

# Phylogeny of Map Tree Frogs, *Boana semilineata* Species Group, with a New Amazonian Species (Anura: Hylidae)

Pedro L. V. Peloso<sup>1,\*</sup>, Renan M. De Oliveira<sup>2</sup>, Marcelo J. Sturaro<sup>1</sup>, Miguel T. Rodrigues<sup>3</sup>,  
Geraldo R. Lima-Filho<sup>1</sup>, Youszef O. C. Bitar<sup>4</sup>, Ward C. Wheeler<sup>5</sup>, Alexandre Aleixo<sup>1</sup>

<sup>1</sup> Museu Paraense Emílio Goeldi, Coordenação de Zoologia, Avenida Perimetral, 1.901, Terra Firme, CEP 66077-530, Belém, PA, Brazil.

<sup>2</sup> Museu Nacional, Departamento de Vertebrados, Universidade Federal do Rio de Janeiro. Quinta da Boa Vista, CEP 20940-040 Rio de Janeiro, RJ, Brazil.

<sup>3</sup> Universidade de São Paulo, Departamento de Zoologia, Rua do Matão, Trav. 14, n 321, Cidade Universitária, Caixa Postal 11461, CEP 05422-970, São Paulo, SP, Brazil.

<sup>4</sup> Universidade Federal do Pará, Faculdade de Ciências Biológicas, Travessa 13, s/n, campus Universitário do Marajó-Soure Pacoval, CEP 68870-000, Soure, PA, Brazil.

<sup>5</sup> American Museum of Natural History, Division of Invertebrate Zoology, Central Park West at 79th Street, 10024, New York, NY, USA.

\* Corresponding author. Email: pedropeloso@gmail.com

**Abstract.** Gladiator Frogs (*Boana*) is a Neotropical group comprised of 92 species sorted into seven species groups. Herein, we present a phylogeny of the *Boana semilineata* species group, including all valid nominal species currently or suspected to be assigned to it— many sequenced for the first time. Parsimony and maximum likelihood analyses of two genes (16S and COI) did not support the monophyly of the *B. semilineata* group as currently defined. To remedy this, *B. secedens*, currently assigned to the *B. puchella* species group, is transferred to the *B. semilineata* group. On the other hand, our analyses corroborate the inclusion of *B. hutchinsi*, *B. pombali* and *B. wavrini* in the *B. semilineata* group. The reinterpretation of the morphology of the lower palpebral membrane in *B. pombali* (previously described as absent, but actually present) corroborates that the reticulated membrane is a synapomorphy of this species group (albeit homoplastic—present in a few species not assigned to this group). A recent paper suggested (based on the analyses of 16S alone) that at least six unnamed taxa (candidate species) are hidden behind the names *B. geographica* and *B. semilineata*. Our analyses resulted in the discovery of additional unnamed lineages, one of which is described and named herein. This new species is diagnosed, among other characters, by having the flanks, anterior and posterior surfaces of thigh with dark brown or blackish stripes, and by the presence of a prepollex forming a projecting spine.

**Keywords.** Amazonia; Biodiversity; Cophomantinae; *Hyla geographica*; Taxonomy.

## INTRODUCTION

*Boana* Gray, 1825 currently comprises 92 species organized in seven species groups. The content of most of these groups was defined by Faivovich et al. (2005) and subsequently corroborated by larger datasets (Wiens et al., 2010; Pyron and Wiens, 2011; Duellman et al., 2016). The *Boana semilineata* group was first proposed by Faivovich et al. (2005) and included fragments of the former *B. geographica* and *B. boans* groups.

Faivovich et al. (2005) suggested that the only putative morphological synapomorphy for this group is the presence of a reticulated palpebral membrane. However, the authors noted that at least one species in the group lacks the reticulated membrane (*Boana pombali* [Caramaschi et al., 2004]) whereas some others, not originally included in the group, do have reticulated eyelids (*B. microderma* [Pyburn, 1977] and *B. hutchinsi* [Pyburn and Hall, 1984]). At the time, the presence of reticulations in

species not included in the group was inferred to be homoplastic. As currently defined, the *B. semilineata* group includes seven valid nominal species: *B. boans* (Linnaeus, 1758), *B. diabolica* (Fouquet et al., 2016), *B. geographica* (Spix, 1824), *B. hutchinsi*, *B. pombali*, *B. semilineata* (Spix, 1824), and *B. wavrini* (Parker, 1936). The species diversity in the *B. semilineata* group is, however, underestimated, as a recent phylogenetic analysis including several samples from the Amazon Basin revealed that many populations represent unnamed taxa (Fouquet et al., 2016). Fouquet et al. (2016), for example, implied that the number of species in the group is likely double the current named diversity.

Inasmuch as only three species of the *Boana semilineata* species group were incorporated into their analyses (*B. boans*, *B. geographica*, and *B. semilineata*), Faivovich et al. (2005) included two additional species in the group (*B. pombali* and *B. wavrini*) based on phenotypic evidence available at that time. Faivovich et al. (2005) tentatively

**How to cite this article:** Peloso P.L.V., Oliveira R.M., Sturaro M.J., Rodrigues M.T., Lima-Filho G.R., Bitar Y.O.C., ... Aleixo A. 2018. Phylogeny of map frogs, *Boana semilineata* species group, with a new Amazonian species (Anura: Hylidae). *South American Journal of Herpetology*, 13:150–169. <http://doi.org/10.2994/SAJH-D-17-00037.1>

included *B. pombali* in the group based on the suggestions made in the original description that the species is similar to *B. semilineata* (Caramaschi et al., 2004). As mentioned above, *B. pombali* is an exception among species of the *B. semilineata* group in not having a reticulated palpebral membrane, according to its original description (Caramaschi et al., 2004). *Boana wavrini* was included in the group because it has a reticulated palpebral membrane and is morphologically and ecologically similar to *B. boans* (Hoogmoed, 1990; Faivovich et al., 2005). To date, neither *B. pombali* nor *B. wavrini* were ever included in any phylogenetic analysis. Although the presence of a reticulated eyelid was suggested as a synapomorphy for the group by Faivovich et al. (2005), some species with reticulated eyelids (e.g., *B. microderma* and *B. hutchinsi*) were not included in the group. At the time, the presence of reticulations in those species was inferred to be a product of convergence (Faivovich et al., 2005).

Subsequent to Faivovich et al. (2005), two additional species were included in the group, *Boana diabolica* and *B. hutchinsi* (Faivovich et al., 2006), for which only the inclusion of *B. diabolica* is based on a phylogenetic analysis. *Boana hutchinsi* (a former member of the *B. geographica* group; Pyburn and Hall, 1984) was included in the *B. benitezi* group by Faivovich et al. (2005) on the basis of its overall similarity to species of that group. Later, however, Faivovich et al. (2006) transferred *B. hutchinsi* to the *B. semilineata* group on the basis of the presence of reticulated palpebral membrane and the state of the prepollex (not forming a projecting spine). Faivovich et al. (2006) commented that the absence of the prepollical spine is unique among *Boana* and shared only with *B. geographica* and *B. semilineata*. The same character state is also present in *B. diabolica* (Fouquet et al., 2016).

The phylogenetic relationships of *Boana secedens* (Lutz, 1963) remain obscure. Despite its high degree of morphological similarity with members of the *B. semilineata* group, *B. secedens* was assigned to the *B. pulchella* group by Caramaschi et al. (2004) on the basis of its overall similarity to *B. bischoffi*. Faivovich et al. (2005) did not sample *B. secedens* but followed the arrangement proposed by Caramaschi et al. (2004) and retained *B. secedens* in the *B. semilineata* group. This assignment is, however, pending phylogenetic test, which is provided herein.

Fouquet et al. (2016) presented a phylogenetic analysis of 57 samples representing several populations commonly referred to *Boana geographica* from across Amazonia. To associate genetic lineages with nominal populations, Fouquet et al. (2016) included a single sample of *B. geographica* from Tefé, Amazonas, Brazil (type locality of *B. geographica*) and two samples of *B. semilineata* from Rio de Janeiro (the type locality for this taxon [Spix, 1824]). Fouquet et al. (2016) carried out the first attempt to evaluate the genetic diversity within *B. geographica* and, despite a limited geographic sample and a single mtDNA

locus (16S), provided many important results, including decisive evidence that *B. geographica* and *B. semilineata* should be treated as separate species, thus corroborating the suggestion by Silveira and Caramaschi (1989). Furthermore, their analysis suggested that at least six candidate species (unnamed taxa) are hidden behind the names *B. geographica* and *B. semilineata*. Fouquet et al. (2016), however, acknowledged the fact that the taxonomic status of some lineages deserved further investigation and that their genetic sampling was far from being exhaustive.

For simplicity, throughout our work we use a similar but simpler terminology than that of Fouquet et al. (2016) when referring to the putative candidate species mentioned by them—*Boana* cf. *geographica* 1 = candidate-G1, and *B. aff. semilineata* 1–5 = candidate-S1–S5, respectively. According to Fouquet et al. (2016) “*B. candidate-G1*” is apparently related to *B. geographica* whereas the other five candidate species, “*B. candidate-S1–S5*”, are related to *B. semilineata* (Fig. 1A). Fouquet et al. (2016) referred to the monophyletic group formed by *B. semilineata* plus candidate species *B. candidate-S1–S5* as the *B. semilineata* clade. Support values and genetic distances inferred for this clade were generally low (Fouquet et al., 2016).

## Study objectives

Herein, we improve upon the taxon and character sampling implemented in Fouquet et al. (2016) by including several samples, all valid nominal species currently assigned to the *Boana semilineata* group, but also improving on the geographical coverage of samples currently identified as *B. geographica* (Amazonia) and *B. semilineata* (Atlantic Forest). Our results corroborate the existence of several unnamed species in the *B. semilineata* group, one of which is described and named herein. Also, we suggest that a reevaluation of the candidate species suggested by Fouquet et al. (2016) is needed.

## MATERIALS AND METHODS

### Phylogenetic analysis

To infer the phylogeny of the *Boana semilineata* group we used a dataset composed of two mitochondrial loci, the Ribosomal Subunit 16S rRNA (16S) and Cytochrome Oxidase 1 (COI). Taxon sampling, and analytical strategies are explained in detail below.

### Outgroup selection and the monophyly of the *Boana semilineata* group

To test the monophyly of the *Boana semilineata* species group with the inclusion of previously unsampled

species, we included a few species from all other species groups in *Boana*, as defined in Faivovich et al. (2005). Taxonomic coverage for the outgroup is as follows: *Boana albopunctata* group (*B. albopunctata* [Spix, 1824], *B. lanciformis* [Cope, 1871]), *Boana benitezi* group (*B. benitezi* [Rivero, 1961], *B. microderma*), *Boana faber* group (*B. faber* [Wied, 1821], *B. albomarginatus* [Spix, 1824]), *Boana pellicens* group (*B. pellicens* [Werner, 1901]), *Boana pulchella* group (*B. pulchella* [Duméril and Bibron, 1841], *B. polytaenia* [Cope, 1870]), and *Boana punctata* group (*B. punctata* [Schneider, 1799], *B. sibleszi* [Rivero, 1972]). All sequences for outgroup taxa were downloaded from GenBank (Benson et al., 2013). A sequence of *Aplastodiscus arildae* [Cruz and Peixoto, 1987] was used to root the tree based on the assumption that *Aplastodiscus* Lutz, 1950 is the sister taxon of *Boana* (Faivovich et al., 2005; Wiens et al., 2010).

### Taxon sampling within the *Boana semilineata* group

Sequences from several representatives of the *Boana semilineata* group were downloaded from GenBank (Benson et al., 2013). Most of these sequences derive from Fouquet et al. (2016), but as many of these sequences are redundant, several were removed. Nonetheless, when available, at least four representatives of each species (including candidate species) from the Fouquet et al. (2016) dataset were maintained and were included in our dataset. New sequences were generated for multiple specimens from additional taxa in the *B. semilineata* group, including several not included in Fouquet et al. (2016). We have included multiple sequences for *B. boans* (versus only one from Fouquet et al., 2016) and multiple sequences of *B. pombali* and *B. wavrini* (not sampled by any molecular phylogenetic study thus far).

***Boana hutchinsi*.** Upon examination of the voucher specimens sequenced by Fouquet et al. (2016), additional material from Vaupés (Colombia) and Amazonas (Brazil), and detailed comparisons with the original description of *B. hutchinsi* (Pyburn and Hall, 1984), we are confident that the specimens sequenced by Fouquet et al. (2016) and identified as *Boana* candidate S5 are *B. hutchinsi*. The specimens share the diagnostic features given by Pyburn and Hall (1984) and were collected near the type locality of *B. hutchinsi* (Vaupés, Colombia).

***Boana secedens*.** Sequences of *Boana secedens* are available on GenBank (accession 16S: KM390785; COI: KU234700). These sequences are not associated with any published paper, but we included them in our analysis as *B. secedens* on the basis of a personal communication from Mariana Lyra (Universidade Estadual Paulista) that these

sequences are both derived from the same sample of *B. secedens* (field number VNBJ 60).

### New sequences and dataset preparation

New sequences were generated at the American Museum of Natural History and Museu Paraense Emílio Goeldi using primers and protocols reported previously (Peloso et al., 2014, 2015). Newly generated sequences were deposited in GenBank with accession numbers MG840844–MG840921. A complete list of all specimens included in the analyses, as well as voucher numbers, locality, and GenBank accession numbers, are in Table S1.

