



UNIVERSIDADE FEDERAL DO AMAZONAS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA

**SISTEMÁTICA E PADRÕES DE DIVERSIDADE DO GRUPO *BOANA*
PUNCTATA (ANURA: HYLIDAE): HISTÓRIA EVOLUTIVA E
IMPLICAÇÕES TAXONÔMICAS**

ALEXANDRE PINHEIRO DE ALMEIDA

Manaus, Amazonas

Maior, 2022



ALEXANDRE PINHEIRO DE ALMEIDA

SISTEMÁTICA E PADRÕES DE DIVERSIDADE DO GRUPO *BOANA PUNCTATA* (ANURA: HYLIDAE): HISTÓRIA EVOLUTIVA E IMPLICAÇÕES TAXONÔMICAS

Tese apresentada ao Programa de Pós-Graduação em Zoologia, da Universidade Federal do Amazonas, como parte dos requisitos para obtenção do título de Doutor em Zoologia.

Orientador: Dr. Marcelo Gordo

Coorientadora: Dra. Izeni Pires Farias

Manaus, Amazonas

Maior, 2022

Ficha Catalográfica

Ficha catalográfica elaborada automaticamente de acordo com os dados fornecidos pelo(a) autor(a).

A447s Almeida, Alexandre Pinheiro de
Sistemática e padrões de diversidade do grupo *Boana punctata*
(Anura: Hylidae): história evolutiva e implicações taxonômicas /
Alexandre Pinheiro de Almeida . 2022
184 f.: il. color; 31 cm.

Orientador: Marcelo Gordo
Coorientadora: Izeni Pires Farias
Tese (Doutorado em Zoologia) - Universidade Federal do
Amazonas.

1. Espécies crípticas. 2. Filogenia. 3. Filogeografia. 4.
Cophomantini. I. Gordo, Marcelo. II. Universidade Federal do
Amazonas III. Título

Sinopse: Nesta tese foram analisadas relações filogenéticas e padrões de diversidade críptica em espécies do grupo *Boana punctata* (Anura: Hylidae) com implicações para a sistemática, taxonomia e história evolutiva do grupo.

Palavras-chave: Espécies crípticas; Filogenia; Filogeografia; 16S rDNA; *Single Nucleotide Polimorphism* (SNPs); Cophomantini; Neotrópico

***Dedico esta tese ao amigo e mentor
Marcelo Menin (In memoriam)***

Agradecimentos

Primeiro agradeço imensamente à minha família. Valéria, minha esposa, melhor amiga e companheira; minha mãe Adelaide e meu padrasto Walter. Sem vocês nada disso seria possível, serei eternamente grato por toda a compreensão, apoio, suporte, paciência e amor principalmente nos momentos difíceis desses últimos seis anos.

Agradeço muito aos meus orientadores, Marcelo Gordo e Izeni P. Farias, não só por esses seis anos do doutorado, mas também, pelos mais de dez anos de parceria e compreensão. Todas as oportunidades, conversas, ensinamentos e projetos compartilhados foram essenciais para chegar nesse momento.

Ainda, agradeço também ao professor Tomas Hrbek, que embora não seja meu orientador formalmente, também fez parte deste caminho profissional e contribuiu de forma significativa na concepção teórica e na interpretação dos resultados obtidos nesta tese.

Ao PPG-ZOOL, em especial à Fabrício Baccaro, Cíntia Cornelli e Gildázio Ferreira (Gil) pela dedicação e empenho em proporcionar as melhores oportunidades possíveis em um período de tão pouco incentivo para se desenvolver pesquisa em nosso país...além, é claro, de todo o suporte durante esse período difícil de incertezas na pandemia.

Agradeço em especial ao amigo, e professor/coordenador Marcelo Menin, que infelizmente não pôde compartilhar deste momento comigo, mais uma das vítimas do descaso nacional com a pandemia do COVID-19. A ele agradeço as palavras de apoio e incentivo para não desistir quando achei que não conseguiria seguir este projeto... serei eternamente grato.

Pela concessão de amostras e sequências agradeço aos doutores: Robson Ávila (UFC), Fernanda Werneck (INPA), Diego Santana (UFMS), Miguel Trefaut (USP), Antoine Fouquet (CRNS), Juan Carlos Chaparro (MUBI), Rommel R. Rojas (UNAP), Teddy Angarita-Sierra (UNAL), Igor J. Roberto (UFC), Célio Haddad (UNESP), Luís Fernando Marin da Fonte (ASA) e Felipe Curcio (UFMT).

Aos curadores e assistentes de coleção, Celio Haddad (CFBH/UNESP-Rio Claro), Nadya Pupin (CFBH/UNESP-Rio Claro), Fernanda Werneck (INPA-H), Ariane Silva (INPA-H), Diego Santana (ZUFMS-UFMS), Felipe Curcio (UFMT-H), Miguel Trefaut (MTR), Juan C. Chaparro (MUBI), Santiago Ron (QCAZA - PUCE), por cordialmente me receberem e auxiliarem na obtenção dos dados morfológicos dos espécimes essenciais a esta tese.

Pela estadia durante as viagens e ajuda na coleta de dados em campo, agradeço à: Robson Ávila (UFC), Moisés Barbosa (UFAC), Dráuzio Moraes (UFU), Vinícius Carvalho

(UFAM), Fabiano Waldez (IFAM-Tabatinga), Wesley Valteran (UEA-Tabatinga), Mariel Acácio (UFAC), Karoline Ceron (UFMS), João Paulo (Santo Antônio do Içá/AM), Nilton Slobodzian (UFAM- Benjamin Constant/AM) e Ivan Junqueira (UFLA), além de outros personagens que engradeceram essa experiência.

Aos amigos, Luciana Frazão, Igor Joventino, Amanda Picelli, Rommel Rojas, Vinicius T. Carvalho, Rafael de Fraga, Robson Ávila, Valeria Machado, Leandro Moraes, Mario Nunes, Rodrigo Araújo pelos inúmeros cafés, conversas e cervejas onde rimos e discutimos sobre os mais diversos temas da vida, invariavelmente desabafando sobre nossas escolhas acadêmicas.

Aos amigos do Projeto Sauim de Coleira, Aline Ramos, Aline Medeiros, Tainara Sobroza, Edson Costa e Raiclicia Nayara pelas conversas, cafés, churrascos e bons momentos compartilhados no nosso querido “sítio”, também conhecido como “macacário”.

Aos amigos do LEGAL, Valéria Machado, Fábio Muniz, Pedro Bittencourt, Mario Nunes, Sandra Hernandez, Ingrid Nunes, Rommel Rojas, Igor Joventino, por toda ajuda nas diferentes fases de obtenção dos dados genéticos, e por compartilhar os momentos de glória e desespero que a rotina de laboratório proporciona.

Aos amigos Érico Polo, Sandra Hernandez, Leandro Moraes, Igor Joventino e Rommel Rojas, agradeço a ajuda na obtenção e análises dos dados moleculares e morfológicos ao longo dos três capítulos desta tese.

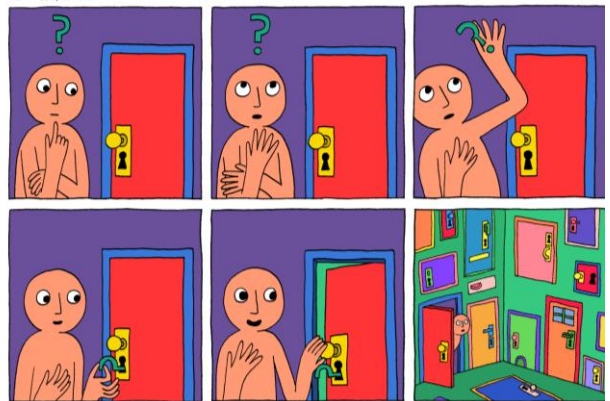
Aos professores Fernanda Werneck (INPA), Rafael de Fraga (ITV) e Danilo Pacheco (INMA) pelas críticas e contribuições significativas durante o exame de qualificação desta tese, que serviram de base para o aprimoramento das questões a serem respondidas.

Aos amigos Filipe, Letícia, Carlos, André, Renato, Antônio, Igo, Júlio, Eduardo, Bernardo, Felipe Chaves, Iuna e Ademar, agradeço imensamente pelos momentos de confraternização e amizade, tornando os últimos dois anos de pandemia um pouco mais leves.

Agradeço imensamente a minha psicóloga Dayana, pela dedicação e profissionalismo que tem me ajudado a lidar com a pressão e ansiedade, possibilitando a finalização deste doutorado.

Às minhas pequenas filhas felinas, Lene Maria, Bagherra e Miamor por me proporcionarem alegria, companhia e paz diariamente.

À FAPEAM pela concessão da bolsa de doutorado e auxílio financeiro para visitar as coleções zoológicas.



Science - Alex Norris

Resumo

A região Neotropical é a mais biodiversa do mundo e os fatores que influenciaram a origem de uma diversidade tão alta são fontes inesgotáveis de pesquisas. Entre os vertebrados terrestres, os anfíbios configuram como um dos grupos mais diversos, e 43% de todas as espécies conhecidas ocorrem nos Neotrópicos. Apesar disso ainda persistem muitas lacunas de conhecimento acerca da riqueza de espécie em função dos altos níveis de diversidade críptica e extensas regiões geográficas desconhecidas. No entanto, o uso de dados moleculares em abordagens de taxonomia integrativa associando métodos de delimitação entre espécies e dados fenotípicos ajudam a esclarecer os limites de distribuição das espécies, ampliando o debate acerca da dos padrões de diversificação, taxonomia e história evolutiva dos anuros neotropicais.

O grupo *Boana punctata* agrega vários destes gargalos de conhecimento; sejam esses relacionados a sistemática do grupo, filogenia e taxonomia; como também sobre os padrões de diversidade críptica em espécies bem conhecidas e de ampla distribuição, como os complexos *Boana cinerascens* e *Boana punctata*. Assim, a ausência de conhecimento refinado acerca das relações filogenéticas das espécies pouco conhecidas do grupo *B. punctata*, e a falta de esclarecimento filogenético e taxonômico no complexo *B. punctata* foram objeto de estudo nesta tese que teve como resultados: **Cap 1-** Com base em sequências de DNA mitocondrial e dados morfológicos, fornecemos novas informações sobre as relações filogenéticas, variações morfológicas e distribuição geográfica. Dentre os principais resultados revelamos que *B. hobbsi* está relacionado com o grupo *Boana benitezi*, polifilético à *B. punctata*. Apoiado nisso, propomos um novo arranjo taxonômico que inclui *B. hobbsi* como membro do grupo *B. benitezi*; **Cap 2-** Utilizando sequências do gene 16S rDNA do complexo *B. punctata* e métodos de delimitação de espécies em uma abordagem de taxonomia integrativa, descobrimos seis linhagens correspondentes a unidades taxonômicas operacionais (OTUs). Entre elas, uma espécie candidata não confirmada, cinco espécies candidatas confirmadas das quais duas correspondem a táxons reconhecidos: dessa forma delimitamos *B. punctata* **Senso Strictu**, *Boana atlantica*, e elevamos *Boana rubrolineata* ao status de espécie plena. **Cap- 3** Com dados genômicos obtidos por sequenciamento de próxima geração (NGS) e análises de descobrimento de espécies, conseguimos delimitar espécies putativas e realizar uma análise de datação molecular. Os resultados revelaram uma diversidade críptica maior do que a encontrada no capítulo anterior desse estudo com sete

espécies candidatas confirmadas pelos dados genéticos e morfológicos. Destas, três representam *B. punctata*, *B. atlantica* e *B. rubrolinetata* e quatro candidatas a espécies. As análises de datação molecular mostram uma diversificação do complexo *B. punctata* que iniciou no Mioceno tardio seguido por eventos subsequentes ao longo do Plioceno e Pleistoceno.

Palavras-chave: Espécies crípticas; Filogenia; Filogeografia; 16S rDNA; *Single Nucleotide Polimorphism* (SNPs); Cophomantini; Neotrópico

Abstract

The Neotropical region is the most biodiverse in the world, and the factors that influenced the origin of such a high diversity are inexhaustible sources of research. Among the terrestrial vertebrates, amphibians are one of the most diverse groups, and 43% of all known species occur in the Neotropics. However, there are still many knowledge gaps regarding species richness due to high levels of cryptic diversity and large unknown geographic regions. However, the use of molecular data in integrative taxonomy approaches associating species delimitation methods and phenotypic data helps to clarify the limits of species distribution, broadening the debate about patterns of diversification, taxonomy and evolutionary history of Neotropical anurans. The *Boana punctata* group has several of these knowledge shortfalls; whether related to group systematics, phylogeny and taxonomy, or to patterns of cryptic diversity in well-known species with wide distribution, such as the *Boana cinerascens* and *Boana punctata* complexes and their taxonomic issues. Thus, the lack of refined knowledge about the phylogenetic relationships of these poorly known species of the *Boana punctata* group, and the lack of phylogenetic and taxonomic clarification in the *B. punctata* complex are object of study in this thesis that had as results: **Cap 1-** Based on mitochondrial DNA sequences, morphological data, we provide new information on phylogenetic relationships, morphological variations and geographical distribution. Such results reveal that *B. hobbsi* is related to the *Boana benitezi* group, polyphyletic to *B. punctata*. Supported by this, we propose a new taxonomic arrangement that includes *B. hobbsi* as a member of the *B. benitezi* group; **Cap 2-** Using 16S rDNA gene sequences from the *B. punctata* and species delimitation methods in an integrative taxonomy approach, we discovered six well supported lineages delimited as operational taxonomic unities (OTUs). Which consists in, one unconfirmed candidate species, five confirmed candidate species of which two correspond to recognized taxa: thus, we delimit *B. punctata* **Sensu Strictu**, *Boana atlantica*, and elevate *Boana rubrolineata* to full species status. **Cap 3-** With genomic data obtained by next-generation sequencing (NGS) and discovery analyses we were able to delimit putative species and perform a molecular dating analysis, revealing a higher cryptic diversity, than observed in our Cap. 2 study. Our results confirmed seven candidate species co by genetic and morphological data. Of these, three represent the nominal taxa *B. punctata*, *B. atlantica* and *B. rubrolineata*; and the remaining lineages correspond to four candidate species. Molecular

dating analyses show a diversification of the *B. punctata* complex beginning in the late Miocene followed by subsequent events throughout the Pliocene and Pleistocene.

Keywords: Cryptic species; Phylogeny; Phylogeography, 16S rDNA; *Single Nucleotide Polimorphism* (SNPs); Cophomantini; Neotropics.

Sumário

Lista de Tabelas.....	xi
Lista de Figuras	xiii
Introdução Geral	17
Capítulo 1. Phylogenetic relationships of the poorly known treefrog <i>Boana hobbsi</i> (Cochran & Goin, 1970) (Anura: Hylidae), systematic implications and remarks on morphological variations and geographic distribution.....	20
Capítulo 2. Unravelling the fluorescent treefrogs' species complex: Reassessing the taxonomic status of <i>Hyla punctata rubro-lineata</i> Lutz, 1951 through integrative taxonomy	66
Capítulo 3. Diversification and phylogeography of the continental <i>Boana punctata</i> species complex (Anura: Hylidae): systematics and evolutionary history.....	125
Considerações Finais	169
Referências Bibliográficas	172

Lista de Tabelas

Capítulo 1.

Table 1. Uncorrected pairwise distances between samples of *Boana hobbsi* (Cochran & Goin, 1970) from the two new localities of occurrence, showing low genetic distances. Specimens from the Japurá River (vicinity of Vila Bittencourt, Amazonas, Brazil) are represented with CZPB-AA vouchers, while the ones from São Gabriel da Cachoeira, Amazonas, Brazil are represented with INPA-H vouchers.....52

Table 2. Uncorrected pairwise distances between samples of *Boana hobbsi* (Cochran & Goin, 1970) and congeneric species of the *Boana benitezi* group (highlighted in bold).....53

Table 3. Uncorrected pairwise distances between samples of *Boana hobbsi* (Cochran & Goin, 1970) and congeneric species of the *Boana punctata* group *sensu lato* (highlighted in bold).....53

Table 4. Morphometric measurements (mm) of *Boana hobbsi* (Cochran & Goin, 1970), based on the newly collected specimens examined in our study (INPA-H and CZPB vouchers) and on information available in the literature. See text for measurement acronyms.....54

Appendix I. List of species included in our phylogenetic analysis, along with the associated voucher and GenBank accession numbers, locality of occurrence, and sequence references. Newly generated sequences are highlighted in bold.....60

Capítulo 2.

Table 1. Genetic distances between and within (values in parenthesis) the major lineages (Clades) recovered in the phylogenetic analysis and bGYMC species delimitation.....106

Table 2. Measures of morphometric variables of *B. punctata* species complex 447 specimens. Columns indicates the group delimited in the bGYMC analysis and the number of specimens examined per clade. Values presented are mean values $\pm$ standard deviation.....106

Table 3. Discriminant Function classification for 447 specimens of *B. punctata* complex for the groups previously designed by bGYMC species delimitation. Bold values represent the total of correctly assignment specimens and the proportion of accuracy of the predictive variables....107

Table 4. Morphometric measurements comparisons between the three nominal species of the *B. punctata* complex. Values presented are the characters variation (mean $\pm$ SD) and in parenthesis the minimum and maximum values.....107

Table S1. List of species included in our phylogenetic and species discovery analysis, along with their identification of Voucher or Field Number, GenBank accession, locality information and sequence references. Newly collected samples and generated sequences are highlighted in bold.....122

Table S2. Summary statistics of Tukey's test performed to body size variable (Snout-vent length – SVL) between the groups delimited in the bGMYC analysis. Asterisk indicates the significant ($p < 0.05$) differences in body-size between the assigned groups.....122

Table S3. Summary statistics of Tukey's test performed to the residuals of morphometric variables PCA performed between the groups delimited in the bGMYC analysis. Asterisk indicates the significant ($p < 0.05$) differences in body-size between the assigned groups.PCA - Tukey test.....123

Table S 4. Character loadings, and eigenvalues of variance explained for for two principal components of PCA for morphometric variables.....123

Table S5. Loadings of variance explained by the discriminant axes of DFA performed for morphometric variables.....124

Capítulo 3.

