standards and Petitions Subcommittee. Available from: http://www.iucnredlist.org/documents/RedListGuidelines.pdf (accessed 11 February 2015).

Katoh, K., K. Misawa, K.-I. Kuma & T. Miyata (2002): MAFFT – a novel method for rapid multiple sequence alignment based on fast Fourier transform. – Nucleic Acids Research, **30**: 3059–3066.

Kok, P. J. R. & M. Kalamandeen (2008): Introduction to the taxonomy of the amphibians of Kaieteur National Park, Guyana. – ABC Taxa, **5**: 1–288.

Köhler, F., K.-J. Schultze, R. Günther & J. Plötner (2008): On the genetic diversity in the mitochondrial 12S rRNA gene of *Platymantis* frogs from western New Guinea (Anura: Ceratobatrachidae). – Journal of Zoological Systematics and Evolutionary Research**, 46**: 177–185.

Köhler, J., D. Koscinski, J. M. Padial, J. C. Chaparro, P. Handford, S. C. Lougheed & I. De la Riva (2010): Systematics of Andean gladiator frogs of the *Hypsiboas pulchellus* species group (Anura, Hylidae). – Zoologica Scripta, **39**: 572–590.

Kolenc, F., C. Borteiro, L. Alcalde, D. Baldo, D. Cardozo & J. Faivovich (2008): Comparative larval morphology of eight species of *Hypsiboas* Wagler (Amphibia, Anura, Hylidae) from Argentina and Uruguay, with a review of the larvae of this genus. – Zootaxa, **1927**: 1–66.

Kime, N. M., W. R. Turner & M. J. Ryan (2000): The transmission of advertisement calls in Central American frogs. –Behavioral Ecology, **11**: 71–83.

Kluge, A. G. (1979): The gladiator frogs of Middle America and Colombia – A reevaluation of their systematics (Anura: Hylidae). – Occasional Papers of the Museum of Zoology, University of Michigan**, 688**: 1–24.

La Marca, E., C. Azevedo-Ramos, S. Silvano, F. Solís, R. Ibáñez, C. Jaramillo, Q. Fuenmayor & J. Hardy (2010): *Hypsiboas crepitans*. The IUCN Red List of Threatened Species 2010: e.T55457A11314699. – http://dx.doi.org/10.2305/IUCN.UK.2010-2.RLTS.T55457A11314699.en. Downloaded on 18 December 2015.

Lescure, J. & C. Marty (2000): Atlas des amphibiens de Guyane. – Patrimoines Naturels, **45**: 1–388.

Lehtinen, R. M. (2014): Confirmation of nest building in a population of the gladiator frog *Hypsiboas crepitans* (Anura, Hylidae) from the island of Tobago (West Indies). – Herpetology Notes, **7**: 227–229.

Liu, C. C. (1935): Types of vocal sac in the Salientia. – Proceedings of the Boston Society of Natural History, **41**: 19–40.

Lynch, J. D. (2006): The tadpoles of frogs and toads found in the lowlands of northen Colombia. – Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales, **30**: 443–457.

Lynch, J. D. & M. A. V. Ramírez (2000): Lista preliminar de anuros del Departamento del Guainía, Colômbia. – Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales, **24**: 579–589.

Lynch, J. D. & A. M. Suarez-Mayorga (2001): The distributions of the gladiator frogs (*Hyla boans* group) in Colombia, with comments on size variation and sympatry. – Caldasia, **23**: 491–507.

Martins, L. B., W. R. Silva & A. A. Giaretta (2009): Distribution and calls of two South American frogs (Anura). – Salamandra, **45**: 106–109.

Melo Santos, A. M. M., D. R. Cavalcanti, J. M. C. da Silva & M. Tabarelli (2007): Biogeographical relationships among tropical forests in north-eastern Brazil. – Journal of Biogeography, **34**: 437–446.

Meyer, C. (2003): Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. – Biological Journal of the Linnean Society, **79**: 401–459.

Myers, C. W. & W. E. Duellman (1982): A new species of *Hyla* from Cerro Colorado, and other tree frog records and geographical notes from western Panama. – American Museum Novitates, **2752**: 1–32.

Napoli, M. F. (2005): A new species allied to *Hyla circumdata* (Anura: Hylidae) from Serra da Mantiqueira, southeastern Brazil. – Herpetologica, **61**: 63–69.

Orrico, V. G. D., M. M. Mongin & A. M. P. T. Carvalho-e-Silva (2007): The tadpole of *Hypsiboas latistriatus* (Caramaschi & Cruz, 2004), a species of the *Hypsiboas polytaenius* (Cope, 1870) clade (Amphibia, Anura, Hylidae). – Zootaxa, **1531**: 25–37.