### Multiple sequence alignment

Sequences were edited and organized using Geneious R9 (Kearse et al., 2012). Sequences for each gene were aligned using MAFFT version 1.3.5 (Katoh et al., 2002), as implemented in the Geneious plugin, with default parameters. GenBank sequences have a conspicuous variation in fragment size and in order to fix this we trimmed all sequences to include only the fragments sequenced by us (AR/BR primer pair for the 16S fragment, COI.PF-A/COI.PR-A for the COI fragment). After trimming, sequences were realigned using the same MAFFT parameters and exported as *fasta* files. *Fasta* files for the 16S and COI alignments were concatenated and converted into *phylip* and *nexus* formats (for RAXML and TNT, respectively) using Sequence Matrix 1.8 (Vaidya et al., 2011). The complete alignment is given (*nexus*) as supplementary information (Data S1).

### Tree inference

The aligned DNA sequences were analyzed with two alternative methods of phylogenetic inference (i.e., optimality criteria): Parsimony (PAR) and Maximum Likelihood (ML). Trees are available for download as supplementary information (Data S2–S3). ML was performed using RAXML v.8.1.15 (Stamatakis, 2006, 2014) under a GTRGAMMA model and unpartitioned sequences. Tree search consisted of 100 replicates with a parsimony random-seed starting tree—additional parameters under the default settings. The General Time Reversible model (Tavaré, 1986: GTR) was selected a priori under the assumption that all other commonly used models are special cases of the GTR, and that overparameterization consistently yields more consistent trees than underparameterization (Lemmon and Moriarty, 2004). Nodal support was assessed by 1,000 bootstrap replicates, with the same sequence evolution model (GTRGAMMA) and remaining parameters set to the default.

PAR was performed in TNT v.1.1 (Goloboff et al., 2008) with the command *xmult*, which implements a variety of search algorithms—Random Addition Sequences

(RAS), Tree Bisection and Reconnection branch swapping (TBR), Parsimony Ratchet (Nixon, 1999), Sectorial Searches, Tree Drifting, and Tree Fusing. Searching continued until the best solution was hit 1,000 times (command: *hits 1000*). Nodal support was assessed by 1,000 Jackknife replicates with a removal rate of  $e^{-1}$  (= 0.36) following the suggestion of Farris et al. (1996) that this is the value most congruent with bootstrapping (command: *resample jak savetrees replications 1000*). For comparability, ML and PAR analyses were conducted with gaps treated as missing data. For both PAR and ML, support values were summarized onto the optimal trees using DendroPy's sumtrees.

### Species description

#### Specimens examined

Institutional abbreviations are **AMNH** (American Museum of Natural History, New York, USA); **ANDES** (Universidad de los Andes, Bogotá, Colombia); **KU** (University of Kansas, Lawrence, USA); **MCP** (Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil); **MCZ** (Museum of Comparative Zoology, Harvard University, Cambridge, USA); **MNRJ** (Museu Nacional, Rio de Janeiro, Brazil); **MPEG** (Museu Paraense Emilio Goeldi, Belém, Brazil); **MZUESC** (Museu de Zoologia, Universidade Estadual de Santa Cruz, Bahia, Brazil); **MZUSP** (Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil); **UTA-A** (Amphibian Collection, University of Texas at Arlington, Texas, USA); and **ZSM** (Zoologische Staatssammlung München, München, Germany). Specimens examined for comparisons are listed in the Appendix.

#### Morphology

For the descriptions, several measurements were taken with an electronic caliper and were recorded to the nearest 0.1 mm. Fourteen measurements were taken from adult males following the methods and landmarks used in Peloso et al. (2014, 2016) and Sturaro and Peloso (2014): **SVL**, snout–vent length; **HL**, head length (snout–angle of jaw); **HW**, head width (between angle of jaws); **ED**, eye diameter (between anterior and posterior corner of eye); **TD**, tympanum diameter (measured at the widest portion); **IOD**, interorbital distance (distance between anterior corner of eyes); **IND**, internarial distance; **END**, eye–nostril distance (anterior corner of eye to posterior margin of nostril); **ESD**, eye–snout distance (anterior corner of eye to tip of snout); **ETD**, eye–tympanum distance (posterior corner of eye to anterior margin of tympanum); **ARM**, arm length (forearm–arm articulation [elbow] to anterior margin of palmar tubercle); **HND**, hand

length (anterior margin of palmar tubercle to tip of Finger III); **THL**, thigh length (middle of cloacal opening to outer edge of flexed knee); **TBL**, tibia length (outer edge of flexed knee to heel); **TAR**, tarsus length (tibio-tarsal articulation to anterior margin of plantar tubercle); **FL**, foot length (anterior margin of plantar tubercle to tip of Toe IV); **3FD**, Finger III disk diameter; **3TD**, Toe III disk diameter; **4FD**, Finger IV disk diameter; and **5TD**, Toe V disk diameter. Webbing formulae follow Savage and Heyer (1967) and Kok and Kalamandeen (2008).

#### Bioacoustics

Advertisement call data were obtained from four specimens (three paratypes and one unvouchered specimen) from Brazil, Pará, Altamira as follows: Paratypes MNRJ 89838 and MNRJ 89839 were recorded on 30 August 2015, air temperature 26.6°C, 80% air humidity. Paratype MNRJ 90998 and one unvouchered male were recorded on 20 September 2016, air temperature, 25.5°C, 85% air humidity. Recordings were made using either a Tascam DR-100 (MNRJ 89838 and MNRJ 89839) or a Tascam DR-22WL (MNRJ 90998 and unvouchered specimen) digital recorder, both with a unidirectional Yoga HT-81 microphone using 44.1 kHz sampling rate and 16-bit encoding. The recordings were made at a distance of about 1 m from males. A total of 23.09 minutes of recordings was obtained from the four males, and 70 calls were analyzed.

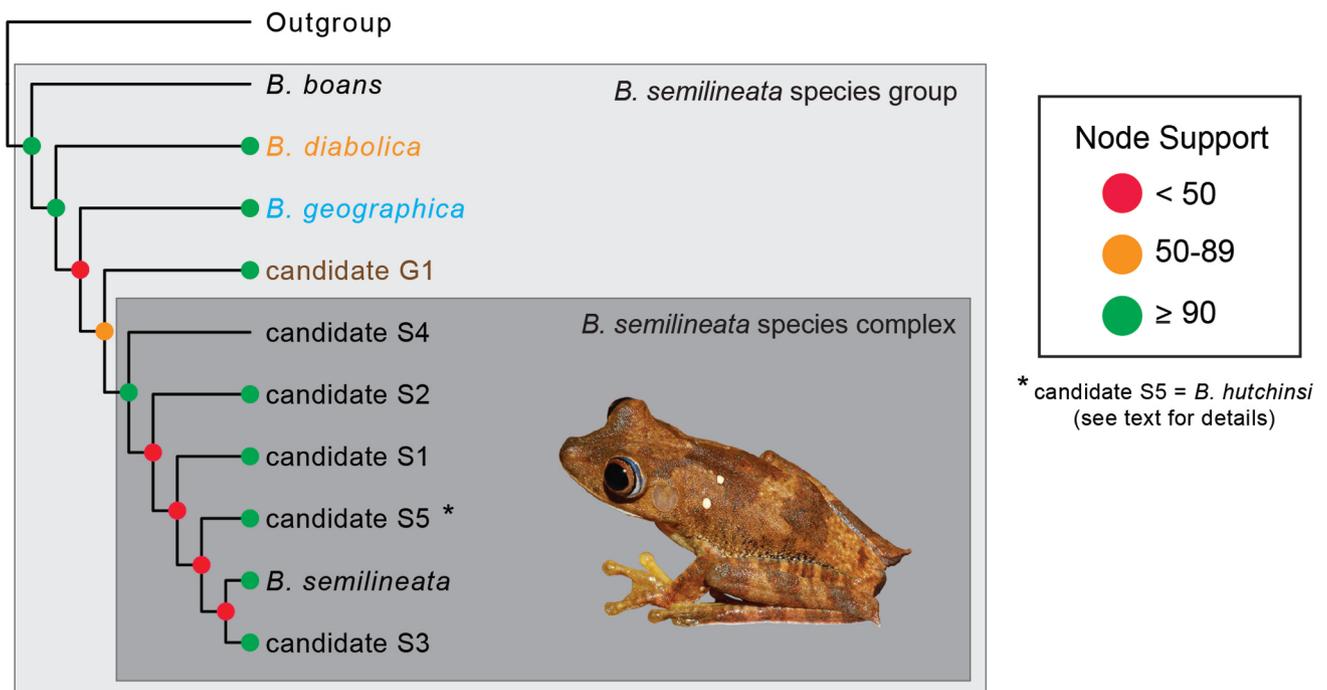
Calls were analyzed in Raven 1.4 (Bioacoustics Research Program, 2011). Temporal traits were measured from the waveform and spectral traits from the spectrogram. Dominant frequency was measured using the “peak frequency” measurement. Waveforms and spectrograms were generated in the same software. Spectrograms were generated using Hann windows type with 256 samples window size and 70% time grid overlap. Advertisement call terminology follows Heyer et al. (1990).

## RESULTS

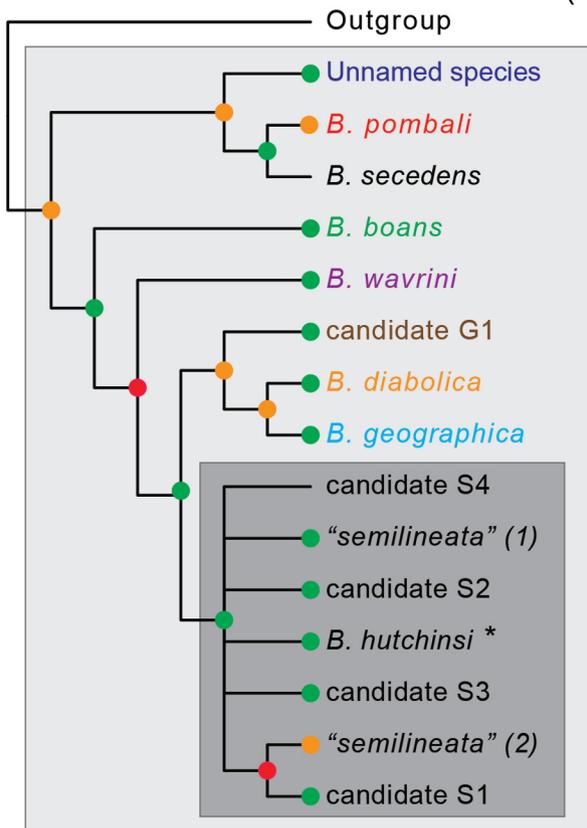
### Phylogeny of map tree frogs (Figs. 1–2)

The PAR analysis resulted in 1,066 equally parsimonious trees of 1,993 steps. The ML analysis obtained final GAMMA-based optimization score of best tree -10,230.475415. Outgroup relationships received very low overall support and are conspicuously different in the ML and PAR analyses. Because our taxon sampling for these outgroup taxa is considerably smaller than previous studies (Faivovich et al., 2005; Wiens et al., 2010; Duellman et al., 2016), we refrain from commenting further on relationships among outgroup taxa. For the present discussion it is important to state that the *Boana semilineata* group is nonmonophyletic in both the ML and

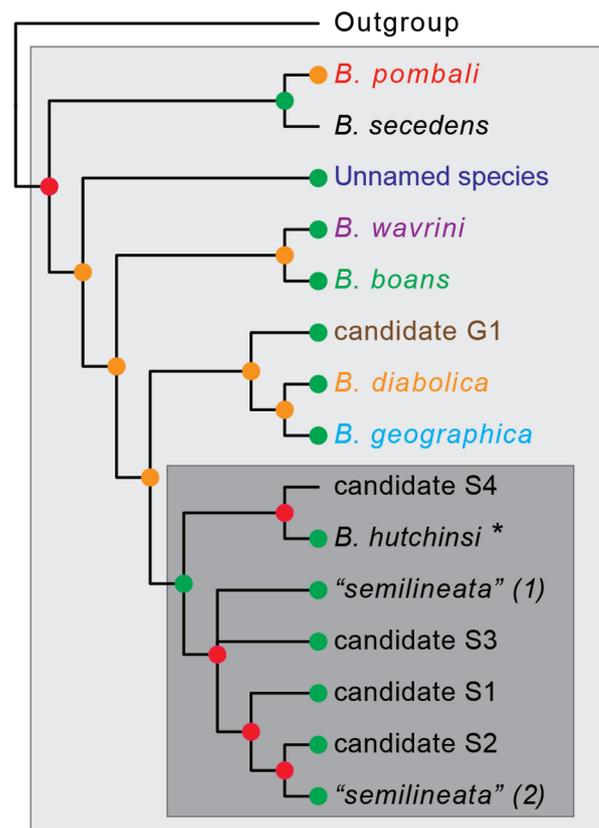
(A) Fouquet et al. (2016)