Table S6. Table S7. List of species included in our phylogenetic tree and *B. punctata* complex samples used in species discovery analysis, along with their identification of Voucher or Field Number, GenBank accession, locality information. All data analysed here were produced

during	this
study.....	163

Table S2. Tabela S2. Geographic association scheme used to define the association between morphological and genetic data to perform the iBPP species delimitation analysis. Columns indicates the samples identification, examined specimens and the geographic range including both genetic and morphological data.....	165
---	-----

Lista de Figuras

Capítulo 1

Figure 1. Bayesian phylogenetic tree of the genus <i>Boana</i> , based on a fragment of the 16S mitochondrial rRNA, highlighting the position of <i>Boana hobbsi</i> (in yellow) in comparison to major clades representing known species groups, and indicating the phylogenetic position of remaining species included in the <i>B. punctata</i> group (in blue). Support values are indicated close to the nodes. Branch scale is indicated as number of substitutions per site.....	55
--	----

Figure 2. Dorsal and ventral views of newly collected preserved specimens of <i>Boana hobbsi</i> (Cochran & Goin, 1970). (A, B) adult male, with dark brown dorsum and little evident dorsolateral stripe (CZPB-AA 1658, SVL= 46.2 mm); (C, D) adult female, with dark brown dorsum and evident dorsolateral stripe (CZPB-AA 1660, SVL= 43.5 mm); (E, F) adult male, with yellowish dorsum and evident dorsolateral stripe (CZPB-AA 1657, SVL= 33.7 mm). All specimens were collected on the right bank of the Japurá River, near to the city of Japurá, state of Amazonas, Brazil.....	56
--	----

Figure 3. Morphological details of newly collected preserved specimens of <i>Boana hobbsi</i> (Cochran & Goin, 1970). (A–C) lateral, dorsal and ventral views of the head of an adult male (CZPB-AA 1658, SVL= 46.2 mm), highlighting the presence of an evident mental gland (red arrow). (D, E) ventral surfaces of the hands and feet of an adult female of <i>B. hobbsi</i> (CZPB-AA 1659; SVL = 45.8 mm).....	57
---	----

Figure 4. Dorsolateral views of newly recorded living specimens of *Boana hobbsi* (Cochran & Goin, 1970): (A) adult female from the right bank of the Japurá River, near to Vila Bittencourt, Amazonas, Brazil (Photo: APA); (B) adult male from the city of São Gabriel da Cachoeira, Amazonas, Brazil (Photo: LJCLM); (C, D) adult and juvenile males from Japurá River, showing variations in dorsal coloration, from tan, to dark brown, to a pale green-grayish, respectively (Photo: VTC).....58

Figure 5. Known geographic distribution of *Boana hobbsi* (Cochran & Goin, 1970) in the northwestern Amazonia. White circles indicate the historical localities of occurrence in Colombia, Venezuela and Brazil (Cochran & Goin 1970; Pyburn 1978; McDiarmid *et al.* 1988; Ruiz-Carranza *et al.* 1996; Acosta-Galvis 2000; Lynch 2005, 2007; Suárez-Mayorga & Lynch 2017; Acosta-Galvis 2018; Barrio-Amorós *et al.* 2019; Simões *et al.* 2019). Yellow circles on the Brazilian territory indicate new distribution records reported at Japurá and Negro river basins. The red star indicates type locality at Caño Guacaya, Apaporis River, Colombia (Cochran & Goin 1970).....59

Capítulo 2

Figure 1. Known geographic distribution of the nominal *Boana punctata* (red circles) and *Boana atlantica* (yellow circles) (left) and sampling effort of molecular (black triangle) and morphologic data (white circles) (right).....108

Figure 2. The 16S ultrametric tree inferred by Bayesian analysis in BEAST and bGYMC species delimitation result in vertical bars. Black dots in the nodes represent posterior probability >0.95, clear nodes represent low supported relationships. The *B. punctata* complex are represented by the well supported clades with no overlapping geographic distribution from the top to the bottom as the following scheme: Clade I (gray bars) *B. punctata sensu stricto*; Clade II (dark green bars); Clade III (red bars) *B. rubrolineata* (stat. nov.); Clade IV (orange bars); Clade V (lime-green bars) *B. atlantica*; Clade VI (yellow bars). Remaining groups correspond to the congener's species of *B. punctata* group, *B. albopunctata* group, *B. semilineata* group and *B. benitezi* group respectively, used as outgroup in our analysis.....109

Figure 3. Morphological analysis of *B. punctata* complex. A) Violin Plot of body size (SVL) differences between the clades delimited in species delimitation. B) Principal components

analysis (PCA) plot of the residuals of morphometric measures (sized-free) from the clades de bGYMC analysis.....110

Figure 4. Discriminant Function Analysis (DFA) plot showing the morphometric variable (sized-free) weight in the separation of the groups delimited in the bGYMC analysis.....110

Figure 5. Overall view of the conservative external morphology of preserved specimens *B. punctata* complex in dorsal view. A) Clade I: MTR 36266-São Pedro, Amazonas, Brazil; B) Clade II: QCAZ-A 8029-Limoncocha, Orellana, Ecuador; C) Clade III, UFMT-A 1084-Campo Grande, Mato Grosso do Sul, Brazil D) Clade IV: ZUFMS-AMP 07949, Araguaína, Tocantins, Brazil, E) Clade V: CFBH 32060 – Ilhês, Bahia, Brazil, F) Clade VI: ZUFMS-AMP 11515, Parque Estadual Nascentes do Rio Taquari, Mato Grosso do Sul, Brazil.....111

Figure 6. Morphological details of *Boana rubrolineata* (**stat nov.**). Dorsal (A) and Ventral (B) views of adult male (CFBH 41004, SVL = 33.66 mm). Semicircular (C) and truncate shapes (D) of the head in dorsal and lateral view respectively (UFMT-A 10128, HW = 12.36 mm, HL = 11.19). Ventral surfaces of hand (E) and foot (F).....112

Figure 7. Living specimens of *B. rubrolineata* and morphological variations in dorsal pattern of coloration through its geographic distribution. (A) Campo Grande – MS; (B) PENRT Taquari – MS; (C - D) Madre de Dios, Peru; (E) Rondônia; (F) Centenário do Sul – PR; (G) Jauru – MT; (H) Corumbá – MS. Photos: (A-B): Matheus Mororti; (C-D): Kevin K. Caldwell; (E): Pedro F. Dias; (F): Guilherme Figueiredo; (G-H) Robson W. Ávila.....113

Figure 8. Geographic distribution of *B. rubrolineata* from south-west of Amazon, Madre de Dios – Madeira Rivers basin, lowlands of Bolivia, Pantanal and wetlands of Paraguay, Argentina, in the Paraguay and Paraná basin. Red circles indicate distribution data from GBIF repository (as *B. punctata*) and white triangles indicates genetic samples used in our phylogenetic analysis. The yellow star indicates the type locality of *Hyla punctata rubrolineata* Lutz, (1951).....114

Figure S1. Linear discriminat histogram of DFA for morphometric variables of *B. punctata* complex specimens, among the groups delimited in bGMYC analysis.....124

Capítulo 3

Figure 1 Time-calibrated tree obtained by the “supertree” in ASTRAL from 30 samples of *B. punctata* complex. Nodes values indicates the support of iBPP species discovery analysis with genetic data only/morphological data only/both data combined respectively Pie charts in the tip of the terminal indicate the populational structure obtained in fineRADstructure, for the delimited lineages. Pie charts and dashed lines indicates the geographic distribution and structure of the lineages. The 95% confidence intervals for delimited lineages nodes are highlighted by horizontal blue bars.....160

Introdução Geral

Entender a riqueza de espécies, como elas se distribuem ao longo das regiões do mundo, e os fatores que levam a esses diferentes padrões de distribuição, foi por muito tempo um dos principais questionamentos entre biólogos e naturalistas em todo mundo, e base de teorias que norteiam o conhecimento científico até hoje (Darwin, 1859; Wallace, 1876). Nesse contexto, a região Neotropical sempre foi alvo de interesse científico, devido seu isolamento e inacessibilidade, e uma biodiversidade única e em relação às demais regiões do mundo. Tais características motivaram grandes expedições científicas que resultaram no descobrimento e descrição formal de dezenas de espécies (Sandwith, 1925; Spix, 1824). Ainda hoje, essa região intriga pesquisadores. E que por sua alta diversidade de organismos, biomas, paisagens, habitats, e história geológica, proporciona a oportunidade de testar modelos que expliquem os processos de diversificação que moldam os padrões de distribuição da diversidade nos Neotrópicos (Antonelli et al., 2018a, 2018b; Bicudo et al., 2019; Hoorn et al., 2010; Jenkins et al., 2013; Morrone, 2014; Werneck, 2011).

Responder questões acerca de processos evolutivos e padrões biogeográficos depende de dados refinados sobre riqueza e distribuição das espécies bem como das comunidades biológicas. No entanto, na região neotropical estes temas ainda permanecem repletos dos chamados “gargalos” de conhecimento (Hortal et al., 2015), entre os quais a ausência de conhecimento a respeito da riqueza de espécies (déficit Linneano), dos limites de suas distribuições geográficas (déficit Wallaceano) (Whittaker et al., 2005) e de suas relações filogenéticas (déficit Darwiniano), afetam grande parte dos grupos taxonômicos (Diniz-Filho et al., 2013; Lomolino, 2004; Whittaker et al., 2005).

Entre os vertebrados terrestres, os anfíbios configuram como um dos grupos mais diversos, com atuais 8.458 espécies descritas formalmente (Frost, 2022), das quais cerca de 43% ocorrem na região Neotropical (Meseger et al., 2020), sendo maior parte da ordem

Anura. Para essa biota, os déficits de conhecimento são significativos, dado que a riqueza de espécie é considerada subestimada em função de altos níveis de diversidade críptica identificada nestes organismos (Fouquet et al., 2007; Vacher et al., 2020) e da ocorrência de grandes lacunas de amostragem (Mayer et al., 2019). Dessa forma, desvendar essas espécies crípticas colabora na melhor definição de estimativas de riqueza e dos limites de distribuição. Consequentemente ajuda a elucidar questões relacionadas a lacunas de conhecimentos (geográficas e filogenéticas) e cria base teórica para resolução de problemas taxonômicos. Por sua vez, essas resoluções contribuem para compreensão dos padrões regionais de diversidade e suas implicações em melhores planejamentos acerca da conservação desses organismos, que estão entre os mais ameaçados do mundo (Angulo & Icochea, 2010; Bickford et al., 2007; IUCN, 2022; Magalhães et al., 2018; Ramos et al., 2019; Trontelj & Fišer, 2009; Vacher et al., 2020).

Na sistemática, a definição e delimitação de espécies como unidade biológica fundamental, é considerada essencial. Essa definição ou delimitação muitas vezes é controversa e tema de debate, devido aos diferentes conceitos utilizados como base teórica, e devido a natureza temporal do processo de especiação, que muitas vezes impede que esta seja observada com clareza (De Queiroz, 2007; Aleixo, 2009). Essas delimitações, e os critérios utilizados para tal, influenciam de forma direta nossas percepções não somente acerca da riqueza de espécies, como também de endemismo e conservação (Aleixo 2009; Vacher et al., 2020). Atualmente, estudos em sistemática, evolução, taxonomia e biogeográfica utilizam-se do conceito unificado de espécies proposto por De Queiroz (2007), onde espécies consistem em linhagens de metapopulações que evoluem separadamente a partir de um ancestral comum. Além disso, as características essenciais que definem uma espécie em conceitos mais clássicos como o biológico (e.g. Mayr, 1942; Dobzhansky, 1970) ou o filogenético (e.g.

Henning, 1966; Cracraft, 1983) consistem em importantes critérios e linhas de evidência para a diferenciação entre as linhagens irmãs.

O uso de marcadores moleculares tornou-se peça fundamental no estudo da diversidade de anfíbios, promovendo avanços no esclarecimento da sistemática e taxonomia dos anuros (e.g. Vences et al., 2005; Frost et al., 2006). Na delimitação de espécies, a análise desses caracteres são fonte de evidência de diversificação, e um passo essencial na taxonomia integrativa, onde diferentes fontes de evidência são utilizadas para a definição se determinada linhagem pode ser definida como uma espécie (Dayrat, 2005; Padial et al., 2010; Carstens et al., 2013). Diferentes bases teóricas, critérios e modelos são utilizados nos métodos moleculares de delimitação de espécies (e.g. conceito filogenético de espécies, DNA-*barcoding*, distância genética etc.) (Yang & Rannala, 2014; Hao et al., 2011; Puillandre et al., 2012; Zhang et al., 2013; Kapli et al., 2017). Atualmente, um dos arcabouços teóricos proeminentes nessas análises é a teoria coalescente, que via modelos probabilísticos infere a história evolutiva dos alelos de um gene compartilhado dentre os indivíduos de uma população até o ancestral comum mais recente (Wakeley, 2008). Essa abordagem permite delimitar espécies de forma robusta pois cobre diferentes critérios de vários conceitos de espécies além de considerar os processos de diversificação das linhagens, passo essencial na taxonomia integrativa (Fujita et al. 2012; Rannala, 2015; Rannala & Yang, 2003).

Os métodos de delimitação podem ser realizados tanto com marcadores moleculares simples (e.g. marcadores mitocondriais como o gene 16S – “*barcoding* dos anfíbios”) como com marcadores múltiplos (e.g. marcadores multi-loci SNPs – *Single Nucleotides Polymorphism*). Atualmente, em estudos de taxonomia integrativa, essas diferentes abordagens são utilizadas como fonte de evidências para a delimitação de espécies, buscando um consenso entre elas. Embora haja bastante debate acerca da utilização desses métodos de delimitação (e.g. Sukumaran & Knowles, 2017; Luo et al., 2018; Hills, 2019; 2021; Burbrink

& Ruane, 2021), essa abordagem tem contribuído significativamente para identificação de diversidade críptica, e complexos de espécies, resultando na descrição formal de novos táxons e arranjos taxonômicos, servindo de base para identificação dos padrões biogeográficos (Caminer & Ron, 2014; 2020; Ferrão et al., 2016; Fouquet et al., 2016; 2021; Funk et al., 2012; Peloso et al., 2018).

Dentre os anuros, os hilídeos (Anura: Hylidae) configuram como um dos grupos mais diversos e taxonomicamente instáveis, com inúmeras mudanças de nomenclatura e sistemática ao longo do tempo. Na última década, a inclusão de amostras genéticas de espécies pouco conhecidas, bem como do aprimoramento das análises filogenéticas, rendera diferentes arranjos sistemáticos, com a descrição de novos gêneros, ampliando o debate acerca da taxonomia e história evolutiva deste grupo (Duellman et al., 2016; Faivovich et al. 2005; 2013; 2018; 2021; Pinheiro et al., 2019; Pyron & Wiens, 2011; Wiens et al., 2005; 2006; 2010).

Embora Hylidae seja uma família bastante estudada nos temas relacionados à filogenia e sistemática, alguns grupos permanecem confusos ou pouco esclarecidos, como o caso do grupo *Boana punctata*. O grupo, quando proposto, reunia apenas duas espécies com relação filogenética próxima comprovada (*Boana punctata* e *Boana cinerascens*), enquanto as demais foram mantidas no arranjo por conta de similaridades morfológicas (Faivovich et al., 2005). No entanto, com a inclusão de amostras de grande parte dessas espécies em novas análises filogenéticas, o grupo *B. punctata* se revelou parafilético (Faivovich et al., 2013; Pinheiro et al., 2019; Pyron & Wiens, 2011; Sturaro et al., 2020) rendendo reformulações neste agrupamento taxonômico. Esse dinamismo, em conjunto com as evidências de diversidade críptica em *B. punctata*, *B. atlantica* e *B. cinerascens* (Caminer & Ron, 2020; Guarnizo et al., 2015; Jansen et al., 2011; Lima et al., 2019; Sturaro et al., 2020), além da, até então, desconhecida relação filogenética das espécies remanescentes, tornam o grupo *B.*

punctata um quebra cabeça interessante a ser estudado. Dessa forma, nesta tese estruturada em três capítulos, busquei 1) esclarecer o posicionamento filogenético de *Boana hobbsi*, uma espécie há muito tempo relacionada à *Boana punctata*, mas pouco conhecida quanto a sua distribuição e posicionamento filogenético. Além disso, busquei 2) esclarecer questões taxonômicas e delimitar possíveis espécies em *B. punctata* utilizando métodos de delimitação e taxonomia integrativa, e por fim 3) ampliar estas análises utilizando dados genômicos e análises de delimitação integradas para inferir sobre a história evolutiva deste complexo de distribuição continental.

CAPÍTULO 1



Boana hobbsi

Phylogenetic relationships of the poorly known treefrog *Boana hobbsi* (Cochran & Goin, 1970) (Anura: Hylidae), systematic implications and remarks on morphological variations and geographic distribution.

Alexandre P. de Almeida, Leandro J.C.L. Moraes, Rommel R. Rojas, Igor J. Roberto, Vinicius Tadeu de Carvalho, Robson W. Ávila, Luciana Frazão, Ariane A.A. Silva, Marcelo Menin, Fernanda P. Werneck; Tomas Hrbek, Izeni P. Farias e Marcelo Gordo. Manuscrito publicado no periódico Zootaxa em 22 de fevereiro de 2021. DOI <https://doi.org/10.11646/zootaxa.4933.3.1>

Phylogenetic relationships of the poorly known treefrog *Boana hobbsi* (Cochran & Goin, 1970) (Anura: Hylidae), systematic implications and remarks on morphological variations and geographic distribution

ALEXANDRE P. DE ALMEIDA^{1,2,7}, LEANDRO J.C.L. MORAES³, ROMMEL R. ROJAS², IGOR J. ROBERTO², VINICIUS TADEU DE CARVALHO^{2,4}, ROBSON W. ÁVILA⁵, LUCIANA FRAZÃO², ARIANE A.A. SILVA³, MARCELO MENIN^{1,6}, FERNANDA P. WERNECK³; TOMAS HRBEK^{1,2}, IZENI P. FARIAS^{1,2} AND MARCELO GORDO^{1,6}

¹*Universidade Federal do Amazonas, Instituto de Ciências Biológicas, Programa de Pós-Graduação em Zoologia, Avenida General Rodrigo Otávio J. Ramos 6200, 69080-900, Manaus, Amazonas, Brazil.*

alexandre.dealmeida@hotmail.com; <https://orcid.org/0000-0003-3938-4198>

²*Universidade Federal do Amazonas, Laboratório de Evolução e Genética Animal, Departamento de Genética, Avenida General Rodrigo Otávio J. Ramos 6200, Japiim, 69080-900, Manaus, Amazonas, Brazil.*

rrojaszamora@gmail.com; <https://orcid.org/0000-0003-3154-8549>

igorjoventino@yahoo.com.br; <https://orcid.org/0000-0003-3268-9597>

luca.frazao@gmail.com; <https://orcid.org/0000-0002-4715-2728>

tomas@evoamazon.net; <https://orcid.org/0000-0003-3239-7068>

izeni@evoamazon.net; <https://orcid.org/0000-0002-1416-4351>

³*Instituto Nacional de Pesquisas da Amazônia (INPA), Coordenação de Biodiversidade, Programa de Coleções Científicas Biológicas, Avenida André Araújo, 69067-375, Petrópolis, Manaus, Amazonas, Brazil.*

leandro.jclm@gmail.com; <https://orcid.org/0000-0001-6704-898X>

ariane.silva@inpa.gov.br

fewerneck@gmail.com; <https://orcid.org/0000-0002-8779-2607>

⁴*Universidade Regional do Cariri, Programa de Pós-Graduação em Diversidade Biológica e Recursos Naturais, Campus do Pimenta, Rua Cel. Antônio Luiz, 1161, 63105-000, Crato, Ceará, Brazil.*

anfios.repteis@gmail.com; <https://orcid.org/0000-0001-7644-6090>

⁵*Universidade Federal do Ceará, Departamento de Biologia, Centro de Ciências, Campus do Pici, Fortaleza, Ceará, Brazil*

robsonavila@gmail.com; <https://orcid.org/0000-0003-3641-8321>

⁶*Universidade Federal do Amazonas, Departamento de Biologia, Avenida General Rodrigo Otávio J. Ramos, 6200, Coroado, 69080-900, Manaus, Amazonas, Brazil.*

menin.m@gmail.com; <https://orcid.org/0000-0002-7209-5083>
 projeto.sauim@gmail.com; <https://orcid.org/0000-0001-5230-9091>
⁷Corresponding author.

Abstract

Boana hobbsi is a poorly known hyliid frog currently placed within the *Boana punctata* group. Yet, morphological, ecological and bioacoustic traits do not support this placement, with no molecular data being available to date to test this hypothesis. Based on newly collected mitochondrial DNA sequences, morphological data review and field observations, we provide new insight into the phylogenetic relationships, morphological variations and geographic distribution of *B. hobbsi*. Our findings reveal that *B. hobbsi* is nested (with strong support) within the *Boana benitezi* group, recovering once more a polyphyletic *Boana punctata* group. Supported by this new genetic, morphological and ecological evidence, we propose a new taxonomic arrangement which includes *B. hobbsi* as a member of the *Boana benitezi* group. Furthermore, we emphasize the importance of conducting biological inventories in remote Amazonian areas, where many taxonomic and geographic knowledge gaps persist with regards to Amphibian diversity.

Keywords: Amazonia, Cophomantini, *B. benitezi* group, *B. punctata* group, Darwinian shortfall, Phylogeny, Wallacean shortfall.

Introduction

Biodiversity research in the Neotropics is a scientific priority across many knowledge areas (Antonelli *et al.* 2018a). However, conducting studies in this region can be particularly challenging due its high species-richness (Moritz *et al.* 2000; Field *et al.* 2009) and vast area covering many biomes (Morrone, 2014; Antonelli *et al.* 2018b). Such a complex system generates knowledge biases on species diversity and on their respective geographic distributions limits, resulting in the so-called Linnean and Wallacean shortfalls (Whittaker *et*

al. 2005; Cardoso *et al.* 2011). Among Neotropical fauna, knowledge involving anurans is particularly affected by these shortfalls. In addition to that, the high levels of cryptic species with morphological conservativeness within this group (Funk *et al.* 2012; Castroviejo-Fisher *et al.* 2017; Rojas *et al.* 2018; Ramos *et al.* 2019; Carvalho *et al.* 2020) and the many gaps on species geographic distributions (Mayer *et al.* 2019) result in a lack of fully resolved phylogenies known as the Darwinian shortfall (Diniz-Filho *et al.* 2013). Those issues persist even in common and widespread anuran taxa, such as the species of *Boana punctata* group.