Ouboter, P. E. & R. Jairam (2012): Amphibians of Surinam. – Koninklijke Brill, Leiden, Netherlands.

Peres-Neto, P. R. & C. R. S. F. Bizerril (1994): The jackknifing of multivariate allometric coefficient (Jolicoeur, 1963): a case study on allometry and morphometric variation in *Corydoras barbatus* (Quoy & Gaimard, 1824) (Siluriformes, Callichthyidae). – Arquivos de Biologia e Tecnologia, **37**: 449–454.

Posada, D. (2008): jModelTest: phylogenetic model averaging. – Molecular Biology and Evolution**, 25**: 1253–1256.

Ron, S. R., P. J. Venegas, E. Toral, M. Read, D. A. Ortiz & A. L. Manzano (2012): Systematics of the *Osteocephalus buckleyi* species complex (Anura, Hylidae) from Ecuador and Peru. – Zookeys, **229**: 1–52.

Ronquist, F., M. Teslenko, P. V. D. Mark, D. Ayres, A. Darling, S. Hohna, B. Larget, L. Liu, M. A. Suchard & J. P. Huelsenbeck (2012): MrBayes 3.2: eficient Bayesian phylogenetic inference and model choice across a large model space. – Systematic Biology, **61**: 539–542.

Sabaj Pérez, M. H. (2010): Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an online reference. Version 2.0 (8 November 2010). – Available at: http://www.asih.org/, American Society of Ichthyologists and Herpetologists, Washington, D.C. Accessed on 15 November 2010.

Dos Santos, S. P., R. Ibáñez & S. R. Ron (2015): Systematics of the *Rhinella margaritifera* complex (Anura, Bufonidae) from western Ecuador and Panama with insights in the biogeography of *Rhinella alata.* – ZooKeys, **501**: 109–145.

Savage, J. M. & W. R. Heyer (1967): Variation and distribution in the tree-frog genus *Phyllomedusa* in Costa Rica, Central America. – Beitrage zur Neotropischen Fauna, **5**: 111–131.

Schmidt, K. P. & R. F. Inger (1951): Amphibians and reptiles of the Hopkins-Branner expedition to Brazil. – Fieldiana Zoology new series, **31**: 439–465.

Spix, J. B. (1824): Animalia nova, species novae testudinum et ranarum quas in itinere per Brasiliam annis 1817–1820 jussu et auspiciis Maximilian Josephi I. Bavaria Regius. – Hübschmann: München.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei & S. Kumar (2011): MEGA5: Molecular evolutionary genetics analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony methods. – Molecular Biology and Evolution, **28**: 2731–2739.

Vences, M., M. Thomas, R. M. Bonett & D. R. Vieites (2005): Deciphering amphibian diversity through DNA barcoding: chances and challenges. – Philosophical Transactions of the Royal Society B-Biological Sciences, **360**: 1859–1868.

Wang, X., A. S. Auler, R. L. Edwards, H. Cheng, P. S. Cristalli, P. L. Smart, D. A. Richards & C.-C. Shen (2004): Wet periods in northeastern Brazil over the past 210 kyr linked to distant climate anomalies. – Nature, **432**: 740–743

Warkentin, K. M. (1999): Effects of hatching age on development and hatchling morphology in the red-eyed tree frog, *Agalychnis callidryas*. – Biological Journal of the Linnean Society, **68**: 443–470.

Wells, K. D. & B. J. Greer (1981): Vocal responses to conspecific calls in a Neotropical hylid frog, *Hyla ebraccata*. – Copeia, **1981**: 615–624.

Werneck, F. P. (2011): The diversification of eastern South American open vegetation biomes: historical biogeography and perspectives. – Quaternary Science Reviews, **30**: 1630–1648.

Wied, M. Prinz zu (1821): Reise nach Brasilien in den Jahren 1815 bis 1817. – Heinrich Ludwig Brönner, Franfurt.

Wied, M. Prinz zu (1824): Abbildungen zur Naturgeschichte Brasiliens. Heft 8. – Landes-Industrie-Comptoir, Weimar.

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

Zar, J. H. (2009): Biostatistical Analysis. – Prentice Hall: New Jersey.

Supplementary material

Supplementary document 1. Examined specimens

Supplementary document 2. Voucher information, localities, and GenBank accession numbers for the specimens analyzed for this study.

Supplementary document 3. Standardized coefficients and factor loadings (r) from a Principal Component Analysis.