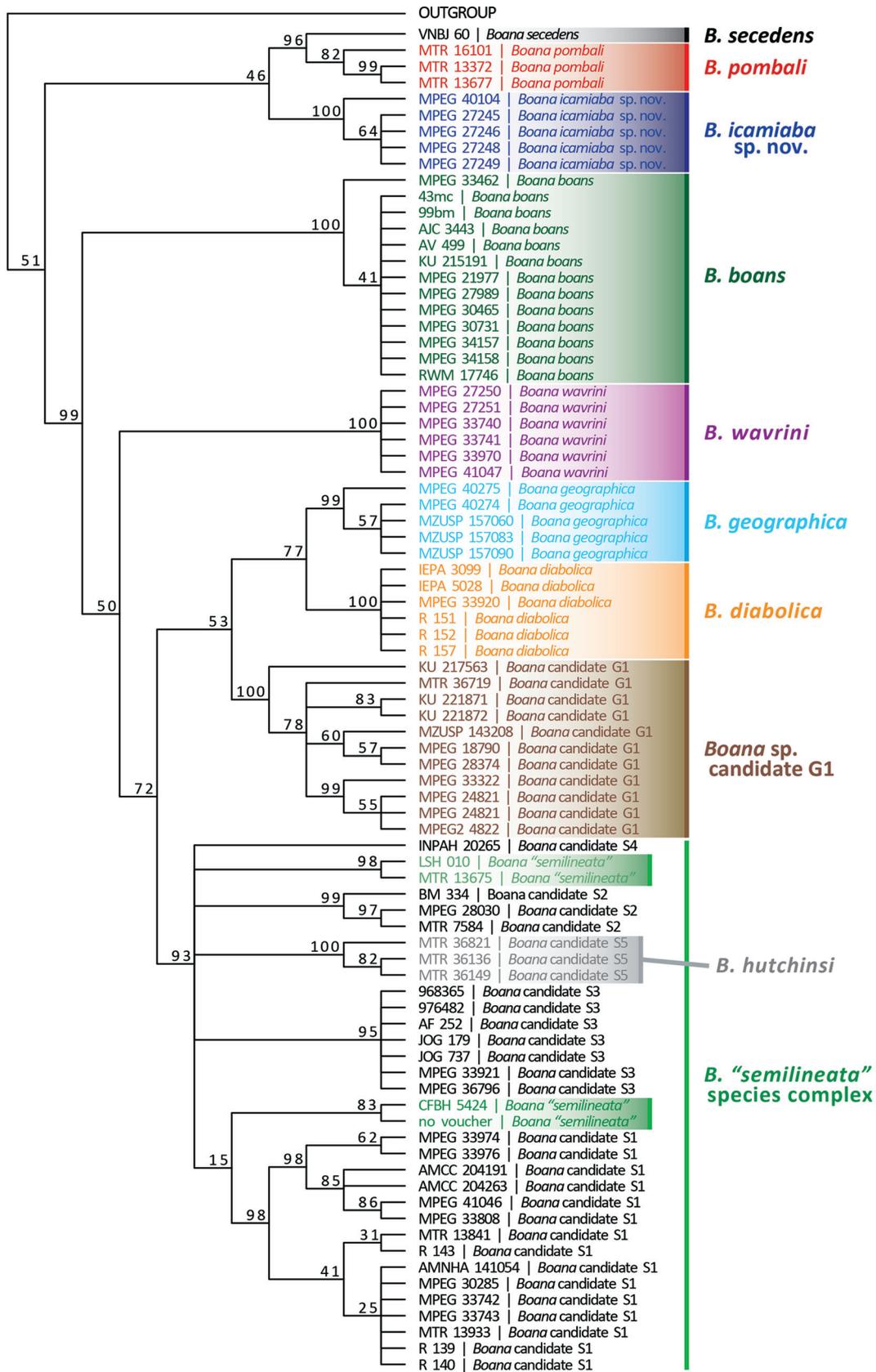
(B) TNT



(C) RAxML



**Figure 1.** Alternative phylogenetic hypotheses inferred for the *Boana semilineata* species group. **(A)** Tree from Fouquet et al. (2016), based on Bayesian Inference—support values are posterior probabilities. **(B)** Parsimony, and **(C)** Maximum Likelihood analyses performed herein—support values are, respectively, jackknife and bootstrap frequencies.



**Figure 2.** Phylogenetic hypothesis for the *Boana semilineata* species group, inferred from two loci (16S and COI) using a parsimony optimization in TNT—strict consensus of 1,066 trees. Outgroup taxa removed for graphic purposes—complete tree given as supplementary information (Data S2). Number above branches are jackknife support values.

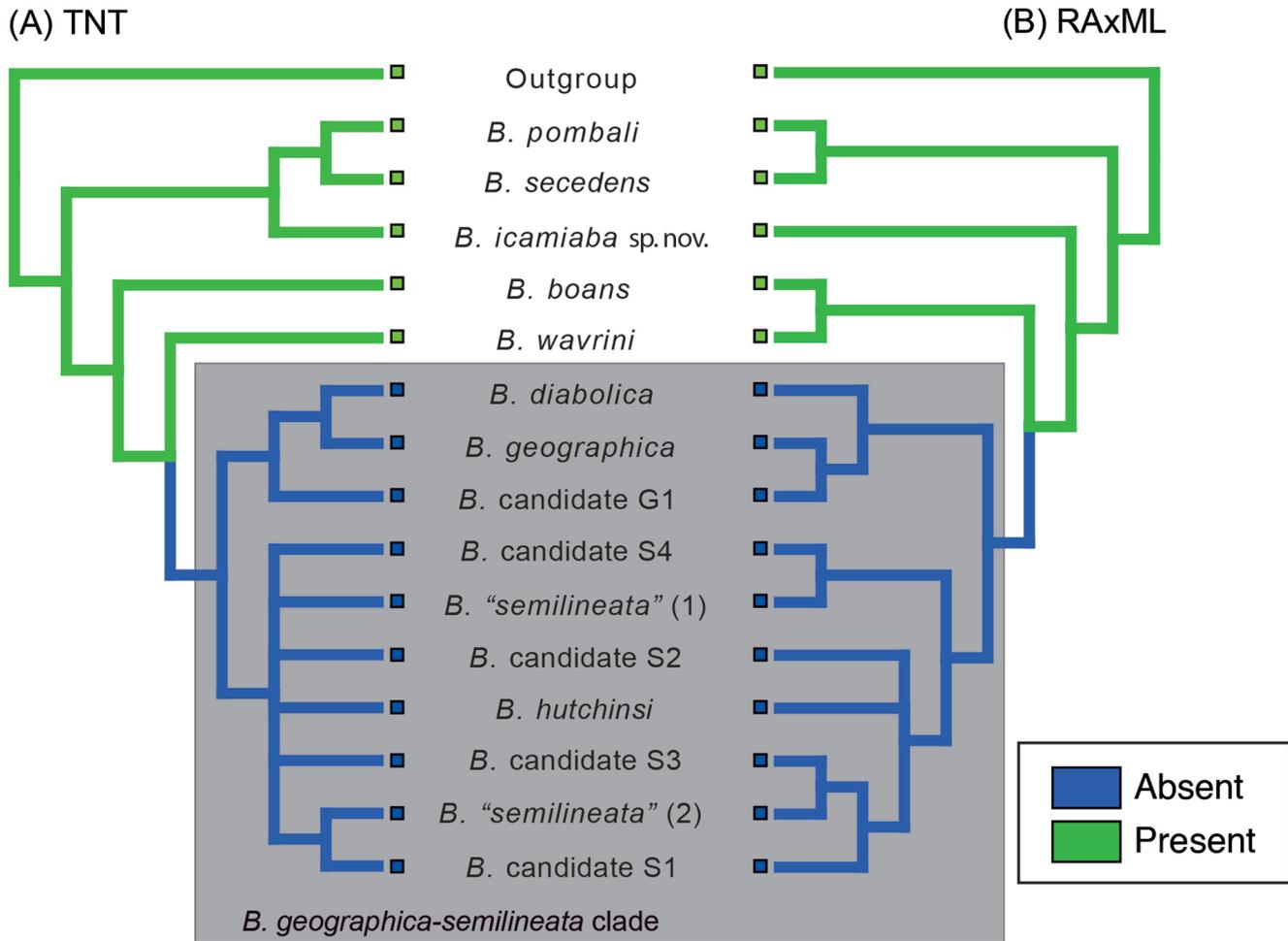
PAR analysis. In both analyses *B. secedens*, currently assigned to the *B. pulchella* species group, is nested inside the *B. semilineata* group where it is strongly supported as the sister species of *B. pombali*. Even with the inclusion of *B. secedens* in the *B. semilineata* group, the clade received low support values in both analyses (ML = 31; PAR = 51). Summaries of the PAR tree are shown in Figs. 1B and 2 and of the ML tree is shown in Fig. 1C. Full PAR and ML trees are available as supplementary information (Data S2–S3).

*Boana secedens* forms a clade with the *B. pombali* samples, but the position of this clade varied between the PAR (Fig. 1B) and ML (Fig. 1C) analyses—forming the sister of an unnamed clade in the PAR tree (Fig. 1B) and the sister of all remaining samples in the ML tree (Fig. 1C). The aforementioned unnamed clade is represented by five samples from eastern Amazonia, Brazil and labeled as *B. geographica* in the MPEG collection (MPEG 27245–27246, MPEG 27248–27249, MPEG 40104; Fig. 2). In addition to its phylogenetic distinctiveness from *B. geographica*, members of this clade have a series of diagnostic phenotypic characters (viz., morphology and advertise-

ment call) that justify their recognition as a distinct species. This confirmed candidate species is described and named below as *B. icamiaba* **sp. nov.**

In the ML tree, *Boana boans* and *B. wavrini* are sister taxa (Fig. 1C), albeit with low support, whereas they do not form a clade in the PAR analysis (Fig. 1B). Samples of *Boana icamiaba* **sp. nov.** were recovered as the sister taxon of the *B. pombali* + *B. secedens* clade in the parsimony analysis (Fig. 1B), whereas they clustered as sister of all species except *B. pombali* + *B. secedens* in the ML analysis (Fig. 1C).

The next more inclusive clade includes a series of named and putatively unnamed taxa (Fouquet et al., 2016) of highly uncertain relationships. The clade includes *Boana geographica*, *B. semilineata*, the recently named *B. diabolica*, and at least six unnamed clades that Fouquet et al. (2016) consider to be candidate species. Both the PAR (Fig. 1B) and ML (Fig. 1C) analyses support the recognition of this clade, which includes topotypic samples of *B. geographica* and *B. semilineata*, the recently described *B. diabolica*, and several putative new species first recog-



**Figure 3.** Parsimony optimization of the presence (green)/absence (blue) of the enlarged prepollux forming a projecting spine in species of the *Boana semilineata* species group. **(A)** TNT, parsimony analysis; and **(B)** RAxML, maximum likelihood topology.

nized by Fouquet et al. (2016: figs. 1–2). Herein, we refer to this clade as the *B. geographica-semilineata* clade, which appears to be supported by at least one morphological synapomorphy: the loss of the prepollical spine in males (Fig. 3). On the other hand, the phylogenetic resolution within the clade is low.

In both PAR and ML *Boana diabolica* is the sister species of *B. geographica*, whereas the two form the sister group of an unnamed clade (candidate G1). This clade (candidate G1 (*B. diabolica* + *B. geographica*)) form the sister clade of all remaining candidate species from Fouquet et al. (2016) plus the samples of *B. hutchinsi* (candidate S5 of Fouquet et al., 2016) and our samples of *B. semilineata*. Here, it is important to highlight that the four samples included in the analyses as *B. semilineata* are not monophyletic in the ML analyses (Fig. 1C; unresolved in the PAR analysis). Two specimens from Espírito Santo do not cluster with topotypic samples of *B. semilineata* from Rio de Janeiro. The resolution of this clade (candidates S1–S4

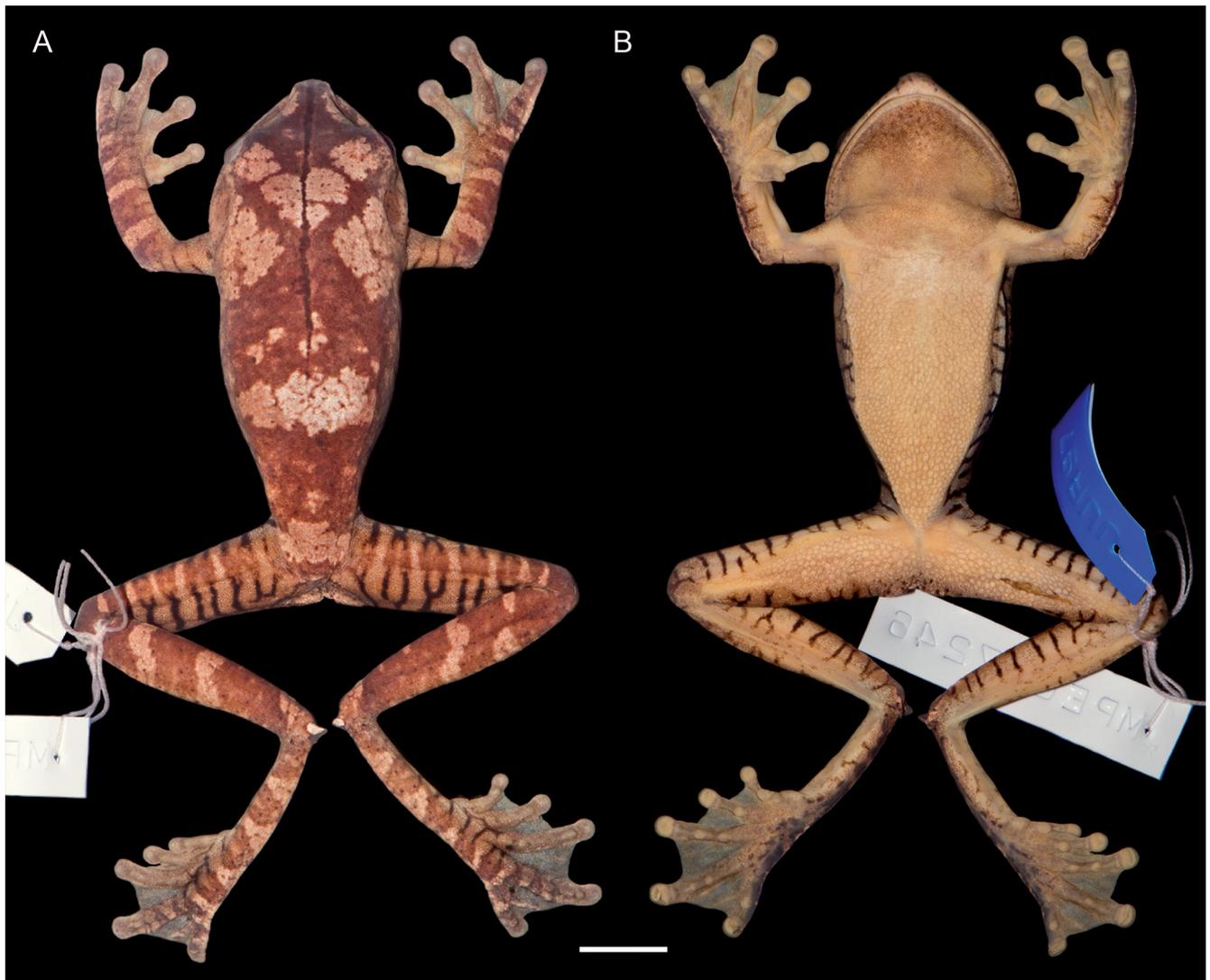
+ *B. hutchinsi* + *H. "semilineata"*) is very low, with a polytomy in the PAR analysis (Figs. 1A, 2) and very low support values in the ML analysis (Fig. 1C). Due to the lack of resolution and consequent taxonomic problems involved with this clade, we refer to it as the *B. semilineata* species complex (Fig. 1B). Our analyses suggest that *B. hutchinsi* is part of this complex.