The *Boana punctata* group (formerly named *Hypsiboas punctatus* group; see Dubois 2017 for nomenclatural changes), originally included eight species (Faivovich *et al.* 2005): *B. alemani* (Rivero, 1964), *B. atlantica* (Caramaschi & Velosa, 1996), *B. punctata* (Schneider, 1799), *B. cinerascens* (Spix, 1824), *B. hobbsi* (Cochran & Goin, 1970), *B. ornatissima* (Noble, 1923), *B. picturata* (Boulenger, 1899), and *B. siblezi* (Rivero, 1972). Two additional species were later included in this group, based on their overall morphological similarities with *B. cinerascens* (Kok 2006; Señaris & Ayarzagüena 2006): *B. liliae* (Kok, 2006) and *B. jimenezi* (Señaris & Ayarzagüena, 2006). However, subsequent studies evidenced this arrangement as non-monophyletic (Pyron & Wiens 2011; Faivovich *et al.* 2013; Duellman *et al.* 2016; Pinheiro *et al.* 2019; Sturaro *et al.* 2020), resulting in the transfer of *B. ornatissima* to the *Boana benitezi* group (Faivovich *et al.* 2013), and of *B. liliae* to the genus *Myersiohyla* Faivovich *et al.* 2005 (Pinheiro *et al.* 2019). These taxonomic uncertainties are also consequences of the uncertain phylogenetic relationships of *B. siblezi*, *B. picturata* and *B. jimenezi*; and of the absence of genetic and refined phenotypic data for some nominal taxa (*B. alemani* and *B. hobbsi* – Wiens *et al.* 2005; Faivovich *et al.* 2013; Duellman *et al.* 2016; Pinheiro *et al.* 2019; Sturaro *et al.* 2020).

Boana hobbsi (Cochran & Goin 1970) is a treefrog with a complex taxonomic history, described for forested habitats of northwestern lowland Amazonia (Cochran & Goin 1970).

Authors describe the presence of dorsolateral folds over the shoulders as a diagnostic character for the species (Cochran & Goin 1970). This apomorphic character, unknown for any other South American hylid frog, precluded the authors from hypothesizing the phylogenetic relationships of *B. hobbsi*. Duellman (1974) reviewed the taxonomic status of some Neotropical frogs and concluded that this unique diagnostic feature was, in fact, an artifact that resulted from the shrinkage of the preserved holotype caused by the high alcohol concentration during storage. As a result, Duellman (1974) synonymized *B. hobbsi* with the widely distributed *B. punctata*. Nevertheless, Pyburn (1978) noticed that divergences in external morphology of living specimens, habitat preferences and advertisement calls did not support this synonymization and restored the full species status of *B. hobbsi* after examining new specimens and the type series. Furthermore, Pyburn (1978) argued based on the evidence at hand that *B. hobbsi* and *B. punctata* may not even be closely related.

In the phylogenetic revision of Hylidae, Faivovich *et al.* (2005) tentatively placed *B. hobbsi* within the *B. punctata* group using this historical taxonomic assessment as support, given the lack of hard data at hand. To date, the phylogenetic placement of *B. hobbsi* within *Boana* remains to be formally tested. After the study of Pyburn (1978), no additional taxonomic or phylogenetic studies included this species. Knowledge advances associated with this species are mostly based only on new distributional records in the rainforests of Colombia (Ruiz-Carranza *et al.* 1996; Acosta-Galvis 2000, 2018; Lynch 2005, 2007; Suárez-Mayorga & Lynch 2017), Venezuela (McDiarmid *et al.* 1988; Barrio-Amorós *et al.* 2019) and Brazil (Simões *et al.* 2019). The latter also presents descriptions of the species advertisement calls (Simões *et al.* 2019).

Based on a new record series of *B. hobbsi* specimens collected in field expeditions at the extreme northwest of Brazil, we assess here, for the first time, the species phylogenetic position, based on molecular data, testing the assignment of *B. hobbsi* to the *Boana punctata*

group. We also provide data on the external morphology of these specimens and compare it with known variations of closely related taxa. In addition, we provide new data on the species geographic distribution in the Brazilian Amazon forest.

Material and methods

We recorded individuals of *B. hobbsi* during night surveys by visual encounter, following Crump & Scott Jr. (1994). Fieldwork was conducted at the forest margins of small streams from two distinct localities in the northwest region of the state of Amazonas, Brazil. Six specimens were collected on the right bank of the Japurá River (1.845994° S, 69.029466° W), city of Japurá, located about 50 km southeast of Vila Bittencourt, at the end of rainy season on September 8th, 2014. Five specimens were collected in the vicinity of the city of São Gabriel da Cachoeira, on November 15th, 2016 (0.130547°S, 67.038947°W). Collected specimens were euthanized with a topical solution of 10% xylocaine, and their femoral muscle or liver tissues were collected and stored in 95% ethanol. Specimens were then fixed with 10% formalin, afterwards preserved in 70% ethanol and deposited at the amphibian section of the Paulo Bührnheim Zoological Collection of the Federal University of Amazonas (CZPB-AA), and at the Amphibians and Reptiles Collection of the National Institute of Amazonian Research (INPA-H), both located in the city of Manaus, Brazil (see voucher numbers at Appendix I). Collecting permits were issued by the Chico Mendes Institute of Biodiversity Conservation (ICMBio/SISBio #11323 and ICMBio/SISBio #44832).

Molecular data collection and phylogenetic analysis

We extracted total DNA using standard phenol/chloroform technique (Sambrook *et al.* 1989) and the Wizard ® commercial kit (Promega, Wisconsin, USA). We selected the mitochondrial 16S ribosomal RNA gene (16S) as a molecular marker due to its recognized relevance as an

amphibian DNA barcode (Vences *et al.* 2005; Fouquet *et al.* 2007; Padial & De la Riva, 2009; Padial *et al.* 2012), and to its efficiency in revealing phylogenetic relationships (e.g., Fouquet *et al.* 2011; Avila *et al.* 2018). The 16S gene was amplified with primers 16Sar and 16Sbr (Palumbi 1996) via Polymerase Chain Reactions (PCR) in 15 μL reaction volumes containing 5.8 μL of ddH₂O, 1.5 μL of 25mM MgCl₂, 1.5 μL of 10X amplification buffer (75mM of Tris HCl, 50 mM of KCl and 20 mM of (NH₄)₂SO₄), 1.5 μL of a 2 μM solution of each primer, 1.5 μL of 10 mM dNTPs (2.5mM of each dNTP), 0.7 μL of Taq DNA polymerase 1 U/ μL (Fermentas, Lithuania) and 1 μL of template DNA (about 40 ng/ μL). The temperature profile consisted of (a) preheating at 90 °C for 60 s, (b) denaturation at 92 °C for 60 s, (c) primer annealing at 50 °C for 50 s, (d) extension at 72 °C for 90 s, and (e) final extension at 72 °C for 5 min. Steps 2–4 were repeated in 35 cycles. PCR products were purified with exonuclease and alkaline phosphatase (Fermentas, Lithuania), and sequenced using standard protocols of ABI BigDye Terminator Cycle Sequencing Kit (ThermoFisher, USA). The resulting single-stranded products were resolved on an ABI 3130xl automatic capillary sequencer and edited using Geneious v.6.1.8. (Kearse *et al.* 2012). Laboratory procedures were conducted at the Laboratory of Animal Genetics and Evolution (LEGAL) – UFAM, and the Thematic Laboratory of Molecular Biology (LTBM) – INPA, both in the city of Manaus, Brazil.

We compiled a dataset containing the new 16S gene sequences of eight specimens of *B. hobbsi*, together with additional 75 homologous sequences downloaded from GenBank (Benson *et al.* 2013); these included 73 sequences of 53 congeneric species, and two sequences of the closely related genus *Aplastodiscus* Lutz, 1950, which were used as outgroup. To embrace the historical knowledge of hyliid frog phylogenies, as well as the most recent taxonomic and systematic changes in its groups, our dataset included sequences from: broader phylogenetic studies (e.g. Salducci *et al.* 2002; Darst & Cannatella, 2004; Faivovich *et al.* 2004; 2005; Köhler *et al.* 2010; Berneck *et al.* 2016; Lyra *et al.* 2017), studies on cryptic

diversity and new species descriptions (e.g. Fouquet *et al.* 2007, 2015, 2016; Antunes *et al.* 2008; Lehr *et al.* 2010; Jansen *et al.* 2011; Funk *et al.* 2012; Caminer & Ron, 2014, 2020; Guarnizo *et al.* 2015; Peloso *et al.* 2018; Motta *et al.* 2018) and recent studies focused in the *B. punctata* group (Lima *et al.* 2019; Sturaro *et al.* 2020). Our dataset (Appendix I) contains representatives of all groups within *Boana* proposed by Faivovich *et al.* (2005). We aligned the dataset using the MAFFT online service, applying the L-INS-i strategy (Katoh *et al.* 2019), and selected the molecular substitution model GTR+I+G based on Akaike Information Criterion, as implemented in PartitionFinder 2.1.1 (Lanfear *et al.* 2017). The phylogenetic tree was inferred under a Bayesian framework, using the software MrBayes 3.2.6 (Ronquist *et al.* 2012), with two independent runs of 30^6 generations. We assessed parameter convergence through stability and convergence between independent and combined runs, and effective sample size (ESS) > 200 using the software Tracer 1.7 (Rambaut *et al.* 2018). We discarded a 10% burn-in of samples and extracted the maximum clade credibility (MCC) tree. Phylogenetic analysis was performed using CIPRES Science Gateway version 3.3 (Miller *et al.* 2010). We additionally estimated uncorrected pairwise distances (p-distances) between the sequences of our newly collected *B. hobbsi* specimens and of closely related congeners (newly evidenced and formerly considered). We estimated uncorrected p-distances using gamma rates and default parameters in MEGA X (Kumar *et al.* 2018).

Morphological data

We obtained the following morphometric measurements of 11 adult specimens: snout-vent length (SVL), head length (HL), head width (HW), interorbital distance (IOD), eye-nostril distance (EN), snout length (SL), eye diameter (ED), tympanum diameter (TD), tibia length (TL), thigh length (THL), tarsus length (TSL), foot length (FL), forearm length (FAL), and hand length (HAL). Measurements were taken with a digital caliper with a precision of 0.01

mm, and the terminology followed Kok & Kalamandeen (2008) and Watters *et al.* (2016). Webbing formula notation followed Savage & Heyer (1967), as modified by Myers & Duellman (1982). Fingers and toes were numbered pre-axially to post-axially, from I–IV and I–V, respectively. Sex was determined by the presence of vocal slits, prepollical spines and body size (Pyburn 1978). Color pattern in life was described based on field notes and photographs of living individuals.

Results

Phylogenetic analysis

Our alignment resulted in a dataset with 558 base pairs (bp) and 81 terminals from the genus *Boana*. Bayesian phylogenetic inferences based on two combined runs (LnL = -6907.7745, ESS = 5334) recovered a tree topology with high support values (posterior probability = 0.95–1.0) for major clades that can be directly associated with previously recognized groups, with the exception of the *Boana pulchella* and *Boana albopunctata* groups, which were recovered with low support values (Fig. 1). Even so, phylogenetic relationships among these major clades were poorly supported (posterior probability < 0.5) most likely due to the limited phylogenetic signal of the 16S gene in deeper nodes.

Our analysis did not recover the *B. punctata* group as monophyletic. Instead, *Boana sibleszi* was recovered with low support as the sister-taxon of a subclade composed by *B. polytaenia* (Cope, 1870) and *B. latistriata* (Caramaschi & Cruz, 2004), therefore nested in the *B. pulchella* group. *Boana picturata* was recovered with low support as the sister-taxon of *Boana heilprini* (Noble, 1923b), therefore nested in the *Boana albopunctata* group. *Boana jimenezi* and *B. hobbsi* were recovered nested in a highly supported clade corresponding to the *Boana benitezi* group (Fig. 1). The other green-colored species *B. atlantica*, *B. punctata*, *B. cinerascens* and *B. gracilis* formed a highly supported clade that comprised the former group

of “green *Hylas* from Amazonian Hylaea” (Lutz, 1948), herein treated as the *Boana punctata* group *sensu stricto* (Fig. 1). *Boana hobbsi* was recovered as monophyletic and the sister-taxon of *Boana tepuiana* (Barrio-Amorós & Brewer-Carias, 2008), although with low support (pp = 0.61). This clade is sister to a clade composed of *B. lemai* (Rivero, 1972) and *B. jimenezi* that also is poorly supported (pp = 0.63). These four species comprise a highly supported clade (pp = 1) within the *B. benitezi* group (Fig.1). Genetic distances (p-distances) between specimens of *B. hobbsi* from the two sampled localities are low, ranging from 0.2% to 1.2% (Table 1). When comparing specimens of *B. hobbsi* to other species of the *B. benitezi* group, the genetic distances ranged from 7% to 15% (Table 2), representing lower values in comparison to that of members of the *B. punctata* group (range 12–16%) (Table 3). Based on such molecular evidence, we propose to include *B. hobbsi* and *B. jimenezi* in the *B. benitezi* group, maintaining the monophyly of this non-morphologically diagnosable group. Our proposal regarding *B. jimenezi* is supported by the systematic review of the *B. punctata* group (Sturaro *et al.* 2020), who found the same result for its phylogenetic position nested in the *B. benitezi* group. To support this new arrangement, we also compare the morphological variations of *B. hobbsi*—which includes the new collected data here presented—with morphological variations of other members of this group.

Systematic account and new data on morphology and distribution of *B. hobbsi*

Family Hylidae Rafinesque, 1815

Tribe Cophomantini (Faivovich, 2005)

Genus *Boana* Gray, 1825

Boana benitezi species group (Faivovich, 2005)

Diagnosis — No known morphological synapomorphy (see Faivovich, 2005). Faivovich *et al.* (2005; 2006) proposed the presence of a flat mental gland as a putative synapomorphy for this

group. However, this character is widely shared among hylid species (Brunetti *et al.* 2012; 2015; Faivovich *et al.* 2013) and its consideration as a synapomorphic character for this group remains dubious (Barrio-Amorós & Brewer-Cárias 2008).

Species content — Ten species. *Boana benitezi* (Rivero, 1961); *Boana hobbsi* (Cochran & Goin, 1970) *Boana jimenezi* (Señaris & Ayarzagüena, 2006); *Boana lemai* (Rivero, 1971); *Boana microderma* (Pyburn, 1977); *Boana nympa* (Faivovich, Moravec, Cisneros-Heredia, & Köhler, 2006); *Boana ornatissima* (Noble, 1923a); *Boana rhythmica* (Señaris & Ayarzagüena, 2002); *Boana roraima* (Duellman & Hoogmoed, 1992); *Boana tepuiana* (Barrio-Amorós & Brewer-Cárias, 2008).

Boana hobbsi (Cochran & Goin, 1970)

Holotype. — MCZ 28052, from Caño Guacayá, a tributary of the Río Apoporis, Amazonas, Colombia.

Morphological variation. —Morphometric data analysis of the newly collected *B. hobbsi* series showed measurements contained within the described variation of the species (Table 3). Other morphological data observed in preserved specimens include: (1) slender body with smooth to weakly shagreened dorsum skin fully covered by melanophores, and a finely granular ventral surface skin; (2) dark brown to pale yellowish dorsum with scattered black spots throughout the dorsal surfaces of body, arms, and shank (Figs. 2A, 2C, 2E); (3) white to cream dorsolateral stripe varying in evidence, beginning at the tip of the snout, crossing the upper eyelid, reaching the sacrum and ending just anterior to the groin (Figs. 2A, 2C, 2E); (4) greenish white to cream belly, with concentration of melanophores on the ventral surfaces of hands, forearms, and feet (Figs. 2B, 2D, 2F); (5) head truncate in lateral view and round in dorsal view, snout truncate in lateral and dorsal views (Figs. 3A, 3B), with an evident mental gland (Fig. 3C); (6) evident prepollex with no spine protruding out of the skin; (7) palmar

tubercle rounded; (8) relative length of fingers $I < II < IV < III$, almost unwebbed ($I - II 2^- - 3^- III 3^- - 3^- IV$) (Fig. 3D); (9) subarticular tubercle rounded in fingers I and II, bifid in fingers III and IV (Fig. 3D); (10) supernumerary tubercles absent; (11) relative length of toes $I < II < III < V < IV$, fully webbed ($I 1^+ - 1^- II 1^+ - 3^- III 1^+ - 1^{1/2} IV 2^+ - 0 V$) (Fig. 3E). Coloration patterns of *B. hobbsi* in life (Fig. 4) include: (1) dark brown to pale green dorsum, with scattered blackish spots; light yellow flanks, fingers, toes and ventral surfaces of forearm and shank; (2) a cream stripe varying in evidence across the dorsolateral regions of head and body; (3) iris golden-yellow, bordered by an intense black ring, with black thin reticulation near the border of the eyelid and a reddish-brown pigmentation around the pupil (Fig 4). Although analyzed specimens were collected from geographically distant localities (nearly 300 km apart), we did not notice any significant difference in morphological characteristics associated with the distinct sampling localities. Our morphological results indicate that despite its relatively wide geographic distribution, the species overall morphology is conserved throughout its range of occurrence.

Comparisons — Data from all other species of the *B. benitezi* group are provided in parenthesis except *Boana pulidoi*, who was recently synonymized. In living and in preserved specimens, *B. hobbsi* differs from all other species of the *B. benitezi* group by the presence of scattered black spots on the dorsal surfaces of body, arms and thighs (absent in all other species), and by a larger body size with mean SVL 41.14 mm (mean SVL < 40.0 mm). By exhibiting a brown dorsal color pattern with a cream dorsolateral stripe, *B. hobbsi* differs from *B. benitezi* (brown, with brown transverse bars varying in width and dorsolateral stripes absent), *B. jimenezi* (green, with large spots in limbs and forearms), *B. lemai* (light yellow to reddish-brown, with dark transversal stains and eventually with white dorsolateral stripes), *B. microderma* (light yellow to orange-brown, with light brown transversal bars), *B. nympha* (bright yellowish green to reddish brown), *B. ornatissima* (green, with an elaborated

ornamentation in the center of the dorsal surface), *B. rhythmica* (reddish brown, with fine black reticulation or black transverse lines, eventually with white dorsolateral stripes), *B. roraima* (light brown with irregular dark brown markings and eventually with middorsal dark stripes), and *B. tepuiana* (in life varying from bright yellow with brown marks, to brown with gray markings, and to dark brown with cream dorsolateral stripes; in preserved specimens a reticulated pattern can also be observed). By its greenish cream-colored ventral surface in living specimens, *B. hobbsi* also differs from *B. benitezi* (bright orange or white) and *B. ornatissima* (varying from greenish yellow to translucid blue). Male specimens of *B. hobbsi* also have an evident mental gland that differs from the condition observed in *B. jimenezi* (absent). In life, *B. hobbsi* can also be distinguished by a unique iris color, with its golden-yellow background, black reticulation and reddish-brown pigmentation around the pupil, from *B. benitezi* (varying from light brown to dark brown), *B. jimenezi* (silvery with a dark brown rim around the pupil and a fine dark reticulation), *B. lemai* (pale green to gray), *B. microderma* (cream to pale yellow with a black peripheric mark), *B. nympa* (varying from cream to creamy-golden with a black peripheric mark), *B. ornatissima* (varying from silver to gray-green background with a reddish-brown area around pupil), *B. rhythmica* (golden without reticulation), *B. roraima* (varying from pale green to yellow without reticulation and a black peripheric mark), and *B. tepuiana* (silvery-gray to dark brown).

Although aforementioned comparisons show that *B. hobbsi* is easily distinguished from congeneric species of the *B. benitezi* group, this species shares greater morphological affinity with this group than when compared to congeneric species of the *B. punctata* group. These morphological similarities include a brown dorsal surface of the body, eventually with highly contrasting white or cream dorsolateral stripes, as observed in *B. lemai*, *B. tepuiana* and *B. benitezi* ; and the dense matrix of chromatophores in the dorsal surface and eventual presence of highly contrasting whitish-cream dorsolateral stripes shared with the greenish

colored *B. jimenezi*. These similarities clearly reflect the closer phylogenetic relationships among species found in our molecular analysis (i.e. the highly supported clade containing *B. hobbsi*, *B. tepuiana*, *B. lemai* and *B. jimenezi*). With remaining members of *B. benitezi* group, *Boana hobbsi* shares a slender body and limbs, eyes bordered by an intense dark ring, and the presence of a mental gland (also present in *B. lemai* and *B. benitezi*).

Geographic distribution. — New sampling localities filled relevant knowledge gaps in the geographic range of *B. hobbsi* (Fig. 5). One of these localities is a small stream inside the forest on the right bank of the Japurá River, near to Vila Bittencourt (1.845994° S, 69.029466° W), located 117 km east of the nearest record on the Caquetá River, and 185 km from the type locality (Caño Guacayá, a tributary of the Río Apoporis, in Amazonas, Colombia – Cochran & Goin [1970]). The second locality is a stream at the road to Cucuí village in the vicinity of São Gabriel da Cachoeira (0.130547°S, 67.038947°W), located 160 km south of the nearest record at Cerro de la Neblina base camp, Venezuela, and 390 km from the type locality (Fig. 5). Our record at São Gabriel da Cachoeira, along with the Cerro de la Neblina Venezuelan record, and the first Brazilian record at Japurá River, all represent the easternmost known limits of the *B. hobbsi* geographic range.