## TAXONOMY

### *Boana icamiaba* sp. nov. (Figs. 4–6, 9A)

#### Holotype (Figs. 4–5)

**MPEG 27246** (field number JUR 91), an adult male, very well preserved. BRAZIL, state of Pará, municipality of Juruti, Capiranga (02°36'34"S, 56°11'46"W), 01 March 2008, collected by S. Neckel-Oliveira et al. Speci-



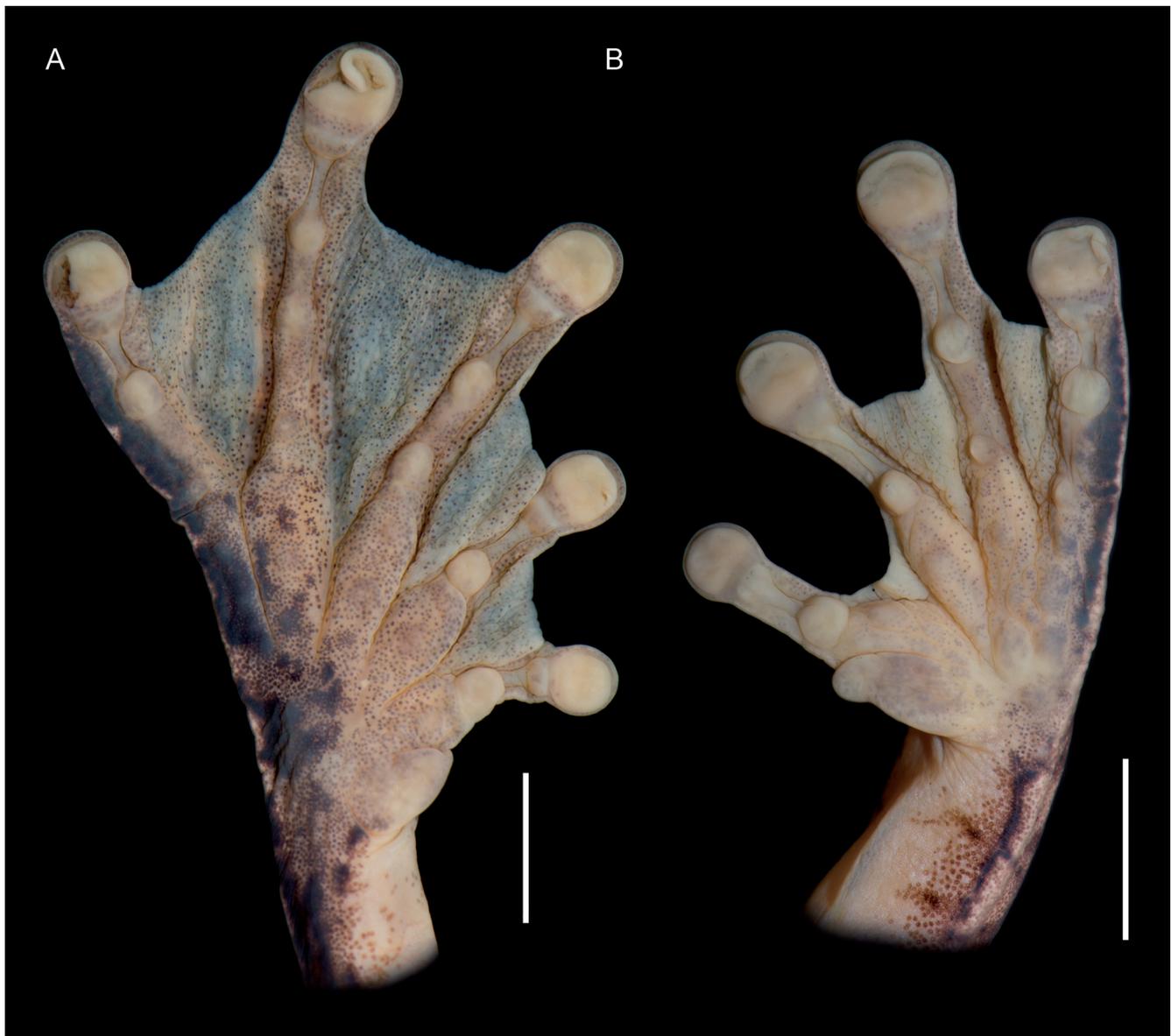
**Figure 4.** Holotype of *Boana icamiaba* sp. nov. (MPEG 27246). (A) Dorsal and (B) ventral views. Scale bar = 10 mm.

men sequenced, with Genbank accession numbers (16S, MG840874; COI, MG840916)

### Paratypes

**MPEG 27245** (Fig. 2; 16S, MG840873, COI, MG840915), **MPEG 27248** (16S, MG840875, COI, MG840917), and **MPEG 27249** (16S, MG840876, COI, MG840918), respectively male, female and male, collected with the holotype. **MPEG 37060**, BRAZIL, Pará, Juruti, Adutora (02°27'09"S, 56°10'54"W), 25 June 2010, collected by F.M. Silva, A. Menks. **MPEG 40108–40110** (MPEG 40108 illustrated in Fig. 6B, MPEG 40100 illustrated in Fig. 6A), adult males, BRAZIL, Pará, municipality of Santarém, Floresta Nacional do Tapajós (03°02'51"S, 54°58'46"W), from 29 February 2012 to 08 March

2012, collected by Y.O.C. Bitar and L. Pinheiro. **MPEG 40100–40104** (MPEG 40104: 16S, MG840877), adult males, BRAZIL, Pará, municipality of Santarém, Reserva Extrativista Tapajós-Arapiuns, Tucuatuba (03°05'37"S, 55°32'08"W), 22 March 2012–13 April 2012, collected by A. D'Angiollala and V. Tasso. **MPEG 40107**, female, BRAZIL, Pará, municipality of Itaituba, Comunidade Jatobá (05°03'23"S, 56°53'04"W). **MPEG 40105–40106**, respectively juvenile and adult female, BRAZIL, Pará, municipality of Itaituba, Vila do Penedo (05°27'17"S, 57°04'42"W). **MNRJ 89837**, female, BRAZIL, Pará, municipality of Altamira (03°19'28"S, 52°15'55"W), 30 August 2015, collected by S. dos Anjos and L. Wronski. **MNRJ 89836, 89838–89839**, adult males, BRAZIL, Pará, municipality of Altamira (03°19'28"S, 52°15'55"W), 30 August 2015, collected by R.M. Oliveira, E. Oliveira,



**Figure 5.** Holotype of *Boana icamiaba* sp. nov. (MPEG 27246). Details of (A) right feet and (B) left hand. Scale bars = 5 mm.

J. Xavier and S. dos Anjos. **MNRJ 90997–91000**, adult males, BRAZIL, Pará, municipality of Altamira, Pedra da Cachoeira (03°19'13"S, 52°19'53"W), 20 September 2015, collected by R.M. Oliveira, L. Wronski, M. Penhacek.

### Etymology

A noun in apposition, given as homage in memory of the legendary Amazonian female warriors, the Icamíabas. These warriors, first described by friar Gaspar de Carvalho (a member of the Francisco Orellana expedition; Medina, 1894), were members of isolated tribes composed of women only. So far as we are aware this is the second taxon to honor to the icamiabas, the other one being a

hooded tickspider, *Cryptocellus icamiabas* Tourinho and Azevedo, 2007.

### Characterization and diagnosis

A member of the *Boana semilineata* group based on its phylogenetic position, and by the presence of a reticulated palpebral membrane. The combination of the following characters can be used as a diagnosis for the new taxon: **(1)** SVL of males 51.5–62.4 mm ( $n = 19$ ), of females 51.2–64.2 mm ( $n = 3$ ); **(2)** presence of reticulated palpebral membrane; **(3)** enlarged prepollex, forming a projecting spine; **(4)** nuptial pads absent; **(5)** fingers III–IV and IV–V extensively webbed; **(6)** calcar appendage well developed; **(7)** presence of a tarsal fringe; **(8)** ab-



**Figure 6.** Paratypes of *Boana icamiaba* **sp. nov.** in life. **(A)** MPEG 40110, and **(B)** MPEG 40108 from Floresta Nacional do Tapajós, Pará, Brazil. **(C–D)** MNRJ 90998, from Altamira, Pará, Brazil.

domen immaculate (uniformly colored, spots absent); **(9)** flanks with numerous dark brown or black stripes; **(10)** anterior and posterior surface of thigh with numerous dark brown or black stripes; **(11)** undersurfaces of body bright orange or reddish in life; **(12)** advertisement call with one or two notes; **(13)** pulses per note varying from 29–74; **(14)** call dominant frequency varying from 1378–2067 Hz.

### Comparison with other taxa

Fouquet et al. (2016) recognized six candidate, unnamed, species in the group and provided a brief morphological characterization of one of them. Inasmuch as we did have access to specimens from most of the unnamed candidate species recognized therein, we feel that detailed comparisons with these unnamed taxa recognized by Fouquet et al. (2016) would not be very useful until they are actually named and formally recognized. For diagnostic purposes, it suffices to say that all candidate species identified in Fouquet et al. (2016) are members of the *Boana geographica-semilineata* clade. The presence of the following characters immediately separates *B. icamiaba* **sp. nov.** from all species in the *B. geographica-semilineata* clade: **(1)** presence of a prepollex developed into a spine (absent in members of the *B. geographica-semilineata* clade); **(2)** absence of nuptial pads (present). Additional pairwise comparisons with named species of the *B. semilineata* group are given below.

### Morphological comparisons

*Boana icamiaba* **sp. nov.** differs from *B. boans* by its much smaller size (SVL of male *B. boans* reaches 132 mm; Kok and Kalamandeen, 2008), flanks with dark stripes (faint, wide bands instead of stripes in *B. boans*), undersurfaces of body bright orange in life (lavender or green in *B. boans*).

The new species differs from *Boana diabolica* by its larger size (SVL 38.5–48.0 mm in males of *B. diabolica*), presence of an enlarged prepollex forming a projecting spine (absent in *B. diabolica*), absence of dark nuptial pads (present in *B. diabolica*), presence of transversal dark stripes on flanks (flanks black with white speckles), and presence of dark stripes on the posterior surface of thigh (absent, uniformly colored in *B. diabolica*).

It differs from *Boana geographica* by its larger size (max SVL 46 mm in males, 48.6 in females *B. geographica*; Fouquet et al., 2016); presence of an enlarged prepollex forming a projecting spine (absent in *B. geographica*), absence of dark nuptial pads (present in *B. geographica*), flanks with dark lines (usually bluish or black with white speckles in *B. geographica*), and abdomen always immaculate (usually presenting brown and black spots in *B. geographica*).

It differs from *Boana hutchinsi* by its larger size (SVL 39.3–47.1 mm in males of *B. hutchinsi*; Pyburn and Hall, 1984), presence of an enlarged prepollex forming a projecting spine (absent in *B. hutchinsi*), absence of nuptial pads (present in *B. hutchinsi*), abdomen always immaculate (abdomen with irregular brown or black spots, often forming a ring), and presence of dark stripes on flanks (blackish with white speckles in *B. hutchinsi*).

It differs from *Boana pombali* in having more extensive webbing on hands (usually not reaching distal subarticular tubercles of Fingers IV and V) and the anterior surface of thigh possessing dark stripes (uniform brown on *B. pombali*). Although there is some overlap in size, the SVL of *B. icamiaba* **sp. nov.** is usually larger (51.2–64.2 mm in males *B. icamiaba*; 49.0–55.6 mm in males of *B. pombali*—the mean size for males *B. icamiaba* **sp. nov.** is 57.5 mm, larger than maximum SVL recorded for *B. pombali*).

It differs from *Boana secedens* by the presence of dark stripes on anterior surface of thigh (absent or only faint lines in *B. secedens*), and extensive webbing between fingers (rudimentary in *B. secedens*). Although there is some overlap in size, the SVL of *B. icamiaba* **sp. nov.** is usually larger (51.2–64.2 mm in males *B. icamiaba*; 55–57.1 mm in males of *B. secedens* [Lutz, 1963]—the mean size for males *B. icamiaba* **sp. nov.** is 57.5 mm).

It differs from *Boana semilineata* by the presence of an enlarged prepollex forming a projecting spine (absent in *B. semilineata*), absence of dark nuptial pads (present in *B. semilineata*), abdomen always immaculate (may have brown and black spots in several specimens of *B. semilineata*), and flanks with dark stripes (usually black with white speckles in *B. semilineata*).

It differs from *Boana wavrini* by its smaller size (SVL 89–113.0 mm in adult males of *B. wavrini*; Hoogmoed, 1990), more extensively webbed hands (less webbing in *B. wavrini*), well-developed calcar appendage (less conspicuous in *B. wavrini*), and flanks with dark stripes (faint, wide bands instead of stripes in *B. wavrini*).

### Bioacoustic comparisons

A higher dominant frequency distinguishes the call of *Boana icamiaba* **sp. nov.** from that of *B. boans* (440–980 Hz; Duellman, 1970, 1978; Zimmerman and Bogart, 1984), *B. diabolica* (1,110–1,190 Hz; Fouquet et al., 2016), *B. geographica* (883–1,023 Hz; Fouquet et al., 2016), *B. semilineata* (865–1,235 Hz; Lingnau and Bastos, 2003), and *B. wavrini* (673–730 Hz; Hoogmoed, 1990). Information about dominant frequency in *B. hutchinsi* calls is not available. Further, advertisement calls of *B. icamiaba* **sp. nov.** have longer notes than *B. geographica* and *B. diabolica* calls (0.04–0.05 s and 0.04–0.07 s, respectively; Fouquet et al., 2016) and shorter notes than *B. boans* and *B. wavrini* calls (0.18–0.28 s and 0.46–0.71 s respectively;

**Table 1.** Advertisement call traits of *Boana icamiaba* sp. nov. from Altamira, Pará, Brazil. Only data for the one-note call type are shown. Data presented as: minimum value–maximum value (mean ± SD).

	MNRJ 89838	MNRJ 89839	Unvouchered	MNRJ 90998	Global
<b>n calls</b>	4	14	23	24	65
<b>SVL (mm)</b>	57.1	54.3	-	53.8	53.8–57.1
<b>Temperature (°C)</b>	26.6	26.6	25.5	25.5	26.6–25.5
<b>Humidity (%)</b>	80	80	85	85	80–85
<b>Call duration (s)</b>	0.1–0.107 (0.104 ± 0.0003)	0.089–0.112 (0.1 ± 0.006)	0.085–0.1 (0.092 ± 0.004)	0.096–0.118 (0.106 ± 0.005)	0.085–0.118 (0.1 ± 0.007)
<b>Pulses/call</b>	44–46 (45 ± 1)	34–41 (37 ± 2)	31–38 (35 ± 2)	29–38 (34 ± 3)	29–46 (36 ± 3)
<b>Interval between calls (s)</b>	29.191–59.327 (10.371 ± 9.084)	4.913–42.29 (14.96 ± 9.228)	3.963–18.853 (7.864 ± 3.06)	2.156–13.471 (6.745 ± 2.789)	2.156–59.327 (10.371 ± 9.084)
<b>Dominant frequency (Hz)</b>	1,722.7	1,378.1–2,067.2 (1,747.2 ± 163)	1,722.7–2,067.2 (1,962.3 ± 162)	1,378.1–2,067.2 (1,880.5 ± 202)	1,378.1–2,067.2 (1,871 ± 192)

**Table 2.** Advertisement call traits of *Boana icamiaba* sp. nov. from Altamira, Pará, Brazil. Summarized data for the two-note call type, emitted sporadically by two males. Data presented as: minimum value–maximum value.

<b>n calls</b>	5
<b>SVL (mm)</b>	54.3
<b>Temperature (°C)</b>	26.6
<b>Humidity (%)</b>	80
<b>Call duration (s)</b>	0.461–0.579
<b>First note duration (s)</b>	0.091–0.098
<b>Second note duration (s)</b>	0.096–0.112
<b>First note pulses</b>	33–39
<b>Second notes pulses</b>	31–40
<b>Pulses/call</b>	67–74
<b>Interval between notes (s)</b>	0.25–0.382
<b>Interval between calls (s)</b>	7.746–16.03
<b>Dominant frequency (Hz)</b>	1,722.7–2,067.2

Duellman, 1970, 1978; Zimmerman and Bogart, 1984; Hoogmoed, 1990). Advertisement calls of *B. hutchinsi* comprise more notes/call (2–8; Pyburn and Hall, 1984).

The only species in the *B. semilineata* species group that has a call that resembles that of *B. icamiaba* sp. nov. is *B. pombali*; however, their calls can be distinguished by the number of pulses per note: 29–36 in *B. icamiaba* sp. nov. and 6–13 in *B. pombali* (Caramaschi et al., 2004). The call of *B. secedens* has not been described. A summary of call parameters of *B. icamiaba* sp. nov. is given in Tables 1 and 2, whereas a comparative summary is given in Table 3.

### Description of holotype (MPEG 27246)

An adult male, SVL 57.2 mm. Head wider than long (HW/HL 1.06), widest at corner of the mouth; snout rounded in both dorsal and lateral views; inter-orbital distance more than three times the distance between the nostrils (IOD/IND 3.25); eye diameter smaller than eye-nostril-distance (END/ED 1.46); *canthus rostralis* slightly concave; loreal region conspicuously concave; nostrils protuberant, directed dorsolaterally; internarial area slightly convex; interorbital region flat. Eyes large and

**Table 3.** Advertisement call parameters in the *Boana semilineata* species group. Data presented as minimum value–maximum value (mean ± SD).

	Call duration (s)	Note duration (s)	Notes/call	Pulses/call	Pulses/note	Interval between calls (s)	Interval between notes (s)	Dominant frequency (Hz)	Source
<i>B. icamiaba</i>	0.08–0.57 (0.13 ± 0.11)	0.08–0.11 (0.1 ± 0.007)	1–2	29–74 (38 ± 9)	29–46 (36 ± 3)	12.15–59.32 (10.57 ± 8.50)	0.25–0.38 (0.32 ± 0.05)	1,378–2,067 (1865 ± 189)	This study
<i>B. boans</i>	0.18–0.28	0.18–0.28	1			0.57–0.68	0.57–0.68	440–980	Duellman (1970, 1978) Zimmerman and Bogart (1984)
<i>B. diabolica</i>	0.24–0.39 (0.33)	0.04–0.07 (0.05)	2–3			3.06–10.72 (6.01)	0.1–0.13 (0.011)	1,110–1,190 (1,160)	Fouquet et al. (2016)
<i>B. geographica</i>	0.36–0.42 (0.39)	0.04–0.05 (0.05)	3–4			2.9–5.2 (4.83)	0.07–0.1 (0.08)	883–1023 (943.5)	Fouquet et al. (2016)
<i>B. hutchinsi</i>	0.25–1.05 (0.68)	0.1	2–8						Pyburn and Hall (1984)
<i>B. pombali</i>	0.08–0.51 (0.43 ± 0.04)	0.07–0.11	1–2		6–13		0.18–0.32 (0.25 ± 0.04)	1,076–2,196	Caramaschi et al. (2004)
<i>B. semilineata</i>	0.10–0.18 (0.15 ± 0.003)	0.01–0.09 (0.04 ± 0.01)	2–3		3–19			865–1,235 (1,013 ± 189)	Lignau and Bastos (2003)
<i>B. wavrini</i>	0.46–0.71 (0.55)	0.46–0.71 (0.55)	1	30–34 (31)	30–34 (31)			673–730 (697)	Hoogmoed (1990)

protuberant, not much larger than tympanum diameter (ED/TD 1.40); inter-orbital region concave; pupil horizontally elliptical; palpebral membrane mostly transparent, with pigmented reticulations, its border pigmented. Supratympanic fold weak; tympanic membrane not visible; completely covered by skin but tympanic annulus is easily distinguishable. Choanae small, elliptical, not concealed by palatal shelf. Vomerine odontophores present; left and right series divided by a much narrow gap (12 odontophores on each side); tongue ovoid, with nearly one third of the posterior end not adhered to floor of mouth. Vocal slits present, extending from anterior base of tongue almost to the angle of the jaw; vocal sac indistinguishable.

Arms robust, slightly hypertrophied; conspicuous dermal fold (fringe) on external margin of forearm and hand, extending from elbow to tip of Finger V; fold more evident on forearm, becoming less pronounced on hand; axillary membrane absent. Finger tips round; enlarged disks present and well developed in all fingers; relative lengths of fingers II < III < V < IV. Subarticular tubercles round, well developed and easily visible; narrower than finger width; one subarticular tubercle on Fingers II and III, two subarticular tubercles on fingers IV and V; distal subarticular tubercles of Fingers IV and V larger, roughly twice as wide as the proximal; prepollex well developed, forming a curved spine, tip not exposed; outer metacarpal indistinguishable. Webbing between Fingers II and III is basal, more extensive between III–IV and between IV–V; webbing formula **II** 2<sup>+</sup>–2<sup>+</sup> **III** 1<sup>+</sup>–2 **IV** 1<sup>½</sup>–1<sup>¾</sup> **V** (left), **II** 2–2<sup>-</sup> **III** 1–2 **IV** 1<sup>½</sup>–1<sup>¾</sup> **V** (right).

Legs long and slender. Calcaneus with a well-developed calcar appendage; tarsal fringe present on external margin of tarsus, extending from calcaneus to tip of Toe V. All toes well developed, bearing expanded, round, well-developed disks; relative lengths of Toes I < II < V < III < IV. Subarticular tubercles round and well developed; one subarticular tubercle on Toes I, II and V; two subarticular tubercles on Toes III and IV; many supernumerary tubercles visible; inner metatarsal tubercle flat, slightly elliptical; outer metatarsal tubercle not visible. Webbing extensive between all toes; webbing formula **I** 0<sup>+</sup>–1<sup>+</sup> **II** 0<sup>+</sup>–1<sup>½</sup> **III** 0<sup>+</sup>–1<sup>½</sup> **IV** 1<sup>¾</sup>–0<sup>+</sup> **V** (left), **I** 0<sup>+</sup>–1<sup>½</sup> **II** 0<sup>+</sup>–1<sup>-</sup> **III** 0<sup>+</sup>–1<sup>½</sup> **IV** 1<sup>¾</sup>–0<sup>+</sup> **V** (right).

Dorsal and lateral skin smooth, undersurfaces granular; more heavily granular in abdominal and femoral regions.

### Variation in type series

The type series is rather homogeneous in general morphology, but specimens vary conspicuously in color patterns. The diagnostic characters are present in all specimens analyzed, including some related to coloration (dark lines on flanks and posterior surface of thigh). The dorsal color pattern varies from almost uniform to con-

spicuously marbled (similar to holotype). A middorsal stripe is always present, extending from the snout and ending at variable point of the dorsum (but never reaching the cloaca). An X-shaped brown mark is often present in the scapular region. Some specimens have large dark brown blotches or white spots. Minute black spots are present all over the dorsum of most specimens. Arms usually have dark stripes posteriorly; forearms have dark bands of variable thickness. The calcar appendage is commonly white but may follow the same pattern present on legs. The lines on the posterior surface of the thigh are always present (dark brown or black), continuous or not with the lines on the anterior surface of the thigh; even when continuous, the lines on the anterior surface may become thicker.

Specimens from Rio Xingu (Altamira) are on average smaller than those from most western populations. Morphometric variation among specimens in the type series is given as supplementary information (Table S2).

### Advertisement call

The new species presents two different call types: a one-note call type ( $n = 65$ ; 92%), and a two-notes call ( $n = 5$ ; 8%). The two-note call type was rarely emitted and heard from two out of four males (MNRJ 89839 and an unvouchered specimen). Below, we describe both call types.

The one-note advertisement call type (Table 1) of *Boana icamiaba* **sp. nov.** consists of multipulsed notes (29–46 [ $36 \pm 3$ ] pulses/call;  $n = 65$ ) emitted at irregular intervals varying from 2.15–59.32 s [ $10.371 \pm 9.084$ ]. Call duration (= note duration) ranges from 0.08–0.12 s [ $0.1 \pm 0.007$ ] and presents a dominant frequency that varies from 1378.1–2067.2 Hz.

Two-notes call (Table 2) duration ranges from 0.46–0.58 s ( $0.53 \pm 0.053$ ;  $n = 5$ ) emitted at intervals which varies from 7.74–16.03 s ( $11.84 \pm 3.19$ ). Notes ranged from 0.09–0.11 s ( $0.1 \pm 0.006$ ) with interval between notes varying from 0.25–0.38 s ( $0.32 \pm 0.06$ ), call dominant frequency from 1,722.7–2,067.2 Hz ( $1,894.9 \pm 77$ ) and pulses per call from 67–74 ( $71 \pm 3$ ).

Calls do not present frequency modulation or harmonic structure. The waveform and spectrogram of one call are given in Fig. 7.