Discussion

The 16S rRNA gene is widely used for reliable species identification (Vences *et al.* 2005; Fouquet *et al.* 2007), for evaluating the monophyly of species, and for discovering divergent lineages (Padial & De la Riva 2009; Padial *et al.* 2010; Padial *et al.* 2012; Motta *et al.* 2018). Our study supports that the variation at the 16S gene, combined with phenotypic data, can be useful to discuss phylogenetic relationships of poorly known anuran species (Fouquet *et al.* 2011; Ávila *et al.* 2018). Moreover, differences in optimality criteria and in number of terminals (Padial *et al.* 2014; Grant 2019) may also explain the disparity of our

results from previously multi locus phylogenetic studies (Faivovich *et al.* 2005; Wiens *et al.* 2005, 2010; Pyron & Wiens 2011; Duellman *et al.* 2016; Pinheiro *et al.* 2019).

The tree topology reported here recovered well-supported major clades representing four of the species groups currently considered within *Boana*: *Boana semilineata* group, *B. pellucens* group, *B. faber* group and *B. benitezi* group (as presently defined). The *Boana punctata* group was recovered here as polyphyletic, due to the phylogenetic position of *B. picturata* within the *B. albopunctata* group, and of *B. sibleszi* within the *B. pulchella* group. However, these relationships were not strongly supported, and in the light of our results, the relationships of *B. picturata* and *B. sibleszi* remain uncertain. Nevertheless, a closer affinity of *B. picturata* with the *B. punctata* group is unlikely given the recent phylogenetic results of Caminer & Ron (2020) and the lack of morphological similarities. Thus, we suggest that phylogenetic approaches focused on the inclusion of non-sampled Cophomantini species may help reveal the relationships of these taxa.

The monophyly of the *B. punctata* group as reported in the literature (e.g. Faivovich *et al.* 2005; Duellman *et al.* 2016; Pinheiro *et al.* 2019; Sturaro *et al.* 2020) has been mostly based on poorly supported phylogenetic relationships of *B. picturata* and *B. sibleszi*, and disregards *B. jimenezi* as part of this group. The lack of molecular affinities of *B. picturata*, *B. sibleszi* and *B. jimenezi* with other species in *B. punctata* group *sensu stricto* seen in our results confirms previous phylogenetic studies (Pyron & Wiens 2011; Pinheiro *et al.* 2019; Caminer & Ron 2020; Sturaro *et al.* 2020). Therefore, green-colored morphological patterns shared between this species is quite common in hylid frogs and constitute cases of evolutionary convergence (Taboada *et al.* 2020). Other studies also reported the non-monophyly of the *B. punctata* group, and even the nominal taxa *B. punctata*, *B. atlantica*, and *B. cinerascens* are paraphyletic and represent species complexes (Jansen *et al.* 2011; Guarnizo *et al.* 2015; Lima *et al.* 2019; Caminer & Ron, 2020; Sturaro *et al.* 2020). After our

phylogenetic positioning of *B. hobbsi*, *B. alemani* remains the only nominal species historically attributed to the *B. punctata* group without a formal phylogenetic test (based on any kind of data). Furthermore, this species taxonomic status is uncertain and seems to be based on a mixed type series, with the holotype being conspecific with *B. punctata* (La Marca, 1992; Camargo *et al.* 2014; Barrio-Amorós *et al.* 2019) and the paratypes likely being juveniles of *Boana xerophylla* (Duméril & Bibron, 1841) (Barrio-Amorós *et al.* 2019). This combination of new evidence points to a stability of the *B. punctata* group as an evolutionary unit, comprising only the species related to the former *Hyla punctata* and *Hyla granosa* groups (the “green *Hylas* from Amazonian Hylaea” *sensu* Lutz [1948]). Nevertheless, a delimitation of the *B. punctata* group is beyond the scope of our study and requires denser taxonomic and molecular sampling.

Phylogenetic affinities of *B. hobbsi* were, until now, hypothesized only based on morphological comparisons (Duellman 1974; Pyburn 1978). Given the historical associations with *B. punctata*, Faivovich *et al.* (2005) provisionally allocated the species in the *B. punctata* group. However, such decision overlooked the divergences already pointed out by Pyburn (1978), who had questioned the phylogenetic affinities of these species due to their remarkable divergences in overall morphology, habitat use and vocalization. After 50 years of being originally described, our study presents the first evidence of the phylogenetic relationships of *B. hobbsi*, based on integrative molecular and morphological approaches, corroborating the statements of Pyburn (1978). In fact, despite advances in our understanding of phylogenetic affinities within the genus *Boana*, a number of issues still remain unclear, particularly regarding the *B. benitezi* group. With the recent taxonomic status updates in the “*B. benitezi*” sequence available at GenBank as representative of *B. tepuiana* (Pinheiro *et al.* 2019), the nominal *B. benitezi* was never included in any molecular phylogenetic analysis. In a similar way, molecular information supporting the placement of *B. rhythmica* within this

group is also missing. Furthermore, in a recent revision of Venezuelan anurans, Barrio-Amorós *et al.* (2019) recognized *Boana pulidoi* (Rivero, 1968) as a junior synonym of *B. benitezi*.

Our results also unraveled an intriguing issue concerning the phylogenetic positioning of a sequence referred as *Boana* sp., from Cerro Guanay, Venezuela (Appendix I). This sequence likely represents a non-described species of the *B. benitezi* group, as already noted by Gorzula & Señaris (1998) and Myers & Donnelly (2008). Morphologically, some issues involving the *B. benitezi* group also remain irresolute, such as diagnosable synapomorphies for the group. Based on current knowledge and on our results, we cannot point to any unambiguous synapomorphy. Still, morphological comparisons within the *B. benitezi* group evidence two groups of similarities: (1) characters shared by *B. hobbsi*, *B. benitezi* (Rivero 1961; Donnelly & Myers 1991; Heyer 1994), *B. lemai* (Rivero 1972; MacCulloch & Lathrop 2005; Señaris *et al.* 2014), *B. rhythmica*, *B. jimenezi* (Señaris & Ayarzagüena, 2006; Señaris *et al.* 2014), *B. ornatissima* and *B. tepuiana* (Barrio-Amorós & Brewer Cárias 2008; Señaris *et al.* 2014), as described above; and (2) characters shared by *B. nympha*, *B. microderma* and *B. roraima*, such as distinctive head, broader than the body, iris creamy golden without reticulation and with a black periphery, and tan to brownish dorsum with dark transversal markings. Studies on the internal morphology of the adults, as well as a refined assessment on the morphology of the species larvae, should shed some light into defining morphological synapomorphies for this group.

Boana hobbsi was known only from records reported in the Colombian and Venezuelan Amazon forest (Cochran & Goin 1970; Pyburn 1978; McDiarmid *et al.* 1988; Ruiz-Carranza *et al.* 1996; Acosta-Galvis 2000; Lynch 2005, 2007; Barrio-Amorós *et al.* 2019) until 2019, when the first record in the Brazilian territory was registered (Simões *et al.* 2019). Our results support the hypothesis that *B. hobbsi* is widely distributed in the northwest

Brazilian Amazonia. This region is known to harbor a unique and endemic amphibian fauna (Azevedo-Ramos & Galatti 2002; Lynch 2005; Menin *et al.* 2017) and has been proposed to be a biogeographic unit (Godinho & Da Silva 2018). Highly restricted geographic distributions are a common pattern for the *B. benitezi* group, as most of its species apparently have small or fragmented ranges within the uplands and highlands of the northern Amazonia (Pantepui region) (Frost 2020). Still, the region is relatively poorly sampled due to the great difficulty of access. The only exceptions to this distribution pattern are *B. nympa*, *B. microderma* and *B. hobbsi*, which occupy the lowlands of western Amazonia (Frost 2020). In fact, we also recorded *B. microderma* syntopically with *B. hobbsi* in the two new localities reported in this study. Based on literature (Pyburn 1978; Simões *et al.* 2019) and on our new records, *B. hobbsi* is associated with small sandy riverbed streams in upland *terra-firme* (non-periodically flooded) forests. Increased fieldwork in these habitats and in this biogeographic region should reveal additional localities of occurrence for *B. hobbsi*, as well for other species from this group, clarifying their geographic ranges. Our results highlight the importance of conducting new biodiversity surveys in remote regions, like the northern and western Amazonia (Azevedo-Ramos & Gallati 2002; Mayer *et al.* 2019), to overcome the biodiversity shortfalls widespread in this mega diverse ecosystem (Bini *et al.* 2006; Brito 2010; Assis 2018).

Acknowledgments

We thank Sebastião Batista, Ivan Junqueira, Elciomar Oliveira, João Danilo, Priscila Azarak, and residents from Taboca village (Villa Bittencourt/AM), for assistance in the sampling. Fieldwork at Japurá River received financial support from: Ministério da Ciência, Tecnologia, Inovação e Comunicações, Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (SISBIOTA Program #563348/2010; Edital Universal #405640/2016-1) and

Fundação de Amparo à Pesquisa do Estado do Amazonas – FAPEAM (Edital FAPEAM/SISBIOTA) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Capes. Fieldwork at Japurá River and São Gabriel da Cachoeira received logistical support from the Comando Militar da Amazônia of Brazilian Army (CMA; Proamazônia program). We thank Victor G.D. Orrico (Zootaxa editor) and two anonymous reviewers who provided suggestions that improved our manuscript. APA thank FAPEAM for PhD fellowship (POSGRAD-UFAM 2016), LJCLM received a fellowship from CNPq (PCI process 313055/2015-7). MM received productivity from CNPq (#303070/2017-0) and financial support by CNPq (#405640/2016-1), IJR thank CAPES for PhD fellowship (88882.156872/2016-01), VTC thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES for fellowship (PNPD), and FPW thanks financial support from CNPq (productivity fellowship #305535/2017-0), Fundação de Amparo à Pesquisa do Estado do Amazonas-FAPEAM (#062.00962/2018 and 062.01110/2017), #and L’Oréal-Unesco For Women in Science Program (Brazil 2016 and IRT 2017).

References

- Acosta-Gálvis, A.R. (2000) Ranas, Salamandras y Caecilias (Tetrapoda: Amphibia) de Colombia. *Biota Colombiana*, 1, 3, 289–319.
- Acosta-Gálvis, A. R. (2018) Anfíbios del Escudo Guayanés en Colombia. In: Lasso, C.A. & Señaris, J.C. (Eds), *Volumen VI. Fauna Silvestre del Escudo Guayanés (Colombia-Venezuela)*. Série Editorial Fauna Silvestre Neotropical, Instituto de Investigación de Recursos Biológicos Alexander Von Humboldt, Bogotá D.C., Colombia.
- Antonelli, A., Ariza, M., Albert, J., Andermann, T., Azevedo, J., Bacon, C., Faurby, S., Guedes, T., Hoorn, C., Lohmann, L.G., Matos-Maraví, P., Ritter, C.D., Sanmartín, I., Silvestro, D., Tejedor, M., ter Steege, H., Tuomisto, H., Werneck, F.P., Zizka, A. & Edwards, S.V. (2018a) Conceptual and empirical advances in Neotropical diversity research. *PeerJ*, 6, e5644.
<https://doi.org/10.7717/peerj.5644>

- Antonelli, A., Zizka, A., Carvalho, F.A., Schran, R., Bacon, C.D., Silvestro, D. & Condamine, F.L. (2018b) Amazonia is the primary source of Neotropical biodiversity. *PNAS*, 115, 23, 6034–6039.
<https://doi.org/10.1073/pnas.1713819115>
- Antunes, A.P., Faivovich, J. & Haddad, C.F.B. (2008) A new species from the Atlantic Forest of Southeastern Brazil (Amphibia: Anura: Hylidae). *Copeia*, 2008, 1, 179–190.
<https://doi.org/10.1643/CH-06-163>
- Assis, L.C.S. (2018) Revisiting the Darwinian shortfall in biodiversity conservation. *Biodiversity and Conservation*, 27, 11, 2859–2875.
<https://doi.org/10.1007/s10531-018-1573-3>
- Ávila, R.W., Pansonato, A., Perez, R., De Carvalho, V.T., Roberto, I.J., Morais, D.H., Almeida, A.P., Rojas, R., Gordo, M. & Farias, I.P. (2018) On *Rhinella gildae* Vaz-Silva, Maciel, Bastos & Pombal 2015 (Anura: Bufonidae): Phylogenetic relationship, morphological variation, advertisement and release calls and geographic distribution. *Zootaxa*, 4462, 274–290.
<http://dx.doi.org/10.11646/zootaxa.4462.2.9>
- Azevedo-Ramos, C. & Galatti, U. (2002) Patterns of amphibian diversity in Brazilian Amazonia: Conservation implications. *Biological Conservation*, 103, 1, 103–111.
[https://doi.org/10.1016/S0006-3207\(01\)00129-X](https://doi.org/10.1016/S0006-3207(01)00129-X)
- Barrio-Amorós, C.L. & Brewer-Carias, C. (2008) Herpetological results of the 2002 expedition to Sarisariñama, a tepui in Venezuelan Guayana, with the description of five new species. *Zootaxa*, 1942, 1–68.
<https://doi.org/10.11646/zootaxa.1942.1.1>
- Barrio-Amorós, C.L., Rojas-Runjaic, F.J.M. & Señaris, J.C. (2019) Catalogue of the amphibians of Venezuela: Illustrated and annotated species list, distribution, and conservation. *Amphibian & Reptile Conservation*, 13, 1, 1–198.
- 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.
<https://doi.org/10.1093/nar/gks1195>
- Berneck, B.V.M., Haddad, C.F.B., Lyra, M.L., Cruz, C.A.G. & Faivovich, J. (2016) The Green Clade grows: A phylogenetic analysis of *Aplastodiscus* (Anura; Hylidae). *Molecular Phylogenetics and Evolution*, 97, 213–223.
<http://dx.doi.org/10.1016/j.ympev.2015.11.014>

- Bini, L.M., Diniz-Filho, J.A.F., Rangel, T.F.L.V., Bastos, R.P. & Pinto, M.P. (2006) Challenging Wallacean and Linnean shortfalls: knowledge gradients and conservation planning in a biodiversity hotspot. *Diversity and Distribution*, 12, 5, 475–482.
<https://doi.org/10.1111/j.1366-9516.2006.00286.x>
- Boulenger, G.A. (1899) Descriptions of new batrachians in the collection of the British Museum (Natural History). *Annals and Magazine of Natural History*, 7, 273–277.
<https://doi.org/10.1080/00222939908678122>
- Brito, D. (2010) Overcoming the Linnean shortfall: Data deficiency and biological surveys priorities. *Basic and Applied Ecology*, 11, 8, 709–713.
<http://dx.doi.org/10.1016/j.baae.2010.09.007>
- Brunetti, A.E., Hermida, G.H. & Faivovich, J. (2012) New insights into sexually dimorphic skin glands of Anurans: The structure and ultrastructure of mental and lateral glands in *Hypsiboas punctatus* (Amphibia: Anura: Hylidae). *Journal of Morphology*, 273, 1257–1271.
<https://doi.org/10.1002/jmor.20056>
- Brunetti, A.E., Hermida, G.H., Luna, M.C., Barsotti, A.M.G., Jared, C., Antoniazzi, M.M., Rivera-Correa, M., Berneck, B.V.M. & Faivovich, J. (2015) Diversity and evolution of sexually dimorphic mental and lateral glands in Cophomantini treefrogs (Anura: Hylidae: Hylinae). *Biological Journal of the Linnean Society*, 114, 1, 12–34.
<https://doi.org/10.1111/bij.12406>
- Camargo, E.G., Rivero, R. & Barrio-Amorós, C.L. (2014) Datos sobre la distribución de cuatro especies de anfibios (Amphibia, Anura) de Venezuela. *Anartia*, 26, 63–68.
- Caminer, M.A. & Ron, S.R. (2014) Systematic of the *Hypsiboas calcaratus* and *Hypsiboas fasciatus* species complex (Anura, Hylidae) with description of four new species. *Zookeys*, 370, 1, 1–68.
<https://doi.org/10.3897/zookeys.370.6291>
- Caminer, M.A. & Ron, S.R. (2020) Systematics of the *Boana semilineata* species group (Anura: Hylidae), with a description of two new species from Amazonian Ecuador. *Zoological Journal of the Linnean Society*, zlaa002.
<https://doi.org/10.1093/zoolinnea/zlaa002>
- Caramaschi, U. & Cruz, C.A.G. (2004) Duas novas espécies de *Hyla* do grupo *H. polytaenia* Cope, 1870 do sudeste do Brasil (Amphibia, Anura, Hylidae). *Arquivos do Museu Nacional do Rio de Janeiro*. 62, 247–254.
- Caramaschi, U. & Velosa, A. (1996) Nova especie de *Hyla Laurenti*, 1768 do leste brasileiro

- (Amphibia, Anura, Hylidae). *Boletim do Museu Nacional, Nova Série, Zoologia*, 365, 1–7.
- Cardoso, P., Erwin, T.L., Borges, P.A.V. & New, T.R. (2011) The seven impediments in invertebrate conservation and how to overcome them. *Biological Conservation*, 144, 2647–2655.
<http://dx.doi.org/10.1016/j.biocon.2011.07.024>
- Carvalho, T.R.D., Moraes, L.J.C.L., Lima, A.P., Fouquet, A., Peloso, P.L.V., Pavan, D., Drummond, L.O., Rodrigues, M.T., Giarretta, A.A., Gordo, M., Neckel-Oliveira, S. & Haddad, C.F.B. (2020) Systematic and historical biogeography of the Neotropical foam-nesting frogs of the *Adenomera heyeri* clade (Leptodactylidae), with description of six new Amazonian species. *Zoological Journal of Linnean Society*, zlaa051.
<https://doi.org/10.1093/zoolinnean/zlaa051>
- Castroviejo-Fisher, S., Köhler, J., De la Riva, I. & Padial, J.M. (2017) A new morphologically cryptic species of *Phyllomedusa* (Anura: Phyllomedusidae) from Amazonian forests of northern Peru revealed by DNA sequences. *Zootaxa*, 4269, 2, 245–264.
<https://doi.org/10.11646/zootaxa.4269.2.4>
- Cochran, D.M. & Goin, C.J. (1970) *Frogs of Colombia*. Smithsonian Institution Press, USA. 655 pp.
<https://doi.org/10.5479/si.03629236.288.1>
- Cope, E.D. (1870) Seventh contribution to the herpetology of tropical America. *Proceedings of the American Philosophical Society*. 11, 147–169.
- Crump, M.L. & Scott Jr., N.J. (1994) Visual encounter survey. In: Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.C. & Foster, M.S. (Eds) *Measuring and monitoring biological diversity: Standard methods for amphibians*. Smithsonian Institution Press, Washington D.C., US, 84–92.
- Darst, C.R. & Canattella, D.C. (2004) Novel relationships among hyloid frogs inferred from 12S and 16S mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 31, 2, 462–475.
<https://linkinghub.elsevier.com/retrieve/pii/S1055790303003464>
- Diniz-Filho, J.A.F., Loyola, R.D., Raia, P., Mooers, A.O. & Bini, L.M. (2013) Darwinian shortfall in biodiversity conservation. *Trends in Ecology and Evolution*, 28, 12, 689–695.
<https://linkinghub.elsevier.com/retrieve/pii/S0169534713002127>

- Donnelly, M.A. & Myers, C.W. (1991) Herpetological results of the 1990 Venezuelan Expedition to the summit of Cerro Guaiquinima, with new tepui reptiles. *American Museum Novitates*, 3017, 1-54.
<http://hdl.handle.net/2246/5042>
- Dubois, A. (2017) The nomenclatural status of *Hysaplesia*, *Hylaplesia*, *Dendrobates* and related nomina (Amphibia, Anura), with general comments on zoological nomenclature and its governance, as well as on taxonomic databases and websites. *Bionomina*, 11, 1, 1.
<https://doi.org/10.11646/bionomina.11.1.1>
- Duellman, W.E. (1974) A reassessment of the taxonomic status of some neotropical hylid frogs. *Occasional Papers of the Museum of Natural History, The University of Kansas, Lawrence, Kansas*, 27, 1–27.
- Duellman, W.E. & Hoogmoed, M.S. (1992) Some Hylid frogs from the Guiana Highlands, Northeastern South America: New species, distributional records and generic reallocation. *Occasional Papers of The Museum of Natural History*, 147, 1–21.
- Duellman, W.E.; Marion, A.B. & Hedges, S.B. (2016) Phylogenetics, classification, and biogeography of the treefrogs (Amphibia: Anura: Arboranae). *Zootaxa*, 4140, 1, 1–109.
<https://doi.org/10.11646/zootaxa.4104.1.1>
- Faivovich, J., Garcia, P.C.A., Ananias, F., Lanari, L., Basso, N.G. & Wheeler, W.C. (2004) Molecular perspective on the phylogeny of *Hyla pulchella* species group (Anura, Hylidae). *Molecular Phylogenetics and Evolution*, 32, 3, 938–950.
<https://linkinghub.elsevier.com/retrieve/pii/S1055790304001058>
- 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, 1–240.
[https://doi.org/10.1206/0003-0090\(2005\)294\[0001:srotff\]2.0.co;2](https://doi.org/10.1206/0003-0090(2005)294[0001:srotff]2.0.co;2)
- Faivovich, J., McDiarmid, R.W. & Myers, C.W. (2013) Two new species of *Myersiophyla* (Anura: Hylidae) from Cerro de la Neblina, Venezuela, with comments on other species of the genus. *American Museum Novitates*, 3792, 1–63.
<https://doi.org/10.1206/3792.1>
- Faivovich, J., Moravec, J., Cisneros-Heredia, D.F. & Köhler, J. (2006) A New Species of the *Hypsiboas benitezi* Group From the Western Amazon Basin (Amphibia: Anura: Hylidae). *Herpetologica*, 62, 1, 96–108.