### Distribution and natural history (Fig. 8)

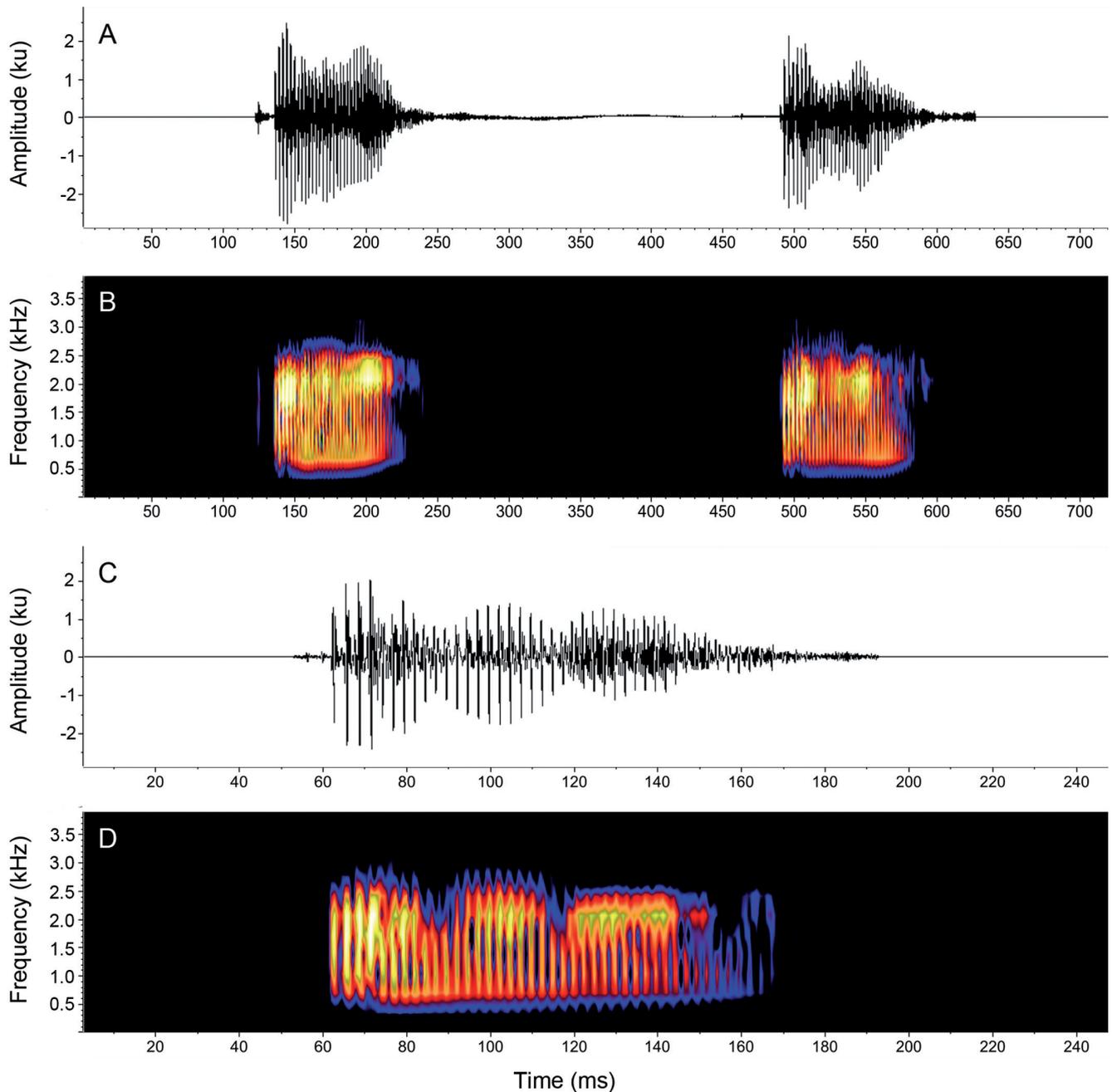
The new species is known from scattered localities in the mid–lower *Rio Madeira–Rio Tapajós* and lower *Rio Tapajós–Rio Xingu* interfluves. At *Rio Tapajós* (Área de Proteção Ambiental do Tapajós, locality D in Fig. 8) specimens were closely associated with streams 12–30 m across. Reproductively active males were observed calling on palm leaves up to 3 m above the water. At *Rio Xingu*

(Altamira, locality G in Fig. 8), the species was usually collected near fast-flowing rocky streams. Males were observed calling from vegetation, usually perched 0.5–2.5 m above the ground.

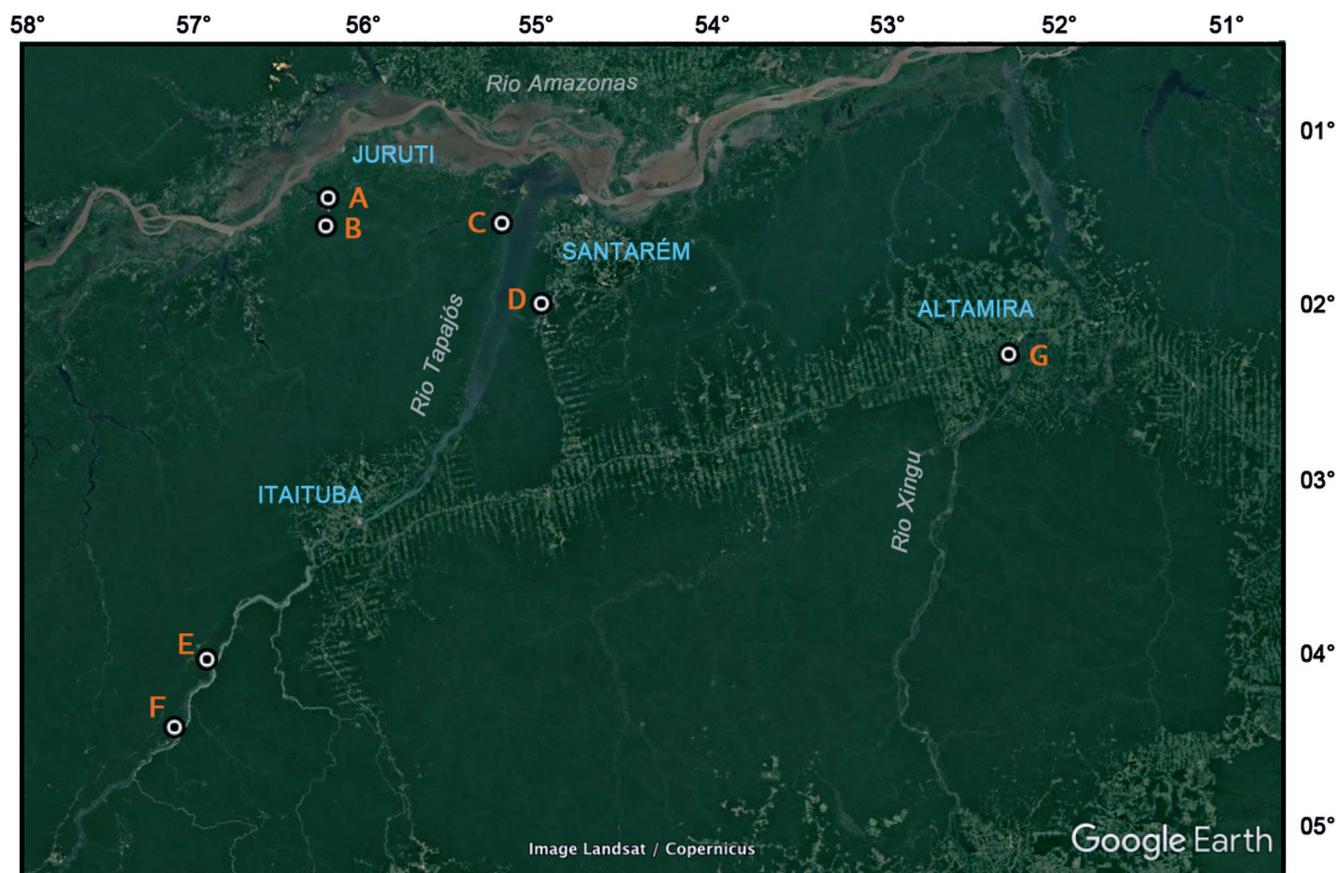
### DISCUSSION

The *Boana semilineata* species group sensu Faivovich et al. (2005) is not monophyletic. Our analyses corroborated the inclusion of *Boana hutchinsi*, *B. pombali* and

*B. wavrini* in the group, as previously suggested on the basis of morphological similarities and tested using molecular data for the first time herein. However, *Boana secedens*, currently referred to the *B. pulchella* species group, was recovered as the sister species of *B. pombali*. Therefore, to remedy the paraphyly of the group, *B. secedens* is transferred from the *B. pulchella* group to the *B. semilineata* group. Faivovich et al. (2005) suggested that the presence of a reticulated palpebral membrane is a possible morphological synapomorphy of this group. Nonetheless, Faivovich et al. (2005) putatively included *B. pombali* in



**Figure 7.** Advertisement call of *Boana icamiaba* sp. nov. (A–B) One-note call type, (A) waveform, and (B) spectrogram. (C–D) Two-notes call type, (C) waveform and (D) spectrogram.



**Figure 8.** Geographic distribution of *Boana icamiaba* **sp. nov.** (A) Capiroanga, *type locality*; (B) Aduatora; (C) Reserva Extrativista Tapajós-Arapiuns; (D) Área de Proteção Ambiental do Tapajós; (E) Comunidade Jatobá; (F) Vila Penedo; (G) Altamira. Map generated using Google Earth (Map data ©2016 Google/Landsat/Copernicus).

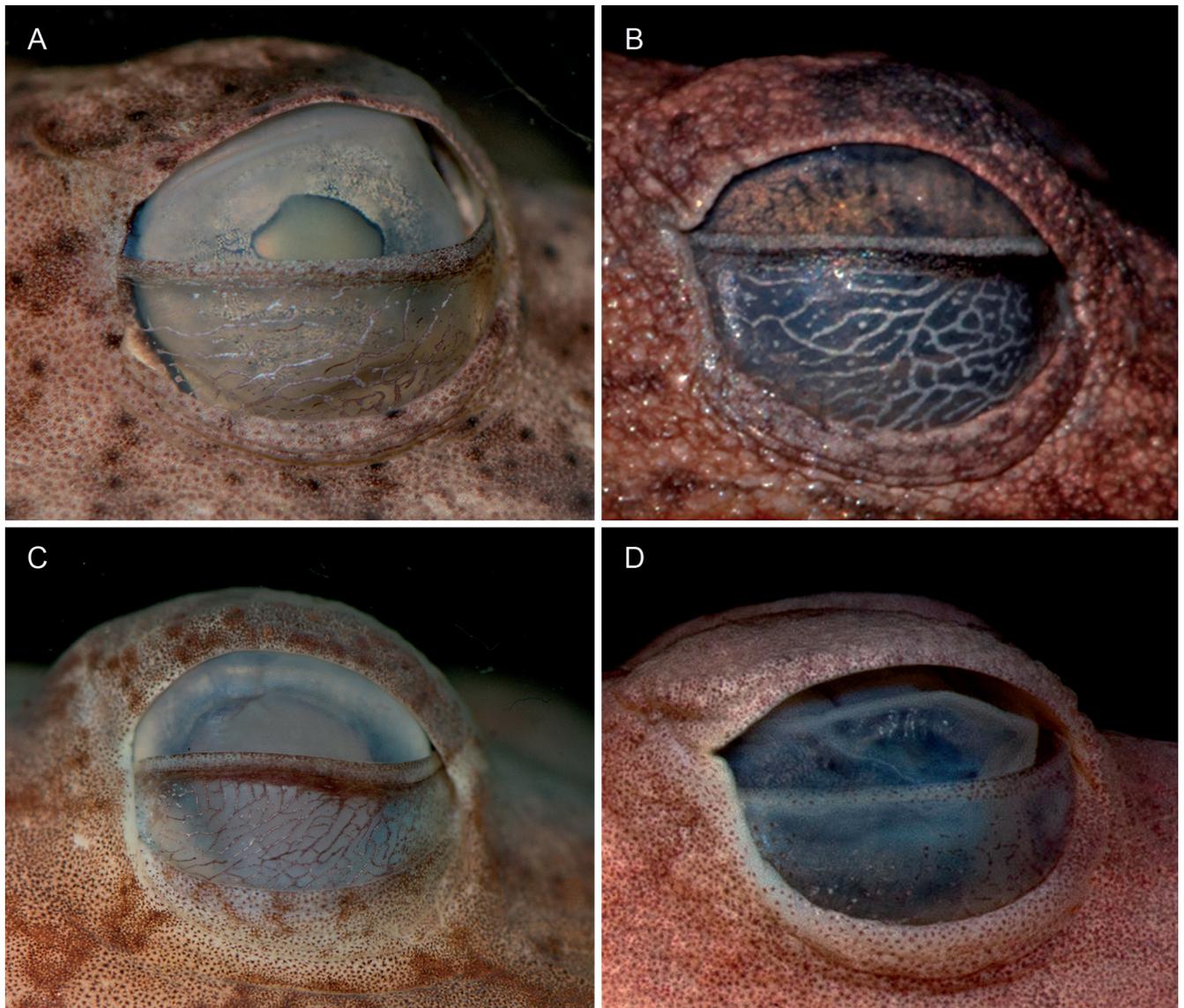
the group “with the caveat that it lacks the reticulated palpebral membrane.” That interpretation by Faivovich et al. (2005) was likely based on the original description of this taxon, which states that *B. pombali* lacks reticulation (“ausência de vermiculações na pálpebra inferior”; Caramaschi et al., 2004: 5). However, examination of several specimens of *B. pombali* (including the holotype: Fig. 9D), revealed that the lower palpebral membrane is reticulated in *B. pombali*, as well as in all in all other species of the group (Fig. 9). Therefore, we corroborate the presence of the reticulated lower membrane as a synapomorphy of this group, as correctly suggested by Faivovich et al. (2005; with instances of homoplasy in other *Boana*, i.e., *B. microderma*).

### Cryptic diversity in the *Boana semilineata* species group

Our analyses support the recognition of several unnamed clades in the *Boana semilineata* species group. Several of these clades were already identified by Fouquet et al. (2016), but one was not—we named this clade as *Boana icamiaba* **sp. nov.** No samples of this taxon were

included in the analysis of Fouquet et al. (2016). Of the additional candidate species reported by Fouquet et al. (2016), our phylogenetic analyses unambiguously corroborate the recognition of candidate G1 as an unnamed clade. Moreover, upon the examination of voucher specimens from Fouquet et al. (2016), we have identified members of their candidate G5 as *B. hutchinsi*. In contrast, candidate species S1–S4 of Fouquet et al. (2016) could not be corroborated as confirmed unnamed species in our analyses. These candidate species were recovered as part of a largely unresolved clade that also involves *B. hutchinsi* and a likely paraphyletic *B. semilineata*. The resolution of this problem will require an improved phylogenetic matrix (e.g., additional samples and genes) and the use of integrative methods (e.g., more robust morphometric and bioacoustic analyses) and might require a thorough systematic revision.

Fouquet et al. (2016) effectively described the taxonomic conundrum involving the two species described and named by Spix (1824): *Boana geographica* and *B. semilineata*. The latter was originally described as a variety (*var. sive*) of the former but has usually been recognized as a distinct taxon (Silveira and Caramaschi, 1989; D’Heursel and de Sá, 1999). The fact that both of Spix’s holotypes



**Figure 9.** Reticulation in the palpebral membrane in members of the *Boana semilineata* species group. The presence of this character state is a synapomorphy of the group. **(A)** *Boana icamiaba* sp. nov., MPEG 27246, holotype; **(B)** *B. geographica*, MZUSP 157091; **(C)** *B. boans*, MCP 11394; **(D)** *B. pombali*, MNRJ 27369, holotype.

(*B. geographica* ZSM 35/0, and *B. semilineata* ZSM 47/0) are now apparently lost (Hoogmoed and Gruber, 1983; Glaw and Franzen, 2006; Fouquet et al., 2016) and that extensive morphological variation exists among populations of *B. geographica* have only added more confusion to the taxonomy of both species (Duellman, 1973; Silveira and Caramaschi, 1989; Fouquet et al., 2016). In their phylogeny, Fouquet et al. (2016) included a specimen recently collected in Tefé (type locality of *B. geographica*) and two samples from the Atlantic Forest (Rio de Janeiro: type locality of *B. semilineata*). The phylogenies of Fouquet et al. (2016) and the one presented herein leave little doubt that *B. geographica* and *B. semilineata* are in fact distinct taxa (Figs. 1–2), but it also highlights that the precise species delimitation of both these taxa is still complicated. We agree with Fouquet et al. (2016) that their sample

from Tefé agrees very well with the description of *Hyla geographica* by Spix (1824) and can be used as a reference of a typical *B. geographica*. On the other hand, the status of *B. semilineata* is more difficult to assess with currently available data. We discuss some of these issues below.

Our analysis recovered, within the *Boana semilineata* species group, a clade including topotypic specimens of *B. geographica* and *B. semilineata*, *B. diabolica*, *B. hutchinsi*, and several putatively unnamed species. This clade (the *B. geographica*-*semilineata* clade) is supported by the loss of the prepollex and might include many unnamed species (Fig. 3). Within the clade there is one important split that leads to two branches with distinct taxonomic problems involved in each of them.

The first branch includes at least three species.

**(1)** *Boana geographica*: This clade includes the topotypic

sample from Tefé, Amazonas, Brazil (MZUSP 157060; GB-058 in Fig. 2) and several other samples that we assign tentatively to this species. Fouquet et al. (2016) suggested some phenotypic diagnostic characters for *B. geographica* including some larval and bioacoustic characters. On the other hand, the precise limits of the geographical distribution of *B. geographica* is not known. We have included three samples recovered as members of *B. geographica* that were not used by Fouquet et al. (2016) and that increase the geographical range of this lineage to Peru (Madre de Dios) and onto the state of Mato Grosso in Brazil (Comodoro). **(2)** *B. diabolica*: This species was recently named and a series of phenotypic diagnostic characters are available for the clade (Fouquet et al., 2016). The new samples included in our study fall within the known range of the species. **(3)** Candidate species G1: Fouquet et al. (2016) suggested the existence of this unnamed candidate species based on four samples which they included in their analysis. We have recovered several additional populations as part of this clade. At this point, without a much-expanded matrix and integrative analyses, it is impossible to assess whether one or multiple unnamed species are involved, although preliminary analyses of voucher specimens are suggest of more than one taxa (PLVP, unpublished data).

The second branch involves additional taxonomic issues. Because this clade includes the topotypic samples of *Boana semilineata* and the possibility of representing several unnamed taxa (see Fouquet et al., 2016), we refer to this clade as the *B. semilineata* species complex. Fouquet et al. (2016) recognized five lineages as potentially unnamed species (candidates S1–S5) in this clade. The monophyly of these lineages recognized by Fouquet et al., (2016) is corroborated by both the PAR and ML analyses. It is therefore possible that the candidate species identified by Fouquet et al. (2016) actually deserve to be treated as full species. One such candidate species was tentatively identified herein as *B. hutchinsi*, although unambiguous identification will only be possible with the inclusion of topotypic material of *B. hutchinsi* in the phylogeny. Furthermore, the unresolved status of *B. semilineata* (not recovered as monophyletic) renders the recognition of most of these species almost impossible at this point. When arguing for the separation of *B. semilineata* from *B. geographica*, Silveira and Caramaschi (1989) implied that the former is restricted to the Atlantic Forest and the latter is an Amazonian species. This geographical separation was reinforced in D’Heursel and de Sá (1999) and has been widely accepted ever since. Fouquet et al. (2016) included two topotypic samples of *B. semilineata* (from Rio de Janeiro, GB-002 and GB-011; Fig. 2) that formed a monophyletic group in their analysis—these were the only two specimens from the Atlantic Forest included in their analysis. We have included two additional samples from the Atlantic Forest (from Espírito Santo) and, surprisingly, these samples do not form a clade with the samples from Rio

de Janeiro (Fig. 3). This result implies that what we currently recognize as *B. semilineata* might not be monophyletic. This finding has both systematic and biogeographic implications: if the polyphyly of the species *B. semilineata* is confirmed, samples of *B. semilineata* from Espírito Santo (northern Atlantic Forest, LSH 010 and MTR 13675; Fig. 2) may be recognized as another candidate species for this species complex. Additionally, this refutes the hypothesis of Fouquet et al. (2016) that the origin of this species seems to be the result of a recent dispersal from Amazonia—at least two dispersal events would be needed to explain the origin of Atlantic Forest populations.

Six samples from scattered localities within the Guiana Shield and which were included de novo in this study (AMCC 204191, 204263; MPEG 33974, 33976, 33808; M3A133) form the sister clade of samples identified as candidate S1 in Fouquet et al. (2016). Analysis of the voucher specimens indicates a similar overall morphology among members of the two clades. Their geographic ranges also overlap, and we therefore tentatively recognize these samples as part of the unnamed candidate species S1.

### Concluding remarks: cryptic amphibian diversity in the Neotropical rainforests

Although the biodiversity of the Neotropics has been the focus of scientific studies for centuries, vast portions of the region are still underexplored. However, as underexplored regions are becoming more widely sampled and museum specimens are revisited, the discovery and description of new taxa is becoming more common. The incorporation of genetic data in taxonomic studies is also becoming routine, and, as a consequence, several widespread species, especially in Amazonia, have been recently shown to represent complexes of many species (Fouquet et al., 2007, 2012, 2014, 2016; Padial and De la Riva, 2009; Funk et al., 2012; Jungfer et al., 2013; Peloso et al., 2014), several of which remain to be named. The recent surge of advancement in the systematics of the *Boana semilineata* group, with the description of two new species (Fouquet et al., 2016; results presented herein) and recognition of several additional unnamed taxa in the group are just another example of how poor our estimates of frog diversity are in the biome. Additional studies, including extensive fieldwork, and the integrative use of bioacoustic, genetic, and morphological data, are urgently needed to unravel the hidden diversity of amphibians in South America.

### ACKNOWLEDGMENTS

For the loan or access of tissues and specimens housed at those institutions, we thank the curators and staff at AMNH (especially David Kizirian and Lauren Von-

nahme), CFBH (Célio Haddad and Nadya Pupin), KU (Rafe Brown and Luke Welson), MNRJ (José P. Pombal-Jr and Marcos Bilate), MPEG (Ana Prudente), and MZUSP (Taran Grant). We thank Carl J. Franklin (UTA) for the photos of type specimens of *Hyla hutchinsi*. PLVP, AA, and MJS were supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq: grant numbers 313680/2014-0 and 400252/2014-7 to PLVP and AA, 400770/2014-8 to MJS) and the National Geographic Society (WGS 430-16). MTR was supported by CNPq and FAPESP (grants 2003/10335-8 and 2011/50146-6). RMO thanks Biota Projetos e Consultoria Ambiental and Norte Energia S.A. for financial support to his fieldwork in Altamira. We are grateful to everyone (too many to mention individually) who helped in the field, collecting specimens, tissues, or advertisement calls used in this study.