<https://doi.org/10.1655/05-12.1>

Field, R., Hawkins, B.A., Cornell, H.V., Currie, D.J., Filho, J.A.F.D., Guégan, J.F., Kaufman, D.M., Kerr, J.T., Mittelbach, G.G., Oberdorff, T., O'Brien, E.M. & Turner, J.R.G. (2009) Spatial species-richness gradients across scales: a meta-analysis. *Journal of Biogeography*, 36, 1, 132–147.

<https://doi.org/10.1111/j.1365-2699.2008.01963.x>

Fouquet, A., Cortois, E.A., Baudain, D., Lima, J.D., Souza, S.M., Noonan, B.P. & Rodrigues, M.T. (2015) The trans-riverine genetic structure of 28 Amazonian frog species is dependent of life history. *Journal of Tropical Ecology*, 31, 361–373.

<https://doi.org/10.1017/S0266467415000206>

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

<https://doi.org/10.1371/journal.pone.0001109>

Fouquet, A., Martinez, Q., Zeidler, L., Courtois, E.A., Gaucher, P., Blanc, M., Lima, J.D., Souza, S.M., Rodrigues, M.T. & Kok, P.J.R. (2016) Cryptic diversity in the *Hypsiboas semilineatus* species group (Amphibia, Anura) with description of a new species from eastern Guiana Shield. *Zootaxa*, 4084, 1, 79–104.

<http://biotaxa.org/Zootaxa/article/view/zootaxa.4084.1.3>

Fouquet, A., Noonan, B.P., Blanc, M. & Orrico, V.G.D. (2011) Phylogenetic position of *Dendropsophus gaucheri* (Lescure and Marty 2000) highlights the need for an in-depth investigation of the phylogenetic relationships of *Dendropsophus* (Anura: Hylidae). *Zootaxa*, 3035, 59–67.

<http://dx.doi.org/10.11646/zootaxa.3035.1.3>

Frost, D.R. (2020) Amphibians Species of the World: an Online Reference. Version 6.1. American Museum of Natural History, New York, USA. Eletronic Database accessible at <https://amphhibiansoftheworld.amnh.org/index.php> (accessed 7 April 2020)

<https://doi.org/10.5531/db.vz.0001>

Funk, W.C., Caminer, M. & Ron, S. (2012) High levels of cryptic species diversity uncovered in Amazonian frogs. *Proceedings of the Royal Society B*, 279, 1806–1814.

<https://doi.org/10.1098/rspb.2011.1653>

Godinho, M.B.D.C. & Da Silva, F.R. (2018) The influence of riverine barriers, climate, and topography on the biogeographic regionalization of Amazonian anurans. *Scientific Reports*, 8, 1, 1–11.

<https://doi.org/10.1038/s41598-018-21879-9>

- Gorzula, S. & Señaris, J.C. (1998) Contribution to the herpetofauna of the Venezuelan Guayana I. A data base. *Scientia Guianae*, 8, xviii–270.
- Guarnizo, C.E, Paz, A., Muñoz-Ortiz, A., Flechas, S.V., Méndez-Narváez, J. & Crawford, A.J. (2015) DNA Barcoding Survey of Anurans across the Eastern Cordillera of Colombia and the Impact of the Andes on Cryptic Diversity. *PLoS ONE*, 10, 5, e0127312.
<https://doi.org/10.1371/journal.pone.0127312>
- Grant, T. (2019) Outgroup sampling in phylogenetics: Severity of test and successive outgroup expansion. *Journal of Zoological Systematics and Evolutionary Research*, 57, 4, 748–763.
<https://doi.org/10.1111/jzs.12317>
- Heyer, W.R. (1994) *Hyla benitezi* (Amphibia: Anura: Hylidae): First Record for Brazil and Its Biogeographical Significance. *Journal of Herpetology*, 28, 4, 497–499.
<https://doi.org/10.2307/1564964>
- Hoogmoed, M. (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 Leiden*, 172, 1–46.
- Jansen, M., Bloch, R., Schulze, A. & Pfenninger, M. (2011) Integrative inventory of Bolivia's lowland anurans reveals hidden diversity. *Zoologica Scripta*, 40, 6, 567–583.
<https://doi.org/10.1111/j.1463-6409.2011.00498.x>
- Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, 20, 4, 1160–1166.
<https://doi.org/10.1093/bib/bbx108>
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 12, 1647–1649.
<https://doi.org/10.1093/bioinformatics/bts199>
- Köhler, J., Koscinski, D., Padial, J.M., Chaparro, J.C., Handford, P., Loughheed, S.C. & De la Riva, I. (2010) Systematic of the Andean gladiator frog of the *Hypsiboas pulchellus* species group. *Zoologica Scripta*, 39, 6, 572–590.
<https://doi.org/10.1111/j.1463-6409.2010.00448.x>

- Kok, P.J.R. (2006) A new species of *Hypsiboas* (Amphibia: Anura: Hylidae) from Kaieteur National Park, eastern edge of the Pakaraima Mountains, Guyana. *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Biologie*, 76, 191–200.
- Kok, P.J.R. & Kalamandeen, M. (2008) Introduction to the taxonomy of the amphibians of Kaieteur National Park, Guyana. *Abc Taxa*, 5, 1–278.
- Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGAX: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, 35, 1547–1549.
<https://doi.org/10.1093/molbev/msy096>
- La Marca, E. (1992) *Catálogo Taxonómico, Biogeográfico y Bibliográfico de las Ranas de Venezuela*. Cuadernos Geográficos ULA 9. Universidade de Los Andes, Mérida, Mérida, Venezuela, 197pp.
- Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T. & Calcott, B. (2017) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, 34, 772–773.
<https://doi.org/10.1093/molbev/msw260>
- Lehr, E., Faivovich, J. & Jungfer, K. (2010) A new andean species of the *Hypsiboas pulchella* group: adults, calls and phylogenetic relationship. *Herpetologica*, 66, 3, 296–307.
<https://doi.org/10.1655/09-026.1>.
- Lima, L.R., Dubeux, M.J.M., Nascimento, F.A.C.D., Bruschi, D.P. & Mott, T. (2019) Uncovering Neotropical treefrog diversity: Integrative taxonomy reveal paraphyly in *Boana atlantica* (Amphibia, Anura, Hylidae). *Amphibia Reptilia*, 40, 4, 511–521.
<https://doi.org/10.1163/15685381-20191109>
- Lutz, B. (1948) Anfíbios anuros da coleção Adolfo Lutz Trabalho do Instituto Oswaldo Cruz. II. Espécies Verdes do Gênero *Hyla* do Leste Meridional do Brasil. *Memórias do Instituto Oswaldo Cruz*, 46, 3, 551–577
- Lutz, B. (1950) Anfíbios anuros da coleção Adolfo Lutz Trabalho do Instituto Oswaldo Cruz.V. *Memórias do Instituto Oswaldo Cruz*, 48, 599–637.
- Lynch J.D. (2005) Discovery of the richest frog fauna in the World - an exploration of the forests to the north of Leticia. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales*, 29, 113, 581-588.
- Lynch J.D. (2007) Diversidad Biológica del sur de la Amazonía colombiana. In: Ruiz S. L., Sánchez E., Tabares E., Prieto A., Arias J. C, Gómez R., Castellanos D., García P. & Rodríguez, L. (Eds.), *Diversidad biológica y cultural del sur de la Amazonia*

- colombiana* - Diagnóstico. Corpoamazonia, Instituto Humboldt, Instituto Sinchi, UAESPNN, Bogotá, pp. 595–600.
- Lyra, M.L., Haddad, C.F.B. & Azeredo-Espin, A.M. L. (2017) Meeting the challenge of the DNA barcoding Neotropical amphibians: polymerase chain reaction optimization and new COI primers. *Molecular Ecology Resources*, 17, 966–980.
<http://doi.wiley.com/10.1111/1755-0998.12648>
- MacCulloch, R.D. & Lathrop, A. (2005) Hylid frogs from Mount Ayanganna, Guyana: New species, redescrptions, and distributional records. *Phyllomedusa*, 4, 1, 17–37.
<https://doi.org/10.11606/issn.2316-9079.v4i1p17-37>
- Mayer, M., da Fonte, L.F.M. & Lötters, S. (2019) Mind the gap! A review of Amazonian anurans in GenBank. *Salamandra*, 55, 2, 89–96.
<http://doi.org/10.5281/zenodo.2840666>
- McDiarmid, R.W., Cocroft, R.B. & Paolillo, A. (1988) Preliminary Field Report. Herpetology Collections - Cerro de la Neblina. In: Brewer-Carias, C. (Ed.), *Cerro de la Neblina. Resultados de la Expedition 1983-1987*. Fundación para el Desarrollo del Comercio Internacional, Caracas, pp. 665–670.
- McDiarmid, R.W. & Donnelly, M.A. (2005) The herpetofauna of Guayana Highlands: Amphibians and reptiles of the Lost World. In: Donnelly, M.A., Crother, B.I., Guyer, G., Wake, M.H. & White, M.E. (Eds) *Ecology and Evolution in the tropics: a herpetological perspective*. University of Chicago press, Chicago, USA, 461–560.
- Menin, M., De Carvalho, V.T., Almeida, A.P., Gordo, M., Oliveira, D.P., Luiz, L.F., Campos, J.V. & Hrbek, T. (2017) Amphibians from Santa Isabel do Rio Negro, Brazilian Amazonia. *Phyllomedusa*, 16, 2, 183–199.
<http://dx.doi.org/10.11606/issn.2316-9079.v16i2p183-199>
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop*, 2010, 1–8.
<https://doi.org/10.1109/GCE.2010.5676129>
- Moritz, C., Patton, J.L., Schneider, C.J. & Smith, T.B. (2000) Diversification of rainforest faunas: an integrated molecular approach. *Annual Review of Ecology and Systematics*, 31, 1, 533–563.
<https://doi.org/10.1146/annurev.ecolsys.31.1.533>
- Morrone, J.J. (2014) Biogeographic regionalization of the Neotropical region. *Zootaxa*, 3782, 1, 1–110.

<http://biotaxa.org/Zootaxa/article/view/zootaxa.3782.1.1>

Motta, J., Menin, M., Almeida, A.P., Hrbek, T. & Farias, I.P. (2018) When the unknown lives next door: A study of central Amazonian anurofauna. *Zootaxa*, 4438, 1, 79–104.

<https://doi.org/10.11646/zootaxa.4438.1.3>

Myers, C.W. & Duellman, W.E. (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.

Myers, C.W. & Donnelly M.A. (2008) The summit Herpetofauna of Ayantepui, Venezuela: Report from The Robert G. Goelet American Museum – Terramar Expedition. *Bulletin of the American museum of Natural History*, 308, 1 –147.

<http://www.bioone.org/doi/abs/10.1206/308.1>

Noble, G.K. (1923 a) New batrachians from the tropical research station British Guiana. *Zoologica*, III, 14, 291–293.

Noble, G.K. (1923 b) Six new batrachians from the Dominican Republic. *American Museum Novitates*. 61, 1–6.

<http://hdl.handle.net/2246/4342>

Padial, J.M., Chaparro, J.C., Castroviejo–Fisher, S., Guyasamin, J., Lehr, E., Delgado, A., Vaira, M., Teixeira, J.R., Aguayo, R. & De la Riva, I. (2012) A revision of species diversity in the Neotropical genus *Oreobates* (Anura: Strabomantidae), with the description of three new species from the Amazonian slopes of the Andes. *American Museum Novitates*, 3755, 1–55.

<http://dx.doi.org/10.1206/3752.2>

Padial, J.M. & De la Riva, I. (2009) Integrative taxonomy reveals cryptic Amazonian species of *Pristimantis* (Anura: Strabomantidae). *Zoological Journal of the Linnean Society*, 155, 97–122.

<https://doi.org/10.1111/j.1096-3642.2008.00424.x>

Padial, J.M., Miralles, A., De la Riva, I. & Vences, M. (2010) The integrative future of taxonomy. *Frontiers in Zoology*, 7, 16.

<https://doi.org/10.1186/1742-9994-7-16>

Padial, J.M., Grant, T. & Frost, D.R. (2014) Molecular systematics of terraranas (Anura: Brachycephaloidea) with an assessment of the effects of alignment and optimal criteria. *Zootaxa*, 3825, 1, 1–132.

<https://doi.org/10.11646/zootaxa.3825.1.1>

- Palumbi, S.R. (1996) Nucleic acids II: the polymerase chain reaction. *In*: Hillis, D.M., Moritz, C. & Mable, B.K. (Eds.) *Molecular Systematics*. Sinauer & Associates Inc., Sunderland, Massachusetts, 205–247 pp.
- Peloso, P.L.V., Oliveira, R.M., Sturaro, M.J., Rodrigues, M.T., Lima-Filho, G.R., Bitar, Y.O.C., Wheeler, W.C. & Aleixo, A. (2018) Phylogeny of map tree frogs, *Boana semilineata* species group, with new Amazonian species (Anura: Hylidae). *South American Journal of Herpetology*, 13, 2, 150–169.
<http://doi.org/10.2994/SAJH-D-17-00037.1>
- Pinheiro, P.D.P., Kok, P.J.R., Noonan, B.P., Means, D.B., Haddad, C.F.B. & Faivovich, J. (2019) A new genus of Cophomantini, with comments on the taxonomic status of *Boana liliae* (Anura: Hylidae). *Zoological Journal of the Linnean Society*, 185, 226–245.
<https://doi.org/10.1093/zoolinnea/zly030>
- Pyburn, W.F. (1977) A New Hylid Frog (Amphibia, Anura, Hylidae) from the Vaupes River of Colombia with Comments on Related Species. *Journal of Herpetology*, 11, 4, 405–410.
<https://doi.org/10.2307/1562722>
- Pyburn, W.F. (1978) The voice and relationship of the treefrog *Hyla hobbsi* (Anura: Hylidae). *Proceedings of the Biological Society of Washington*, 91, 123–131.
- Pyron, R.A. & Wiens, J.J. (2011) A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Biology and Evolution*, 61, 543–583.
<https://doi.org/10.1016/j.ympev.2011.06.012>
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67, 5, 901–904.
<https://doi.org/10.1093/sysbio/syy032>
- Ramos, E.K.S., Magalhães, R.F., Marques, N.C.S., Baêta, D., Garcia, P.C.A. & Santos, F.R. (2019) Cryptic diversity in Brazilian endemic monkey frogs (Hylidae, Phyllomedusinae, *Pithecopus*) revealed by multispecies coalescent and integrative approaches. *Molecular Phylogenetics and Evolution*, 132, 105–116.
<https://doi.org/10.1016/j.ympev.2018.11.022>
- Rivero, J.A. (1961). Salentia of Venezuela. *Bulletin of the Museum of Comparative Zoology*, 26, 1, 1–207.
- Rivero, J.A. (1964) Salientios (Amphibia) en la Coleccion de la Sociedad de Ciencias Naturales la Salle de Venezuela. *Caribbean Journal of Science*, 4, 1, 297–305.

- Rivero, J.A. (1972) Notas sobre los anfibios de Venezuela. I. Sobre los hilídos de la Guayana Venezolana. *Caribbean Journal of Science*, 11, 181–193.
- Rojas, R.R., Fouquet, A., Ron, S.R., Hernández-Ruz, E.J., Melo-Sampaio, P.R., Chaparro, J.C., Vogt, R.C., Carvalho, V.T., Pinheiro, L.C., Ávila, R.W., Farias, I.P., Gordo, M. & Hrbek, T. (2018) A Pan-Amazonian species delimitation: high species diversity within the genus *Amazophrynella* (Anura: Bufonidae). *PeerJ*, 6, e4941.
<https://peerj.com/articles/4941>
- Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61, 539–542.
<https://doi.org/10.1093/sysbio/sys029>
- Ruiz-Carranza, P.M., Ardila-Robayo, M.C. & Lynch, J.D. (1996) Lista actualizada de la fauna de Amphibia de Colombia. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales*, 20, 77, 365–415.
- Salducci, M., Marty, C., Chappaz, R. & Gilles, A. (2002) Molecular phylogeny of French Guiana Hyliinae: implications for the systematic and biodiversity of the Neotropical frogs. *Comptes Rendus – Biologies*, 325, 2, 141–153.
<https://linkinghub.elsevier.com/retrieve/pii/S1631069102014233>
- Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, USA.
- Savage, J.M. & Heyer, R.W. (1967) Variation and distribution of the tree-frog genus *Phyllomedusa* in Costa Rica, Central America. *Beiträge zur Neotropischen Fauna*, 5, 111–131.
- Schneider, J.G. (1799) *Historia Amphibiorum Naturalis et Literariae. Fasciculus Primus. Continens Ranas, Calamitas, Bufones, Salamandras et Hydros in Genera et Species Descriptos Notisque suis Distinctos. Friederici Frommanni, Jena*, 264 pp.
<https://doi.org/10.5962/bhl.title.78757>
- Señaris, J.C. & Ayarzagüena, J. (2002) A New Species of *Hyla* (Anura: Hylidae) from the Highlands of Venezuelan Guayana. *Journal of Herpetology*, 36, 4, 634–640.
<https://doi.org/10.2307/1565934>
- Señaris, J.C. & Ayarzagüena, J. (2006) A new species of *Hypsiboas* (Amphibia; Anura; Hylidae) from the Venezuelan Guayana, with notes on *Hypsiboas sibleszi* (Rivero 1972). *Herpetologica*, 62, 309–319.

[https://doi.org/10.1655/0018-0831\(2006\)62\[308:ANSOHH\]2.0.CO;2](https://doi.org/10.1655/0018-0831(2006)62[308:ANSOHH]2.0.CO;2)

- Señaris, J.C., Lampo, M., Rojas-Runjaic, F.J.M. & Barrio-Amorós, C.L. (2014) *Guía ilustrada de los anfibios del Parque Nacional Canaima, Venezuela*. Ediciones IVIC, Instituto Venezolano de Investigaciones Científicas (IVIC). Caracas, Venezuela, 264 pp.
- Simões, P.I., Rojas-Runjaic, F.M., Gagliardi-Urrutia, G. & Castroviejo-Fisher, S. (2019) Five new country records of Amazonian anurans for Brazil, with notes on morphology, advertisement calls, and natural history. *Herpetology Notes*, 12, 211–219.
- Spix, J.B. (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*, 1–53 pp.
<https://doi.org/10.5962/bhl.title.63182>
- Suárez-Mayorga, A.M. & Lynch, J.D. (2017) Myth and truth on the herpetofauna of Chiribiquete: from the lost world to the last world. *Revista Colombia Amazónica*, 10, 177–190.
- Sturaro, M.J., Costa, J.C.L., Maciel, A.O., Lima-Filho, G.R., Rojas-Runjaic, F.J.M., Mejia, D.P. & Peloso, P.L.V. (2020) Resolving the taxonomic puzzle of *Boana cinerascens* (Spix, 1824), with resurrection of *Hyla granosa gracilis* Melin, 1941 (Anura:Hylidae). *Zootaxa*, 4750, 1, 1–30.
<https://doi.org/10.11646/zootaxa.4750.1.1>
- Taboada, C., Brunetti, A.E., Lyra, M.L., Fitak, R.R., Soverna, A.F., Ron, S.R., Lagorio, M.G., Haddad, C.F.B., Lopes, N.P., Johsen, S., Faivovich, J., Chemes, L.B. & Bari, S.E. (2020) Multiple origins of green coloration in frogs mediated by a novel biliverdin-binding serpin. *Proceedings of the National Academy of Sciences*, 117, 31, 18574–18581.
<http://www.pnas.org/lookup/doi/10.1073/pnas.2006771117>
- Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680.
- Vences, M., Meike, T., Van der Meijden, A., Chiari, Y. & Vieites, D. (2005) Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology*, 2, 7–12.
<https://doi.org/10.1186/1742-9994-2-5>

- von May, R., Catenazzi, A., Santa-Cruz, R., Gutierrez, A.S., Moritz, C. & Rabosky, D.L. (2019) Thermal physiological traits in tropical lowland amphibians: Vulnerability to climate warming and cooling. *PLoS ONE*, 14, 8, 1–18.
<https://dx.plos.org/10.1371/journal.pone.0219759>
- Watters, J.L., Cummings, S.T., Flanagan, R.L. & Siler, C.D. (2016) Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. *Zootaxa*, 4072, 4, 477–495.
<https://doi.org/10.11646/zootaxa.4072.4.6>
- Whittaker, R.J., Araújo, M.B., Paul, J., Ladle, R.J., Watson, J.E.M. & Willis, K.J. (2005) Conservation biogeography: Assessment and prospect. *Diversity and Distribution*, 11, 3–23.
<https://doi.org/10.1111/j.1366-9516.2005.00143.x>
- Wiens, J.J., Fetzner Jr.J.W., Parkinson, C.L. & Reeder, T. (2005) Hylid frogs phylogeny and sampling strategies for speciose clades. *Systematic Biology*, 54, 5, 719–748.
- 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.

Table 1. Uncorrected pairwise distances between samples of *Boana hobbsi* (Cochran & Goin, 1970) from the two new localities of occurrence, showing low genetic distances. Specimens from the Japurá River (vicinity of Vila Bittencourt, Amazonas, Brazil) are represented with CZPB-AA vouchers, while the ones from São Gabriel da Cachoeira, Amazonas, Brazil are represented with INPA-H vouchers.

Specimens	1	2	3	4	5	6	7	8
1 <i>B. hobbsi</i> CZPB-AA 1657								
2 <i>B. hobbsi</i> CZPB-AA 1659	0.002							
3 <i>B. hobbsi</i> CZPB-AA 1660	0.005	0.007						
4 <i>B. hobbsi</i> INPA-H 37272	0.002	0.005	0.007					
5 <i>B. hobbsi</i> INPA-H 37273	0.002	0.005	0.007	0.001				
6 <i>B. hobbsi</i> INPA-H 37274	0.007	0.010	0.012	0.004	0.004			
7 <i>B. hobbsi</i> INPA-H 37275	0.005	0.007	0.010	0.002	0.002	0.006		
8 <i>B. hobbsi</i> INPA-H 37276	0.002	0.005	0.007	0.001	0.001	0.004	0.002	

Table 2. Uncorrected pairwise distances between samples of *Boana hobbsi* (Cochran & Goin, 1970) and congeneric species of the *Boana benitezi* group (highlighted in bold).

	Taxon	1	2	3	4	5	6	7	8	9
1	<i>Boana hobbsi</i>									
2	<i>Boana jimenezi</i>	0.07								
3	<i>Boana lemai</i>	0.07	0.04							
4	<i>Boana microderma</i>	0.11	0.11	0.12						
5	<i>Boana nympha</i>	0.12	0.12	0.11	0.09					
6	<i>Boana ornatissima</i>	0.15	0.13	0.14	0.18	0.17				
7	<i>Boana roraima</i>	0.12	0.11	0.10	0.10	0.10	0.16			
8	<i>Boana</i> sp.	0.13	0.10	0.10	0.12	0.12	0.18	0.10		
9	<i>Boana tepuiana</i>	0.07	0.06	0.05	0.11	0.12	0.17	0.11	0.11	

Table 3. Uncorrected pairwise distances between samples of *Boana hobbsi* (Cochran & Goin, 1970) and congeneric species of the *Boana punctata* group *sensu lato* (highlighted in bold).

	Taxon	1	2	3	4	5	6	7	8
1	<i>Boana hobbsi</i>								
2	<i>Boana atlantica</i>	0.16							
3	<i>Boana</i> cf. <i>atlantica</i>	0.16	0.04						
4	<i>Boana cinerascens</i>	0.16	0.10	0.10					
5	<i>Boana gracilis</i>	0.15	0.08	0.09	0.11				
6	<i>Boana picturata</i>	0.14	0.17	0.17	0.15	0.16			
7	<i>Boana punctata</i>	0.16	0.04	0.03	0.11	0.09	0.17		
8	<i>Boana sibleszi</i> .	0.12	0.13	0.13	0.13	0.14	0.13	0.14	

Table 4. Morphometric measurements (mm) of *Boana hobbsi* (Cochran & Goin, 1970), based on the newly collected specimens examined in our study (INPA-H and CZPB vouchers) and on information available in the literature. See text for measurement acronyms.

[illegible]

Figure 1. Bayesian phylogenetic tree of the genus *Boana*, based on a fragment of the 16S mitochondrial rRNA, highlighting the position of *Boana hobbsi* (in yellow) in comparison to major clades representing known species groups, and indicating the phylogenetic position of remaining species included in the *B. punctata* group (in blue). Support values are indicated close to the nodes. Branch scale is indicated as number of substitutions per site.

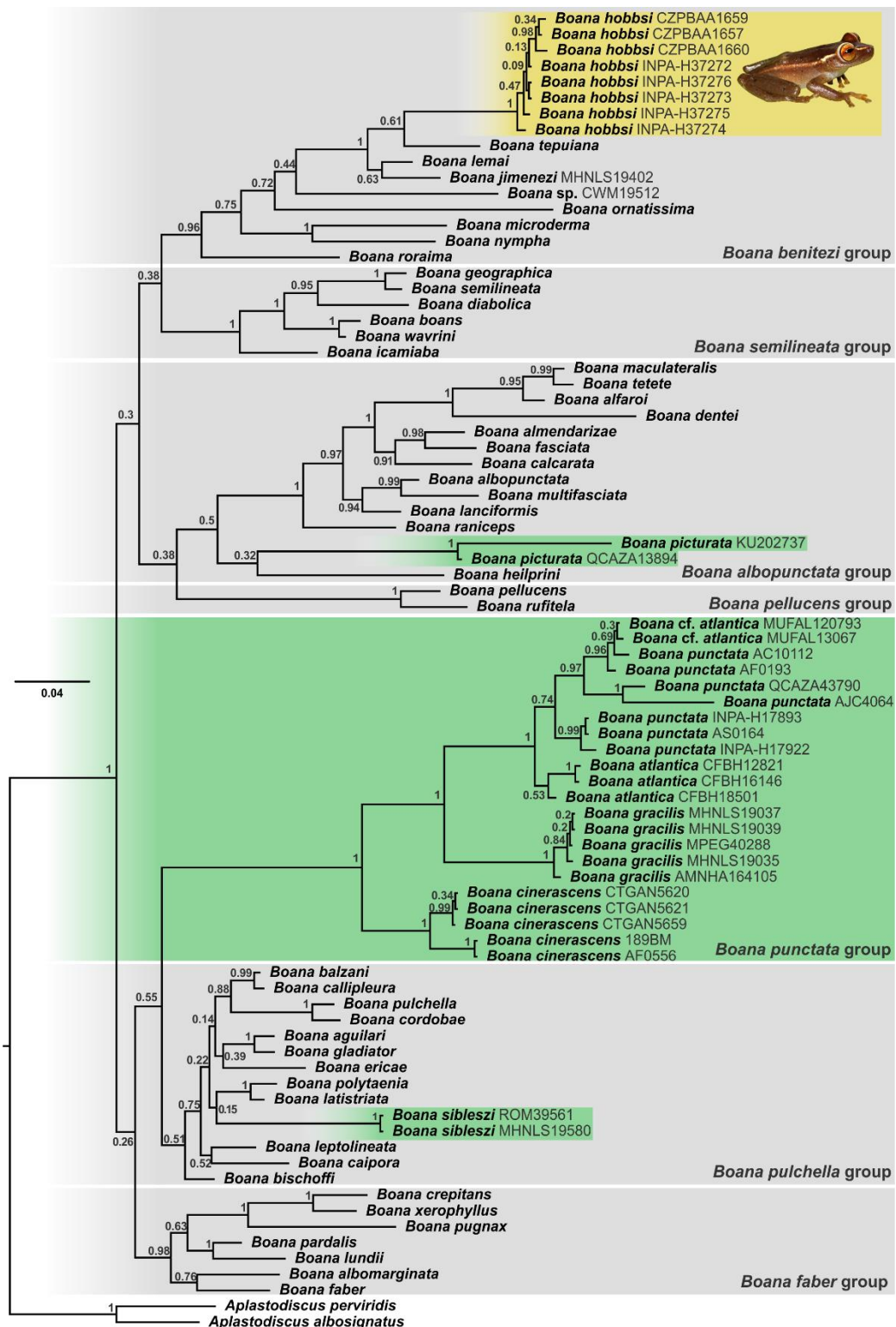


Figure 2. Dorsal and ventral views of newly collected preserved specimens of *Boana hobbsi* (Cochran & Goin, 1970). (A, B) adult male, with dark brown dorsum and little evident dorsolateral stripe (CZPB-AA 1658, SVL= 46.2 mm); (C, D) adult female, with dark brown dorsum and evident dorsolateral stripe (CZPB-AA 1660, SVL= 43.5 mm); (E, F) adult male, with yellowish dorsum and evident dorsolateral stripe (CZPB-AA 1657, SVL= 33.7 mm). All specimens were collected on the right bank of the Japurá River, near to the city of Japurá, state of Amazonas, Brazil.

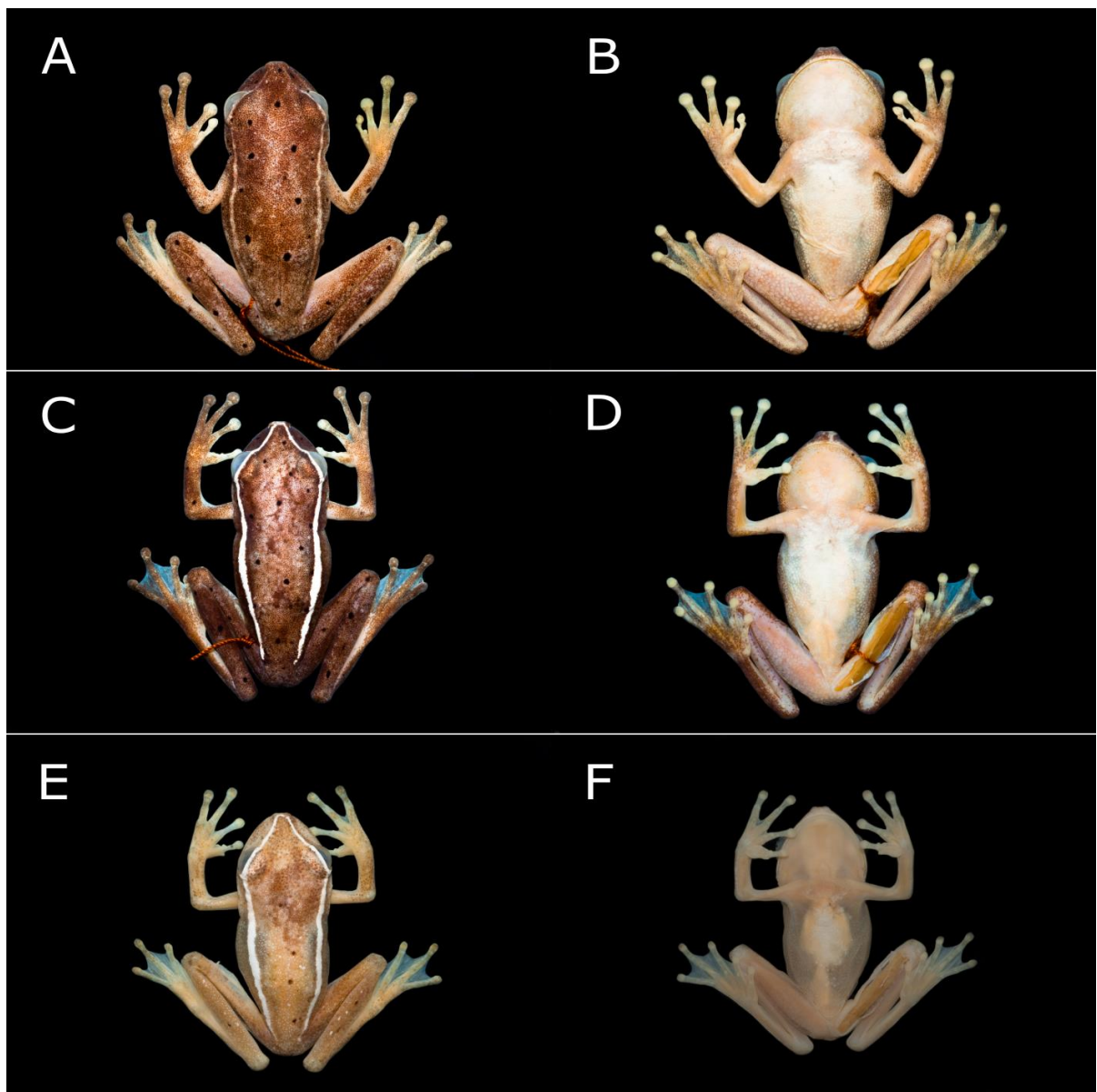


Figure 3. Morphological details of newly collected preserved specimens of *Boana hobbsi* (Cochran & Goin, 1970). (A–C) lateral, dorsal and ventral views of the head of an adult male (CZPB-AA 1658, SVL= 46.2 mm), highlighting the presence of an evident mental gland (red arrow). (D, E) ventral surfaces of the hands and feet of an adult female of *B. hobbsi* (CZPB-AA 1659; SVL = 45.8 mm).

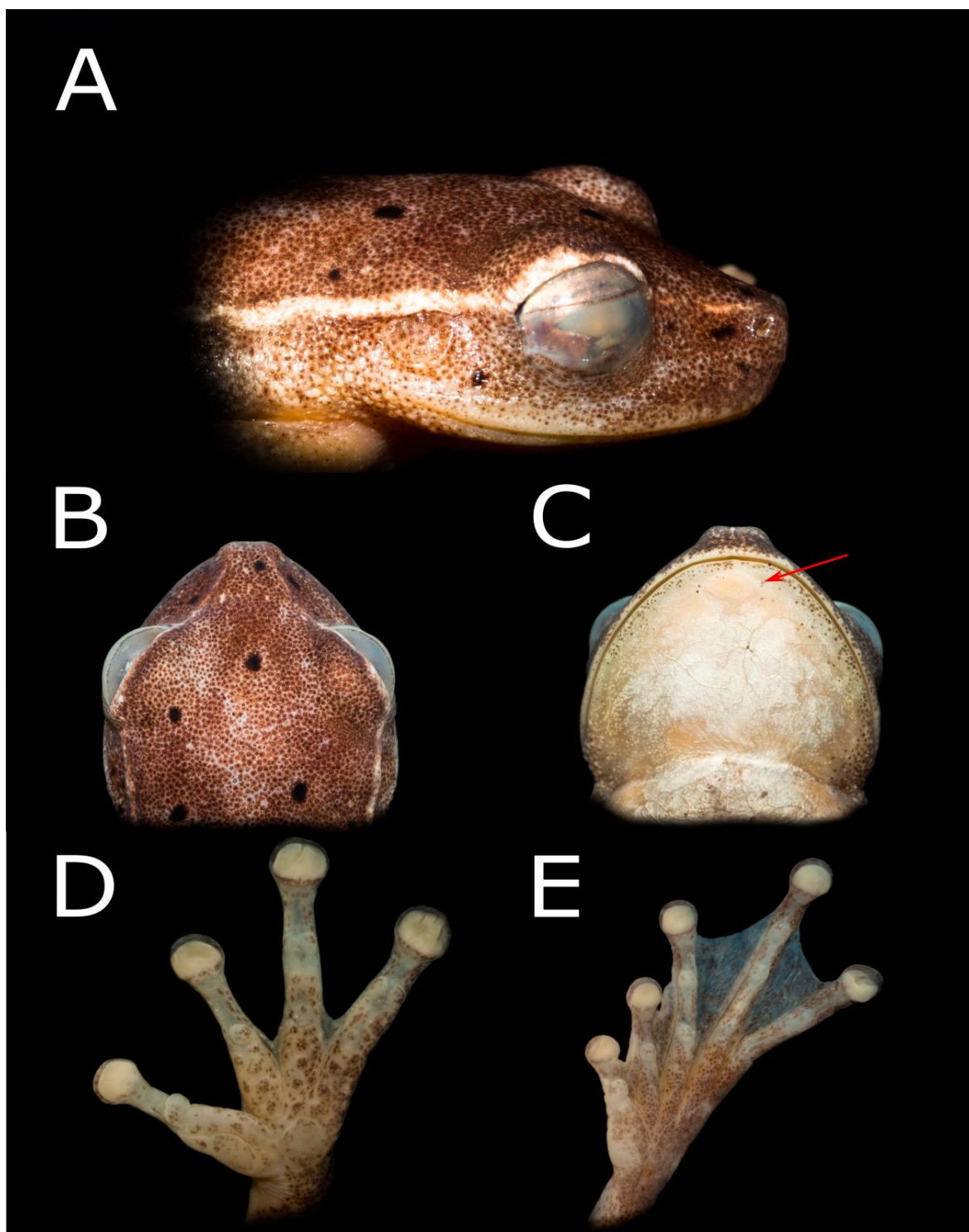


Figure 4. Dorsolateral views of newly recorded living specimens of *Boana hobbsi* (Cochran & Goin, 1970): (A) adult female from the right bank of the Japurá River, near to Vila Bittencourt, Amazonas, Brazil (Photo: APA); (B) adult male from the city of São Gabriel da Cachoeira, Amazonas, Brazil (Photo: LJCLM); (C, D) adult and juvenile males from Japurá River, showing variations in dorsal coloration, from tan, to dark brown, to a pale green-grayish, respectively (Photo: VTC).

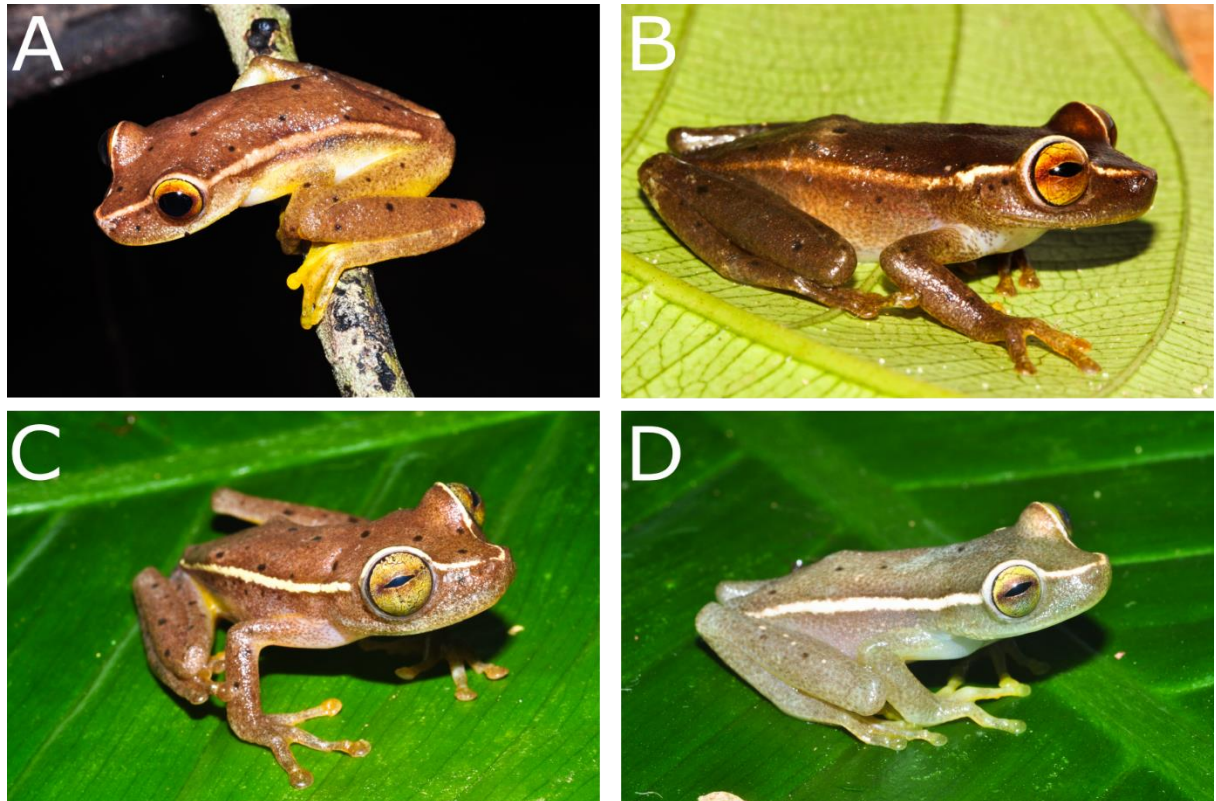
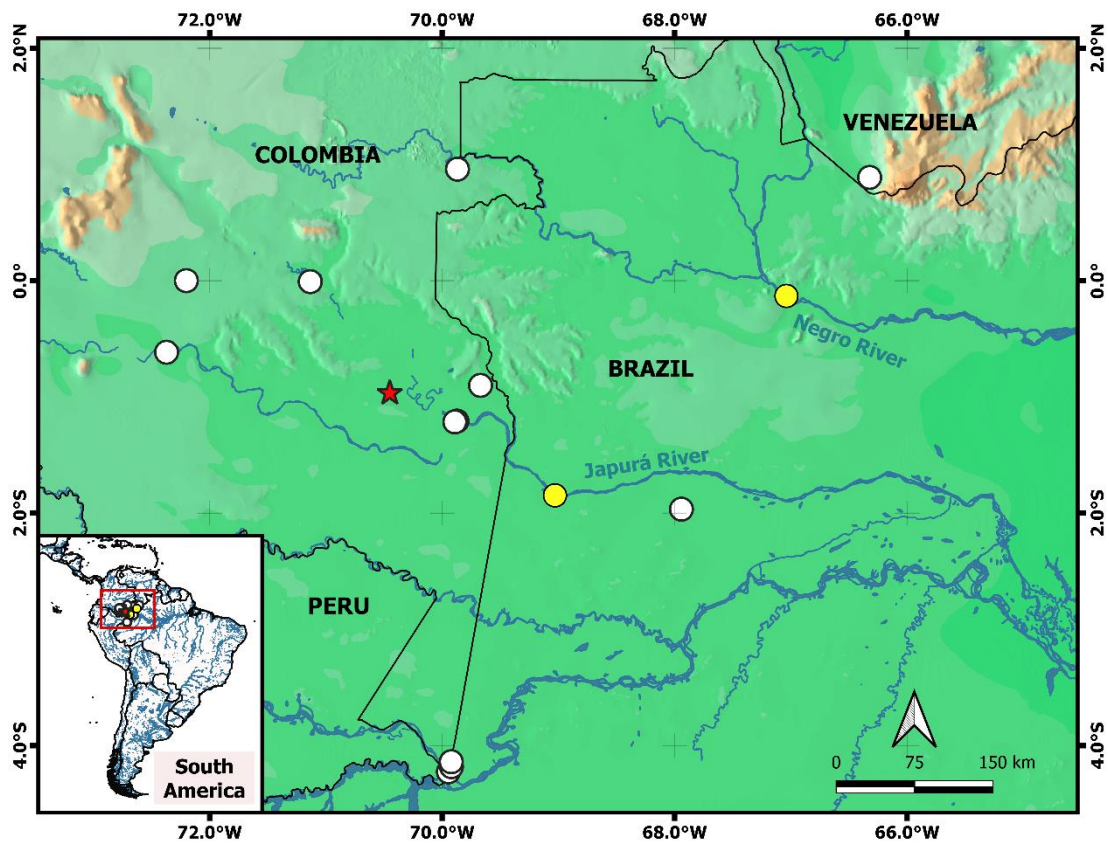