## REFERENCES

- Benson D.A., Cavanaugh M., Clark K., Karsch-Mizrachi I., Lipman D.J., Ostell J., Sayers E.W. 2013.** GenBank. *Nucleic Acids Research* 41:D36–D42. [DOI](#)
- Bioacoustics Research Program. 2011.** Raven Pro: Interactive Sound Analysis Software, Version 1.4. Available from [www.birds.cornell.edu/raven](http://www.birds.cornell.edu/raven).
- Caramaschi U., Pimenta B.V.S., Feio R.N. 2004.** Nova espécie do grupo de *Hyla geographica* Spix, 1824 da floresta Atlântica, Brasil (Amphibia, Anura, Hylidae). *Boletim do Museu Nacional, Rio de Janeiro: Nova Serie, Zoologia* 518:1–14.
- Cope E.D. 1870.** Seventh contribution to the herpetology of tropical America. *Proceedings of the American Philosophical Society* 11:147–169.
- Cope E.D. 1871.** Eighth contribution to the herpetology of tropical America. *Proceedings of the American Philosophical Society* 11:553–559.
- Cruz C.A.G., Peixoto O.L. 1987.** Espécies verdes de *Hyla*: o complexo “*albofrenata*” (Amphibia, Anura, Hylidae). *Arquivos de Universidade Federal Rural do Rio de Janeiro* 8:59–70.
- D’Heursel A., de Sá R.O. 1999.** Comparing tadpoles of *Hyla geographica* and *Hyla semilineata*. *Journal of Herpetology* 33:353–361. [DOI](#)
- Duellman W.E. 1970.** Hylid frogs of Middle America. *Monographs of the Museum of Natural History* 1–2:1–753. [DOI](#)
- Duellman W.E. 1973.** Frogs of the *Hyla geographica* group. *Copeia* 1973:515–533. [DOI](#)
- Duellman W.E. 1978.** The biology of an equatorial herpetofauna in Amazonian Ecuador. *Miscellaneous Publication of the Natural History Museum, The University of Kansas* 65:1–352.
- Duellman W.E., Marion A.B., Hedges B. 2016.** Phylogenetics, classification, and biogeography of the treefrogs (Amphibia: Anura: Arborescens). *Zootaxa* 4104:1–109. [DOI](#)
- Duméril A.M.C., Bibron G. 1841.** *Erpétologie Générale ou Histoire Naturelle Complète des Reptiles*. Tome Huitième. Librairie Encyclopedique de Roret, Paris. [DOI](#)
- Faivovich J., Haddad C.F.B., Garcia P.C.A., Frost D.R., Campbell J.A., Wheeler W.C. 2005.** Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. *Bulletin of the American Museum of Natural History* 294:1–240. [DOI](#)
- Faivovich J., Moravec J., Cisneros-Heredia D., Köhler J. 2006.** A new species of the *Hypsiboas benitezii* group from the Wertern Amazon Basin (Amphibia: Anura: Hylidae). *Herpetologica* 62:96–108. [DOI](#)
- Farris J.S., Albert V.A., Källersjö M., Lipscomb D., Kluge A.G. 1996.** Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12:99–124. [DOI](#)
- Fouquet A., Cassini C.S., Haddad C.F.B., Pech N., Rodrigues M.T. 2014.** Species delimitation, patterns of diversification and historical biogeography of the Neotropical frog genus *Adenomera* (Anura, Leptodactylidae). *Journal of Biogeography* 41:855–870. [DOI](#)
- Fouquet A., Gilles A., Vences M., Marty C., Blanc M., Gemmill N.J. 2007.** Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. *PLoS ONE* 2:e1109. [DOI](#)
- Fouquet A., Loebmann D., Castroviejo-Fisher S., Padial J.M., Orriero V.G.D., Lyra M.L., ... Rodrigues M.T. 2012.** From Amazonia: to the Atlantic forest: molecular phylogeny of Physelaphryninae frogs reveals unexpected diversity and a striking biogeographic pattern emphasizing conservation challenges. *Molecular Phylogenetics and Evolution* 65:547–561. [DOI](#)
- Fouquet A., Martinez Q., Zeidler L., Courtois E.A., Gaucher P., Blanc M., ... Kok P. 2016.** Cryptic diversity in the *Hypsiboas semilineatus* species group (Amphibia, Anura) with the description of a new species from the eastern Guiana Shield. *Zootaxa* 4084:79–104. [DOI](#)
- Funk W.C., Caminer M., Ron S.R. 2012.** High levels of cryptic species diversity uncovered in Amazonian frogs. *Proceedings of the Royal Society B* 279:1806–1814. [DOI](#)
- Glaw F., Franzen M. 2006.** Type catalogue of amphibians in the Zoologische Staatssammlung München. *Spixiana* 29:153–192.
- Goloboff P.A. 1999.** Analysing large datasets in reasonable times: solutions for composite optima. *Cladistics* 15:415–428. [DOI](#)
- Goloboff P.A., Farris J.S., Nixon K.C. 2008.** TNT, a free program for phylogenetic analysis. *Cladistics* 24:774–786. [DOI](#)
- Gray J.E. 1825.** A synopsis of the genera of reptiles and Amphibia, with a description of some new species. *Annals of Philosophy, London, Series 2* 10:193–217.
- Heyer W.R., Rand A.S., Cruz C.A.G., Peixoto O.L., Nelson C.E. 1990.** Frogs of Boracéia. *Arquivos de Zoologia* 231–410. [DOI](#)
- Hoogmoed M.S. 1990.** Resurrection of *Hyla wavrini* Parker (Amphibia: Anura: Hylidae), a gladiator frog from northern South America. *Zoologische Mededelingen* 64:71–93.
- Hoogmoed M.S., Gruber U. 1983.** Spix and Wagler type specimens of reptiles and amphibians in the Natural History Museum in Munich (Germany) and Leiden (the Netherlands). *Spixiana Supplement* 9:319–415.
- Jungfer K.-H., Faivovich J., Padial J.M., Castroviejo-Fisher S., Lyra M.L., Berneck B.V.M., ... Haddad C.F.B. 2013.** Systematics of spiny-backed treefrogs (Hylidae: *Osteocephalus*): an Amazonian puzzle. *Zoologica Scripta* 42:351–380. [DOI](#)
- Katoh K., Misawa K., Kuma K., Miyata T. 2002.** MAFFT: a novel method for rapid multiple sequence alignment based on Fast Fourier transform. *Nucleic Acids Research* 30:3059–3066. [DOI](#)
- Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., ... Drummond A. 2012.** Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. [DOI](#)
- Kok P.J.R., Kalamandeen M. 2008.** Introduction to the taxonomy of the amphibians of Kaieteur National Park, Guyana. *ABCTaxa*, Brussels.
- Lemmon A., Moriarty E. 2004.** The importance of proper model assumption in Bayesian phylogenetics. *Systematic Biology* 53:265–277. [DOI](#)
- Linnaeus C. 1758.** *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentialibus, synonymis, locis, Tomus I. Editio decima, reformata*. Laurentiis Salvii, Holmiae. [DOI](#)
- Lingnau R., Bastos R.P. 2003.** Vocalizações de duas espécies de anuros do Sul do Brasil. *Arquivos do Museu Nacional, Nova Série, Zoologia* 61:203–207.
- Lutz B. 1950.** Anfíbios anuros da coleção Adolpho Lutz do Instituto Oswaldo Cruz, V. *Memórias do Instituto Oswaldo Cruz*. Rio de Janeiro 48:599–637.
- Lutz B. 1963.** New species of *Hyla* from southeastern Brazil. *Copeia* 1963:561–562.
- Medina, J.T. 1894.** Descubrimiento del Río de las Amazonas. Imprenta de E. Rasco, Sevilla.

- Nixon K.C. 1999.** The Parsimony Ratchet, a new method for rapid parsimony analysis. *Cladistics* 15:407–414. [DOI](#)
- Padial J.M., De la Riva I. 2009.** Integrative taxonomy reveals cryptic Amazonian species of *Pristimantis* (Anura). *Zoological Journal of the Linnean Society* 155:97–122. [DOI](#)
- Parker H.W. 1936.** A collection of reptiles and amphibians from the Upper Orinoco. *Bulletin du Musée Royal d'Histoire Naturelle de Belgique* 12:1–4
- Peloso P.L.V., Frost D.R., Richards S.J., Rodrigues M.T., Donnellan S., Matsui M., ... Wheeler W.C. 2015.** The impact of anchored phylogenomics and taxon sampling on phylogenetic inference in narrowmouthed frogs (Anura, Microhylidae). *Cladistics* 32:113–140. [DOI](#)
- Peloso P.L.V., Orrico V.G.D., Haddad C.F.B., Lima-Filho G.R., Sturaro M.J. 2016.** A new species of clown tree frog, *Dendropsophus leucophyllatus* group, from Amazonia (Anura, Hylidae). *South American Journal of Herpetology* 11:66–80. [DOI](#)
- Peloso P.L.V., Sturaro M.J., Forlani M.C., Motta A.P., Gaucher P., Wheeler W.C. 2014.** Phylogeny, taxonomic revision, and character evolution of the genera *Chiasmocleis* and *Syncope* (Anura, Microhylidae) in Amazonia, with descriptions of three new species. *Bulletin American Museum of Natural History* 386:1–111. [DOI](#)
- Pyburn, W.F. 1977.** A new hylid frog (Amphibia, Anura, Hylidae) from the Vaupés River of Colombia with comments on related species. *Journal of Herpetology* 11:405–410.
- Pyburn W.F., Hall D.H. 1984.** A new stream inhabiting treefrog (Anura: Hylidae) from southeastern Colombia. *Herpetologica* 40:366–372.
- Pyron R.A., Wiens J.J. 2011.** A large-scale phylogeny of Amphibia including over 2,800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution* 61:543–583. [DOI](#)
- Rivero J.A. 1961.** Saliencia de Venezuela. *Bulletin of the Museum of Comparative Zoology* 126:1–207.
- Rivero J.A. 1972.** Notas sobre los anfibios de Venezuela: 1. Sobre los hilidos de la Guayana Venezolana. *Caribbean Journal of Science* 11:181–193.
- Savage J.M., Heyer W.R. 1967.** Variation and distribution in the treefrog genus *Phyllomedusa* in Costa Rica, Central America. *Beitrage zur Neotropischen Fauna* 5:111–131. [DOI](#)
- Schneider J.G. 1799.** *Historiae Amphibiorum naturalis et literariae. Fasciculus primus continens continens Ranas, Calamitas, Bufones, Salamandras et Hydros in genera et species descriptos notisque suis distinctos. Friederici Frommanni, Ienae.* [DOI](#)
- Silveira S.R., Caramaschi U. 1989.** Revalidação de *Hyla semilineata* Spix, 1824 (Anura, Hylidae). Resumos XVI Congresso Brasileiro de Zoologia. Universidade Federal da Paraíba, João Pessoa, Paraíba.
- Spix J.B.von. 1824.** *Animalia nova sive species novae Testudinum et Ranarum, quas in itinere per Brasiliam annis MDCCCXVII–MDCCCXX jussu et auspiciis Maximiliani Josephi I. Bavariae Regis. Typis Franc. Seraph. Hübschmanni, Monachii.* [DOI](#)
- Stamatakis A. 2006.** RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690. [DOI](#)
- Stamatakis A. 2014.** RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. [DOI](#)
- Sturaro M.J., Peloso P.L.V. 2014.** A new species of *Scinax* Wagler, 1830 (Anura: Hylidae) from the middle Amazon River basin, Brazil. *Papéis Avulsos de Zoologia* 54:9–23. [DOI](#)
- Tavaré S. 1986.** Some probabilistic and statistical problems in the analysis of DNA sequences. Pp. 57–86, in Miura R.M. (Ed.), *Some Mathematical Questions in Biology—DNA Sequence Analysis*. American Mathematical Society, Providence.
- Tourinho A.L., Azevedo C.S. 2007.** A new Amazonian *Cryptocellus* Westwood (Arachnida, Ricinulei). *Zootaxa* 1540:55–60. [DOI](#)
- Vaidya G., Lohman D.J., Meier R. 2011.** SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27:171–180. [DOI](#)
- Werner F. 1901.** Ueber Reptilien und Batrachier aus Ecuador und Neu Guinea. *Verhandlungen des Zoologisch-Botanischen Vereins in Wien* 51:593–614. [DOI](#)
- Wied M. 1821.** Reise nach Brasilien in den Jahren 1815 bis 1817. Volume 2. Henrich Ludwig Bröner, Frankfurt. [DOI](#)
- Wiens J.J., Kuczynski C.A., Hua X., Moen D.S. 2010.** An expanded phylogeny of treefrogs (Hylidae) based on nuclear and mitochondrial sequence data. *Molecular Phylogenetics and Evolution* 55:871–882. [DOI](#)
- Zimmerman B.L., Bogart J.P. 1984.** Vocalizations of primary forest frog species in the Central Amazon. *Acta Amazonica* 14:473–520. [DOI](#)

## ONLINE SUPPORTING INFORMATION

The following Supporting Information is available for this article online:

**Table S1.** Taxon sampling. Complete list of terminals included in the phylogenetic analyses, including metadata and GenBank accession numbers.

**Table S2.** Variation in measurements in the type series of *Hypsiboas icamiaba* sp. nov. Abbreviations are explained in Materials and Methods, all measurements in millimeters (mm).

**Data S1.** Alignment (nexus) used for the phylogenetic analyses.

**Data S2.** Complete parsimony tree. Numbers above branches are jackknife support values.

**Data S3.** Complete maximum likelihood tree. Numbers above branches are bootstrap support values.

## APPENDIX

### Specimens examined for comparisons

**Boana boans (n = 27).** BRAZIL: **Amapá:** Mazagão, BR 156 (MPEG 34157–34158); **Pará:** Barcarena, Vila dos Cabanos (MPEG 27989); **Pará:** Vitória do Xingu, Bom Jardim. Rio Xingu, Usina Hidroelétrica de Belo Monte MPEG 21978–27989); **Pará:** Itaituba, Área de Proteção Ambiental do Tapajós, Mina do Tocantinzinho (MPEG 33462–33463); **Pará:** Parauapebas, Floresta Nacional de Carajás, Igarapé Bahia (MCP 11394); **Pará:** Almeirim: Floresta Estadual do Pará (MPEG 30463–30465); **Pará:** Óbidos: Estação Ecológica Grão Pará, Centro (MPEG 30731–30732). PERU: **Madre de Dios:** Lago Sandoval, 12 km east of Puerto Maldonado (KU 215191–215193). VENEZUELA: **Bolívar:** Mount Auyan-Tepui (AMNH 46043).

**Boana diabolica (n = 10).** BRAZIL: **Amapá:** Floresta Nacional do Amapá (MPEG 39804–39807, 39984–39988); **Pará:** Almeirim (MPEG 33920).

**Boana hutchinsi (n = 05).** BRAZIL: **Amazonas:** Rio Içá (MTR 36136, 36141). COLOMBIA: **Vaupés:** Caño Kuduyari. Comunidad Pirasemo (ANDES, field number ACJ 2264); 2 km southwest of Umañapito (UTA-A 24819, *holotype*, UTA-A 24830, *paratype*, UTA-A 24835, *paratype*; all examined from photographs).

**Boana geographica (n = 12).** BRAZIL: **Acre:** Mâncio Lima, Parque Nacional da Serra do Divisor (MZUSP 157090–157091); **Amazonas:** Municipality of Beruri, Lago Chaviana, Rio Purus (MZUSP 157082–157084, 157086–157089); **Amazonas:** Tefé (MZUSP 157060). PERU: **Madre de Dios:** Lago Sandoval, ca. 12 km east of Puerto Maldonado (KU 215121).

**Boana secedens (n = 1).** BRAZIL: **Rio de Janeiro:** Barro Branco (MNRJ 3591, *holotype*, examined from photographs).

**Boana “semilineata” (n = 19).** BRAZIL: **Bahia:** Camamu, Zumbi dos Palmares (CFBH 27815, 27819, 27823); **Espírito Santo:** Linhares (CFBH 9969, 19452, 26298); **Minas Gerais:** Teófilo Otoni, Fazenda Nossa Senhora de Fátima (CFBH 11475–11480); **Rio de Janeiro:** Duque de Caxias (CFBH 5423–5425); **Santa Catarina:** Itapema (CFBH 8459); São Paulo: Iguape (CFBH 15762–15764).

**Boana pombali (n = 6).** BRAZIL: **Bahia:** Mata de São João, Reserva Sapiranga (CFBH 27804); **Bahia:** Ilhéus (CFBH 13270–13273) **Minas Gerais:** Salto da Divisa, Fazenda Alto Cariri (MNRJ 27369, *holotype*, examined from photographs).

**Boana wavrini (n = 10).** BRAZIL: **Amazonas:** Itacoatiara: Lindóia. Linha de Transmissão Oriximiná-Itacoatiara-Cariri (MPEG 33970–33971); **Amazonas:** Maués: Floresta Nacional de Pau-Rosa (MPEG 28620–28621); **Pará:** Juruti, Capiranga (MPEG 27250–27252); **Pará:** Faro, Floresta Estadual de Faro (MPEG 23118); **Pará:** Oriximiná, Comunidade Casinha. Lago Sapucuá. (MPEG 33740–33741).