Figure 5. Known geographic distribution of *Boana hobbsi* (Cochran & Goin, 1970) in the northwestern Amazonia. White circles indicate the historical localities of occurrence in Colombia, Venezuela and Brazil (Cochran & Goin 1970; Pyburn 1978; McDiarmid *et al.* 1988; Ruiz-Carranza *et al.* 1996; Acosta-Galvis 2000; Lynch 2005, 2007; Suárez-Mayorga & Lynch 2017; Acosta-Galvis 2018; Barrio-Amorós *et al.* 2019; Simões *et al.* 2019). Yellow circles on the Brazilian territory indicate new distribution records reported at Japurá and Negro river basins. The red star indicates type locality at Caño Guacaya, Apaporis River, Colombia (Cochran & Goin 1970).



Appendix I. List of species included in our phylogenetic analysis, along with the associated voucher and GenBank accession numbers, locality of occurrence, and sequence references. Newly generated sequences are highlighted in bold.

Species	Voucher/Field Number	GenBank (Accession number)	Locality	References
<i>Aplastodiscus albosignatus</i>	CFBHT 14205	KU184018	Brazil: São Paulo, São Luis do Paraitinga	Berneck <i>et al.</i> (2016)
<i>Aplastodiscus perividis</i>	AAG 1259	KY213874	Brazil: São Paulo, Atibaia	Berneck <i>et al.</i> (2016)
<i>Boana aguilari</i>	MUSM 19804	HM444783	Peru: Junin, Pampa Hermosa	Lehr <i>et al.</i> (2010)
<i>Boana albopunctata</i>	ZUEC 12053	AY549317	Brazil: São Paulo, Campinas	Faivovich <i>et al.</i> (2004)
<i>Boana albomarginata</i>	USNM 284519	AY549316	Brazil: Pernambuco, near Caraurucu, on the way to Serra dos Cavalos	Faivovich <i>et al.</i> (2004)
<i>Boana alfaroi</i>	QCAZ A50785	KF955305	Ecuador: Provincia de Sucumbios, Panacocha	Camminer & Ron (2014)
<i>Boana almendarizae</i>	QCAZ A31452	JN970618	Ecuador: Tungurahua, Rio Pastaza	Funk <i>et al.</i> (2012)
<i>Boana atlantica</i>	CFBH 12821	MK348482	Brazil: Bahia, Camumu	Lima <i>et al.</i> (2019)
<i>Boana atlantica</i>	CFBH 16146	MK348487	Brazil: Bahia, Uruçuca	Lima <i>et al.</i> (2019)
<i>Boana atlantica</i>	CFBH 18501	MK348492	Brazil: Bahia, Uruçuca	Lima <i>et al.</i> (2019)
<i>Boana cf. atlantica</i>	MUFAL 12079_3	MK348503	Brazil: Alagoas, Maceió	Lima <i>et al.</i> (2019)
<i>Boana cf. atlantica</i>	MUFAL 13067	MK348506	Brazil: Pernambuco, Paulista	Lima <i>et al.</i> (2019)
<i>Boana balzani</i>	DLR 41193	AY549323	Bolivia: Departamento La Paz, Provincia Noryungas, Serrania Bellavista	Faivovich <i>et al.</i> (2004)
<i>Boana bischoffi</i>	CFBH 3356	AY549324	Brazil: Santa Catarina, Rancho Queimado	Faivovich <i>et al.</i> (2004)
<i>Boana boans</i>	RWM 17746	AY843610	Venezuela: Amazonas, Caño Agua Blanca, 3.5 km SE to Neblina Base Camp on Rio Mawarinuma	Faivovich <i>et al.</i> (2005)

<i>Boana caipora</i>	CFBH 5738	EU077268	Brazil: São Paulo, Serra do Mar, Serra de Paranapiacaba, Município de Pilar do Sul	Antunes <i>et al.</i> (2008)
<i>Boana calcarata</i>	NMP6V 71250	AY843613	Peru: Anguilla 50 km of Iquitos	Faivovich <i>et al.</i> (2005)
<i>Boana callipleura</i>	MNCN 4112	HM480426	Bolivia: Cochabamba, between Los Guácharos and El Palmar	Kohler <i>et al.</i> (2010)
<i>Boana cinerascens</i>	AF 0556	KR811175	French Guiana: Savane Virginie	Fouquet <i>et al.</i> (2015)
<i>Boana cinerascens</i>	189bm	EU201113	French Guiana: Trinité	Fouquet <i>et al.</i> (2007)
<i>Boana cinerascens</i>	CTGA_N5620	MG806013	Brazil: Amazonas, FEX-CAMPUS, Manaus	Motta <i>et al.</i> (2018)
<i>Boana cinerascens</i>	CTGA_N5621	MG806016	Brazil: Amazonas, FEX-CAMPUS, Manaus	Motta <i>et al.</i> (2018)
<i>Boana cinerascens</i>	CTGA_N5659	MG806018	Brazil: Amazonas, FEX-CAMPUS, Manaus	Motta <i>et al.</i> (2018)
<i>Boana cordobae</i>	MLP 2139	AY549329	Argentina: Cordoba, Tanti	Faivovich <i>et al.</i> (2004)
<i>Boana crepitans</i>	CFBHT 12841	KU495262	Brazil: Bahia, Camumu	Lyra <i>et al.</i> (2016)
<i>Boana dentei</i>	13mc	AF467270	French Guiana: Guiana Mountain of Kaw (3)	Salducci <i>et al.</i> (2002)
<i>Boana diabólica</i>	MTB 136	KU168891	French Guiana: Gaa Kaba	Fouquet <i>et al.</i> (2016)
<i>Boana ericae</i>	CFBH 3599	AY549332	Brazil: Goiás, Alto Paraíso de Goiás	Faivovich <i>et al.</i> (2004)
<i>Boana faber</i>	CFBHT 279995	KU495264	Brazil: São Paulo, Nazaré Paulista	Lyra <i>et al.</i> (2016)
<i>Boana fasciata</i>	QCAZ 17030	JN970535	Ecuador: Morona Santiago, Tiink, Rio Zamora	Funk <i>et al.</i> (2012)
<i>Boana geographica</i>	AMNH A141054	AY843628	Guyana: Warniabo Creek, 4 mi SW Dubulay Ranch house	Faivovich <i>et al.</i> (2005)
<i>Boana gladiator</i>	MNCN 5523	HM480413	Peru: Cusco, Valle de Marcapata	Kohler <i>et al.</i> (2010)
<i>Boana gracilis</i>	AMNH	AY549336	Guyana: Iwokrama, Muri	Faivovich <i>et al.</i>

	A164105		Scrub camp, 80m	(2004)
<i>Boana gracilis</i>	MHNLS 19035	MN781658	Venezuela: Amazonas: San José de Chipiro, boca del río Ventuari, municipio Atapabo, alto Orinoco	Sturaro <i>et al.</i> (2020)
<i>Boana gracilis</i>	MHNLS 19037	MN781659	Venezuela: Amazonas: San José de Chipiro, boca del río Ventuari, municipio Atapabo, alto Orinoco	Sturaro <i>et al.</i> (2020)
<i>Boana gracilis</i>	MHNLS 19039	MN781660	Venezuela: Amazonas: San José de Chipiro, boca del río Ventuari, municipio Atapabo, alto Orinoco	Sturaro <i>et al.</i> (2020)
<i>Boana gracilis</i>	MPEG 40288	MN781661	Brazil: Amazonas: Novo Airão: Parque Nacional do Jaú	Sturaro <i>et al.</i> (2020)
<i>Boana heilprini</i>	AMNH A168405	AY843632	Pet trade	Faivovich <i>et al.</i> (2005)
<i>Boana hobbsi</i>	CZPB-AA1657	To be submitted	Brazil: Amazonas, rio Japurá, Japurá, near Villa Bittencourt	This study
<i>Boana hobbsi</i>	CZPB-AA1659	To be submitted	Brazil: Amazonas, rio Japurá, Japurá, near Villa Bittencourt	This study
<i>Boana hobbsi</i>	CZPB-AA1660	To be submitted	Brazil: Amazonas, rio Japurá, Japurá, near Villa Bittencourt	This study
<i>Boana hobbsi</i>	INPA-H37272	To be submitted	Brazil: Amazonas, São Gabriel da Cachoeira	This study
<i>Boana hobbsi</i>	INPA-H37273	To be submitted	Brazil: Amazonas, São Gabriel da Cachoeira	This study
<i>Boana hobbsi</i>	INPA-H37234	To be submitted	Brazil: Amazonas, São Gabriel da Cachoeira	This study
<i>Boana hobbsi</i>	INPA-H37235	To be submitted	Brazil: Amazonas, São Gabriel da Cachoeira	This study
<i>Boana hobbsi</i>	INPA-H37236	To be submitted	Brazil: Amazonas, São Gabriel da Cachoeira	This study
<i>Boana icamiaba</i>	MPEG 27245	MG840873	Brazil: Para, Juruti, Capiiranga	Peloso <i>et al.</i> (2018)
<i>Boana jimenezi</i>	MHNLS 19402	MN688742	Venezuela: Bolívar: Gran Sabana, Campamento Guayaraca, Sabana de	Sturaro <i>et al.</i> (2020)

Guayaraca, P.N. Canaima				
<i>Boana lanciformis</i>	AJC 3973	KP149465	Colombia: Casañare, Sabanalarga, Sabanalarga	Guarnizo <i>et al.</i> (2015)
<i>Boana latistriata</i>	MZUSP 111556	AY549360	Brazil: Minas Gerais: Município Itamontes	Faivovich <i>et al.</i> (2004)
<i>Boana lemai</i>	ROM 39570	AY843637	Guyana: Mount Ayanganna	Faivovich <i>et al.</i> (2005)
<i>Boana leptolineata</i>	CFBH 3848	AY549341	Brazil: Santa Catarina: Município de São Domingos	Faivovich <i>et al.</i> (2004)
<i>Boana lundii</i>	CFBHT 01902	KU495270	Brazil: Minas Gerais: São Roque de Minas	Lyra <i>et al.</i> (2016)
<i>Boana maculateralis</i>	WED 60015	JN970627	Peru: Loreto: San Jacinto	Funk <i>et al.</i> (2012)
<i>Boana microderma</i>	NMP6V 71258/1	AY843644	Peru: Anguilla, 50 km of Iquitos	Faivovich <i>et al.</i> (2005)
<i>Boana multifasciata</i>	ROM 22756	JN970642	Guyana: Baramitã	Funk <i>et al.</i> (2012)
<i>Boana nympha</i>	NMP6V 71202/2	AY843670	Peru: 50 km W of Iquitos	Faivovich <i>et al.</i> (2005)
<i>Boana ornatissima</i>	51mc	EF376056	French Guiana: Chutes Voltaires	Salducci <i>et al.</i> (2002)
<i>Boana pardalis</i>	CFBHT 04366	KU495275	Brazil: Rio de Janeiro: Petrópolis	Lyra <i>et al.</i> (2016)
<i>Boana pellucens</i>	248804	JN970652	Ecuador: El Oro Avanzada-Playón road, km 8	Funk <i>et al.</i> (2012)
<i>Boana picturata</i>	KU 202737	AY326055	Pichincha, Tinalandia, Santo Domingo de Colorados, Ecuador	Darst & Cannatella (2004)
<i>Boana picturata</i>	QCAZ A13894	MN921776	Pichincha, Amanecer Campesino, Rio Santiago, Ecuador	Caminer & Ron (2020)
<i>Boana polytaenia</i>	CFBHT 06606	KU495277	Brazil: São Paulo, São Luis do Paraitinga, Santa Virginia	Lyra <i>et al.</i> (2016)
<i>Boana pugnax</i>	AJC 3529	KP149296	Colombia: Santander: San Vicente de Chucuri, Vereda las Margaritas, Reserva el arboretum,	Guarnizo <i>et al.</i> (2015)

Quebrada los Cauchos				
<i>Boana pulchella</i>	MNCN 37790	AY549350	Argentina: Buenos Aires, Escobar, El Cazador	Faivovich <i>et al.</i> (2004)
<i>Boana punctata</i>	AC_101_12	MN172521	Pilcopata, Distrito Kosnipata, Provincia Pauacartambo, Cusco, Peru	von May <i>et al.</i> (2019)
<i>Boana punctata</i>	AF193	EU201112	French Guiana: Mana	Fouquet <i>et al.</i> (2007)
<i>Boana punctata</i>	AJC 4064	KP149397	Colombia: Casañare, Sabanalarga, Sabanalarga	Guarnizo <i>et al.</i> (2015)
<i>Boana punctata</i>	AS0164	JF790122	Bolivia: Santa Cruz: Ñuflo de Chavez, San Sebastian	Jansen <i>et al.</i> (2011)
<i>Boana punctata</i>	APL17893	MK348507	Brazil: Rondônia	Lima <i>et al.</i> (2019)
<i>Boana punctata</i>	APL17922	MK348508	Brazil: Rondônia	Lima <i>et al.</i> (2019)
<i>Boana punctata</i>	QCAZ A43790	MN921797	Ecuador: Orellana, Rio Napo, La Primavera	Caminer & Ron (2020)
<i>Boana raniceps</i>	N/A	AF467269	Guyane: Yi-Yi's Creek	Salducci <i>et al.</i> (2002)
<i>Boana roraima</i>	ROM 39624	AY843660	Guyana: Mount Ayanganna	Faivovich <i>et al.</i> (2005)
<i>Boana rufitela</i>	KRL 798	AY843662	Panama: Cocle, El Cope, Parque Nacional 'Omar Torrijos'	Faivovich <i>et al.</i> (2005)
<i>Boana semilineata</i>	CFBH 5424	AY843779	Brazil: Rio de Janeiro, Duque de Caxias	Faivovich <i>et al.</i> (2005)
<i>Boana sibleszi</i>	MHNLS 19580	MN688743	Venezuela: Bolívar: Gran Sabana, Sierra Lema, Troncal 10.6 km al S de la alcabala	Sturaro <i>et al.</i> (2020)
<i>Boana sibleszi</i>	ROM 39561	AY843667	Guyana: Mount Ayanganna	Faivovich <i>et al.</i> (2005)
<i>Boana</i> sp.	CWM 19512	AY843671	Venezuela: Bolivar, Cerro Guanay	Faivovich <i>et al.</i> (2005)
<i>Boana tepuiana</i>	USNM 302435	AY843606	Venezuela: Brazil, Roraima, Villa Pacaraima, border marker BV (Brazil)	Faivovich <i>et al.</i> (2005)

<i>Boana tetete</i>	QCAZ A40080	JN970539	Ecuador: Napo, Comunidad Santa Rosa	Funk <i>et al.</i> (2012)
<i>Boana wavrini</i>	MTR_ACLX12 0P8	KU495289	Brazil: Mato Grosso, Guaranta do Norte	Lyra <i>et al.</i> (2016)
<i>Boana xerophylla</i>	AJC 4116	KP149478	Colombia: Casañare, Sabanalarga, Sabanalarga	Guarnizo <i>et al.</i> (2015)

CAPÍTULO 2



*Hyla punctata
rubro-lineata*

Unriddling the fluorescent treefrogs' species complex: Reassessing the taxonomic status of *Hyla punctata rubro-lineata* Lutz, 1951 through integrative taxonomy.

Alexandre P. Almeida; Camila Mattedi; Erico Polo; Juan C. Chaparro; Antoine Fouquet; Robson W. Ávila; Igor J. Roberto; Rommel R. Rojas; Teddy Angarita; Vinicius T. de Carvalho; Diego J. Santana; Miguel T. Rodrigues; Fernanda P. Werneck; Tomas Hrbek; Izeni P. Farias; Ulisses Caramaschi, Marcelo Gordo. Manuscrito submetido no periódico Systematics and Biodiversity em 18 de maio de 2022.

Title:

Unriddling the fluorescent treefrogs' species complex: Reassessing the taxonomic status of *Hyla punctata rubro-lineata* B. Lutz, 1951 through integrative taxonomy.

^{1,2,*}Alexandre P. Almeida; ³Camila Mattedi; ²Erico Polo; ⁴Igor J. Roberto; ⁵Juan C. Chaparro; ⁶Antoine Fouquet; ⁷Robson W. Ávila; ⁸Luciana Frazão; ⁹Rommel R. Rojas; ¹⁰Teddy Angarita; ^{2,11}Vinicius T. de Carvalho; ¹²Diego J. Santana; ¹³Miguel T. Rodrigues; ¹⁴Fernanda P. Werneck; ²Tomas Hrbek; ²Izeni P. Farias; ³Ulisses Caramaschi, ^{1, 15}Marcelo Gordo

1. Programa de Pós-graduação em Zoologia, Instituto de Ciências Biológicas, Universidade Federal do Amazonas, Manaus, AM, Brazil.
2. Laboratório de Evolução e Genética Animal, Departamento de Genética, Universidade Federal do Amazonas, Manaus, AM, Brazil.
3. Universidade Federal do Rio de Janeiro, Museu Nacional, Departamento de Vertebrados, Rio de Janeiro, RJ, Brazil.
4. Laboratório de Biologia e Ecologia de Animais Silvestres, Universidade Federal do Cariri Brejo Santo, CE, Brazil.
5. Departament of Herpetology, Museo de Biodiversidad del Perú (MUBI), Cusco, Peru.
6. Laboratoire Evolution et Diversité Biologique, UMR 5174, CNRS, IRD, Université Paul Sabatier, Toulouse, France.
7. Departamento de Biologia, Centro de Ciências, Campus do Pici, Universidade Federal do Ceará, Fortaleza, CE, Brazil.
8. Centre for Functional Ecology - Science for People & the Planet, UNESCO CHAIR in Biodiversity Safeguard for Sustainable Development, Department of Life Sciences, University of Coimbra, Coimbra, Portugal.
9. Universidad Nacional de la Amazonía Peruana, Iquitos, Loreto, Peru.
10. Universidad Nacional de Colombia, sede La Paz, Cesar, Colombia.
11. Programa de Pós-graduação em Diversidade Biológica e Recursos Naturais, Universidade Regional do Cariri, Crato, CE, Brazil
12. Instituto de Biociências, Cidade Universitária, Universidade Federal do Mato Grosso do Sul, Campo Grande, MS, Brazil.
13. Universidade de São Paulo, Instituto de Biociências, Departamento de Zoologia, São Paulo, SP, Brazil.
14. Coordenação de Biodiversidade, Programa Coleções Científicas Biológicas, Instituto Nacional de Pesquisas da Amazonia (INPA), Manaus, AM, Brazil.
15. Instituto de Ciências Biológicas, Departamento de Biologia, Coordenação de Aperfeiçoamento Pessoal de Nível Superior – CAPES, Universidade Federal do Amazonas, Manaus, AM, Brazil -

*Corresponding author - alexandre.dealmeida@hotmail.com

Abstract

Uncovering cryptic biodiversity has been an increasing topic in taxonomy and evolutionary research, contributing effectively for conservation of new species that have been neglected due to a conservative morphology. Anurans are good examples of this trend, possessing high levels of cryptic diversity in several species complex, especially in the tropic regions. The *Boana punctata* species group is composed of green tree-frogs with very conservative external morphology, widespread distribution across different river basins across South America. *Boana punctata* has the most extensive distribution, with many taxonomy uncertainties, leading to the description of several taxa under his name, constituting a great taxonomic challenge over the years. Here we investigate the cryptic diversity of lineages within *Boana punctata* species complex, using an extensive dataset of 16S rDNA sequences through most of its geographic distribution. Through integrative taxonomic framework, we use morphometric measures, external morphology, and Bayesian generalized Yule coalescent model to discovery potential new candidate species, as well as to make new taxonomic decisions. Six operational taxonomic unities were discovered: *Boana punctata sensu stricto* with widespread distribution throughout the Amazon and northern Atlantic Forest, *Boana atlantica* with a restricted range to the Atlantic Forest of the state of Bahia, Northeast Brazil, two confirmed candidate species, one unconfirmed candidate species. We elevate *Boana rubrolineata* to full species status, with distribution in the Madeira-Bolivia and Paraguay-Paraná Rivers basins. We discuss the potential drivers of this cryptic diversity within this group and conservation threats.

Keywords: *Boana punctata* group, Cryptic diversity, Conservative morphology, Bioacoustics, Species delimitation, Cophomantini.

Introduction

Amphibians are one of the most biodiverse groups of vertebrate in the world, with about 43% of known species occurring in the Neotropical region (Meseguer, Antoine, Fouquet, Delsuc, & Condamine, 2020; Wake & Koo, 2018). Nevertheless, knowledge on amphibia species richness and its geographic boundaries are widely underestimated in the neotropics, and despite the increasing research effort, those biodiversity shortfall persists (Guerra, Jardim, Llusia, Márquez, & Bastos, 2020; Moura & Jetz, 2021). However, overlooked groups of amphibians became more well studied, and the improvement of genetic data acquisition and phylogenetic analysis has revealed hidden species complex in many well-known species (Gehara et al., 2014; Seger et al., 2021). Indeed, reveal these pattern of diversity became a first step to clear our understanding of the species limits, providing insights of the patterns of diversification which leads to improvement of taxonomy and perhaps conservations efforts for these species (Angulo & Icochea, 2010; Bickford et al., 2007; Fouquet et al., 2021; Rojas et al., 2018).

Well-resolved taxonomy is fundamental to understand diversity processes and patterns, however, likewise other taxonomic groups, among Neotropical treefrogs several taxonomic issues remain not properly addressed (e.g. Araujo-Vieira et al., 2020; Sturaro et al., 2020). The *Boana punctata* species group (sensu Almeida et al. 2021) is a remarkable example. While *Boana sibleszi* (Rivero, 1971) phylogenetic relationship remains uncertain, *Boana alemani* (Riveiro, 1961) has been suggested to be conspecific with *B. punctata* (Barrio-Amorós, Rojas-Runjaic, & Señaris, 2019). On the other hand, the widespread species *Boana punctata* (Schneider, 1799), and *Boana cinerascens* (Spix, 1824), *Boana gracilis* (Melin, 1941) and *Boana atlantica* (Caramaschi & Velosa, 1996) represent species complex

with many synonyms to be evaluated (Caminer & Ron, 2020; Guarnizo et al., 2015; Jansen, Bloch, Schulze, & Pfenninger, 2011; Lima, Dubeux, do Nascimento, Bruschi, & Mott, 2019; Motta, Menin, Almeida, Hrbek, & Pires Farias, 2018; Sturaro et al., 2020).

Broadly distributed in open wetlands areas through South America (Frost, 2022), *Boana punctata* species complex comprises two nominal species, *Boana punctata* and *Boana atlantica* (Caramaschi & Velosa, 1996). Both are notable green treefrog known for their remarkable colour patterns, for fluorescent reflectance under UV light (Taboada et al., 2017a; Taboada et al., 2017b), and for sharing several similarities in morphology and natural history (Brunetti, Taboada, & Faivovich, 2014, 2015; Camurugi & Juncá, 2013; Duellman, 1978; Napoli & Cruz, 2005). Historically, morphology of preserved specimens of *B. punctata* complex is quite problematic, because they lose most of their colour in life pattern becoming whitish and difficult to distinguish from other related species of the *B. punctata* group (Hoogmoed, 1979). Because of this, conservative external morphology and wide geographic range, there are many taxonomic problems regarding *B. punctata* and related species, rendering several synonyms along the years.

Schneider (1799) described *B. punctata* under the name *Calamita punctata*, based on specimens from Suriname, illustrated by Seba (1734). Subsequently, Spix (1824) named *Hyla papillaris* and *Hyla variolosa* from the Amazon basin, and Günther (1869) named *Hyla rhodoporus* from Surinam (Upper Amazon); all three species were latter synonymized with *B. punctata* by Peters (1872) and confirmed by Günther (1872). Ruthven (1922) described *Hylella pearsei* (= *Hyla pearsei* Dunn, 1944) from the region of the Sierra Nevada de Santa Marta, an isolated massif located in the continental Caribbean region of Colombia which was synonymized by Cochran & Goin (1970) whom argued that the holotype of *H. pearsei* is an “immature of *Hyla punctata*”. In this same publication, Cochran & Goin (1970) described *Hyla rubeola* from Serranía de La Macarena, Meta, an isolated massif located between eastern

slope of the Cordillera Oriental and the northern end of the Amazon basin of Colombia. However, Duellman (1974) also considered it as junior synonym of *H. punctata*. In an extensive revision of the *B. punctata* group, calling them as “green Hylas from Amazonian Hylaea and Gran Chaco”, B. Lutz (1951) designed the subspecies *Hyla punctata rubro-lineata* to the populations from Buena Vista, departamento Santa Cruz in Bolívia, and adjacent region in Argentina, Paraguay and central Brazil, which also was latter synonymized by Duellman (1974) due overlapping of diagnostic characters. Later *Hyla atlantica* Caramaschi & Velosa, 1996 was described for the populations from Atlantic Forest in the state of Bahia, Brazil.

The taxonomic status of most of these synonyms remains unchanged. However, findings of hidden diversity on genetics, tadpoles morphology and acoustic data for *B. punctata* and *B. atlantica* (Brunetti et al., 2015; Brusquetti & Lavilla, 2006; Cei, 1980; Guarnizo et al., 2015; Jansen et al., 2011; Kolenc et al., 2008; Lima et al., 2019; Napoli & Cruz, 2005 Schulze et al. 2015), highlights the necessity of a carefully revision of its taxonomy and made dubious the decision to keep *B. punctata rubrolineata* as a synonym of *B. punctata*.

Thus, including those multiple lines of evidence (e.g. DNA sequences, morphology, bioacoustics geographic distribution) already reported, on a congruent framework of integrative taxonomy (Padial, Miralles, De La Riva, & Vences, 2010) in addition with recent species delimitation methods (Hillis, 2019) could help to solve historic issues, rendering a more stable taxonomy in this frog species (Brusquetti, Jansen, Barrio-Amorós, Segalla, & Haddad, 2014; Mângia et al., 2020; Orrico et al., 2017).

In this study we investigated the phylogenetic diversity and phylogeography of *B. punctata* species complex and reassessed through an integrative taxonomic framework (mitochondrial DNA, morphology, and bioacoustics) the taxonomic status of the subspecies

B. punctata rubrolineata. Based in our finding we propose to elevate it to a full species status and discuss its systematic, geographic distribution and conservation.

Materials and Methods

Molecular Sampling

To properly cover most of *B. punctata* species complex's geographic distribution (Figure 1), we gathered a total of 152 sequences of *B. punctata* and 27 samples of *B. atlantica* from newly collected specimens in the field, samples deposited in research institutes, and sequences deposited in GenBank (Table S1).

All collected specimens were euthanized with a topical solution of 2% xylocaine (CMFV, 2013), and then fixed with 10% formalin and stored in 70% ethanol. Muscular or liver tissues samples were taken and stored in 96% ethanol. We deposited all collected specimens at the amphibians and reptiles collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil and all tissue samples in the collection of Laboratório de Evolução e Genética Animal – CTGA-LEGAL, Universidade Federal do Amazonas (UFAM), Manaus, Brazil. Collect permits were issued in Brazil by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/SISBio#), and in Peru by Servicio Nacional Forestal y de Fauna Silvestre (#241-2017-SEFOR/DGGSPFFS).

Laboratory procedures

We extracted genomic DNA from tissue samples following the CTAB 2% (Doyle & Doyle, 1987) and phenol/chloroform (Sambrook et al. 1989) protocols. Then, we amplified a locus of the mitochondrial 16S rRNA gene via Polymerase Chain Reactions (PCR). To amplify the 16S molecular marker we using the primers 16Sar and 16Sbr (Palumbi, 1996), and L2508-16S (Kawaguchi, Miya, & Nishida, 2001) and S-LA-16S-H (Thom et al., 2018;

Miya & Nishida, 2000). PCR conditions were performed in 15µl reaction volumes containing 5.8 µl of dd H₂O, 1.5 µl of 25mM MgCl₂, 1.5 µl of 10X amplification buffer (75mM of Tris HCl, 50mM of KCl and 20mM of (NH₄)₂SO₄), 1.5 µl of 2 µM solution of both primer, 1.5 of 10mM dNTPs (2.5mM of each dNTP), 0.7 µl of Taq DNA polymerase 1 U/µl (Fermentas, Lithuania) and 1 µl of template DNA (about 50 ng/µl). These reactions cycling program consist in a preheating cycle at 90°C for 60 s, followed by denaturation at 92°C for 60 s (repeated 35x), primer annealing at 50°C for 50 s, extension at 72°C for 90 s (repeated 35x), and final extension at 72°C for 5 min. The reaction products were purified with exonuclease and alkaline phosphatase (Fermentas, Lithuania) and sequenced using standard protocols of ABI BigDye Terminator Cycle Sequencing Kit (ThermoFisher, USA). Subsequently sequencing reaction were resolved in ABI 3500 automatic sequencer (Applied Biosystems). We conducted all laboratories procedures in Laboratório de Evolução e Genética Animal (LEGAL) – UFAM, Manaus, Brazil.

Phylogenetic analysis, species delimitation methods

In order to perform our phylogenetic analysis we included sequences of mitochondrial 16S rRNA gene from specimens of *B. punctata* and *B. atlantica* from previous studies (Caminer & Ron, 2020; Faivovich et al., 2005; Guarnizo et al., 2015; Jansen et al., 2011; Lima et al., 2019; Vacher et al., 2020; von May et al., 2019) deposited in GenBank (Benson et al. 2013) (Table S1). All sequences were checked, assembled, and edited in the software Geneious v7.0.6 (Biomatters) (Kearse et al., 2012). As outgroup we selected homologous sequences from the congener's species: *Boana gracilis* (Melin, 1941) and *Boana cinerascens* (Spix, 1824) of the *B. punctata* group; *Boana lanciformis* (Cope, 1871) and *Boana albopunctata* (Spix, 1824) of the *B. albopunctata* group; *Boana hobbsi* (Cochran & Goin, 1970), *Boana jimenezi* (Señaris & Ayarzagüena, 2006) and *Boana tepuiana* (Barrio-Amarós & Brewer-Carias, 2008) from *Boana benitezi* group; and latter *Boana geographica* (Spix, 1824),

Boana semilineata (Spix, 1824) and *Boana diabolica* (Fouquet, Martinez, Zeidler, Courtois, Gaucher, Blanc, Lima, Souza, Rodrigues & Kok, 2016) from *B. semilineata* group. Accession number and all details of samples and sequences used in our molecular analysis can be found in supplemental material (Table S1).

Then we aligned our dataset using MAFFT version 7 online server under the E-INS-i strategy (Katoh et al., 2019). Before the phylogenetic analysis, we comprising our dataset reducing to unique haplotypes, using the function hapCollapse (<http://github.com/legalLab/protocols-scripts>). Our final dataset includes 100 sequences with 565 bp with 211 variables sites. The nucleotide substitution model, GTR+I+G, was inferred in PartitionFinder 2.1.1 (Lanfear et al., 2017) under the Akaike Information Criterion. Subsequently, we performed the phylogenetic reconstruction, and generated a ultrametric tree in BEAST 2.5.1 (Bouckaert et al., 2014) using uncorrelated relaxed log normal clock and coalescent constant population size evolutionary model (following Monaghan et al., 2009). We ran three independent runs of 50,000,000 MCMC generations with trees and parameters sampled every 5,000 generations, and after verifying convergence we combined the runs in LogCombiner 2.5.1 (Rambaut & Drummond, 2018). We checked for parameter convergence and effective sample size (ESS > 200) in Tracer 1.7 (Rambaut et al., 2018). A maximum clade credibility tree was obtained after burn-in of 5,000 trees in TreeAnnotator (Bouckaert et al., 2014).

To define our operational taxonomic units (OTU), we applied the Bayesian implementation of generalized mixed Yule-coalescent model – bGYMC (Reid & Carstens, 2012), that consists in a species discovery method, that takes in account potential phylogenetic errors in the model parameter estimates via Markov chain of Monte Carlo (MCMC). Here, we refrain to apply other species delineation methods based on genetic-distance (e.g. Automatic Barcode Gap Discovery - ABGD) due its conservativeness that may

underestimate the species diversity detection in datasets with short fragments of 16S (Chan, Hertwig, Neokleous, Flury, & Brown, 2022; Puillandre et al., 2012). Instead, we calculated genetic distances (uncorrected p-distance) in MEGA v.7 (Kumar et al., 2016) and used these data as another source of evidence, due to the largely use to infer diversification in frog species.

Species concept and integrative taxonomy workflow

Here we follows the general metapopulation lineage species concept, recognizing as species when there is evidence for the separation of metapopulation lineages (De Queiroz, 2007). In addition, we follow an integrative taxonomy workflow to infer the candidate species and applying a consensus protocol for taxonomic decisions (Padial et al., 2010).

Morphological data

We assessed standard morphometric measures and external morphology from 447 specimens, 419 of *B. punctata* and 28 specimens of *B. atlantica*. Only males were prioritised in our analysis. The specimens' localities cover most of the species complex geographic distribution (Figure 1). We took linear measures from 14 morphometric characters: Snout-vent length (SVL); Head length (HL); Head width (HW); Snout length (SL); Eye-nostril distance (END); Interorbital distance (IOD); Eye diameter (ED); Tympanum diameter (TD) Thigh length (ThL); Tibia length (TiL); Tarsus length (TaL); Foot length (FL); Forearm length (FaL) and Hand length (HaL); terminologies and acronym follows Watters, Cummings, Flanagan, & Siler (2016). Measures were taken by A.P. Almeida, I.J. Roberto, and C. Mattedi with digital callipers (to the nearest 0.01 mm). We also examined discrete features for external morphology according to standard definitions of Duellman, (1970) and Heyer, Rand, Cruz, Peixoto, & Nelson, (1990). Webbing formulae follow Savage & Heyer (1967) definitions as subsequently modified by Myers & Duellman (1982). Colour patterns of living specimens

were taken by field notes and observed in high quality photography taken by ours and external collaborators. All examined specimens and their respective collections are in Appendix I.

Bioacoustics data and vocal repertoire

Differences in advertisement call of frog species are often used as a trait that represent evidence of lineage divergence and taxonomic distinctness (Köhler et al., 2017). However, in a taxonomic context, it is mandatory to take care at the interpretation of such differences in species with high intraspecific variation of acoustic traits, and complex vocal repertoire (Köhler et al., 2017; Wells, 1977; Wells & Schwartz, 2006). For *Boana punctata* acoustic repertoire, seven types of vocalizations with particular biological contexts were known (Brunetti et al., 2015), and at least two types of advertisement call were described for *B. atlantica* (Brunetti et al., 2015; Camurugi & Juncá, 2013; Napoli & Cruz, 2005). To avoid misinterpretation of the acoustic parameters we included in our analysis only the vocalizations that fits within the two types of advertisement call as defined by Brunetti et al. (2015): the long advertisement call (LAC) and short advertisement call (SAC).

We accessed 103 advertisement call records of *B. punctata* complex, from populations representing most of both species' distribution, from newly recorded specimens and from online repositories. We recorded calling males in our fieldwork with a digital audio-recorder ZOOM – H1 *hand recorder*, equipped with external microphones or eventually connected with a Sennheiser unidirectional microphone (MKE 600). We also obtained records from the sound collection of the 'Fonoteca Neotropical Jacques Vielliard (FNJV), and from the Macaulay Library at the Cornell Lab of Ornithology, (<http://macaulaylibrary.org>). In addition, we accessed unpublished records from external collaborators. All information about the locality, authorship, and host collection of the records can be found in supplementary data. Recordings were digitalized at 44.1 kHz, with a resolution of 16 bits. Calls were analyzed in

Raven Pro v1.6.1 (Center for Conservation Bioacoustics 2019), and Spectrograms were produced with Fast Fourier Transform (FFT) length of 1,024 samples, overlap 89.8 %, window Hanning, 3-dB filter bandwidth of 496 Hz, and window size of 256 (Taucce et al. 2020). Spectrogram and oscillogram figures were produced with the Seewave package version 1.6.4 (Sueur et al. 2008) in the R platform version 3.3.3 (R Core Team 2019).

To make the intraspecific comparison we analysed the following acoustic traits for both types of advertisement call (LAC-SAC): Call rate; Call duration; Silent interval; Number of Notes; Note repetition rate; Note duration; Internote interval; Frequency minimum (Frequency 5%); Frequency maximum (Frequency 95%); Dominant frequency; Bandwidth 90% . All acoustic analysis were conducted in Raven Pro 1.5, 64-bit version (Bioacoustic Research Program, 2014). Definitions and as terminology of call description follows the note centred approach, as well as framework of analysis, as well suggested by Köhler et al., (2017).

Phenotype comparisons and statistical analysis

To assess whether the phenotypic variation present significant differences between the groups delimited in our molecular analysis, we performed a principal component analysis (PCA) associated with an analysis of variance (ANOVA). Afterwards we performed a Tukey's test to identify which groups differed from each other. In addition, we performed a Discriminant Function Analysis (DFA) to identify the variables that most contribute to the clustering of the specimens and test their predictive power in classifying it into genetic groups. To analyse morphological data, we removed the size effect by performing a linear regression each variable on SVL. Then the residuals obtained by those regression were used in the PCA and DFA. All statistical analysis were performed in R version 4.1.1 (R Core Team 2020) environment in R Studio interface (R Studio Team 2020).

Geographic distribution

We assessed the total geographic distribution of *B. punctata* and *B. atlantica* through analysis of georeferenced occurrence database on: Global Biodiversity Information Facility (GBIF) (www.gbif.org), Vert-Net (www.vertnet.org), SpeciesLink (www.specieslink.org.br), Anfíbios del Ecuador (Ron & Read, 2019), Lista de los Anfíbios de Colombia (Acosta Galvis, 2021 in: <http://www.batrachia.com>). We also included georeferenced data from literature, and when it was not available, we inferred it based on locality description. In addition, we included data from unpublished records of technical reports with photographic evidence.

Results

Phylogenetics, phylogeography and species delimitation

The tree topology obtained from the Bayesian analysis recovered clades representing the *Boana punctata* group (pp = 0.98), *B. albopunctata* group (pp = 1.0), *B. benitezi* group (pp = 0.99) and *B. semilineata* group (pp = 1.0). The analysis shows *B. punctata* species group as a well-supported clade (pp = 0.98) including the *B. punctata*, *B. atlantica*, *B. gracillis* and *B. cinerascens*. *Boana cinerascens* was recovered as the sister taxon of the remaining species of a clade (Figure 2).

We recovered 16 OTUs as result of bGYMC analysis, of which ten correspond to the nominal species used as outgroup in the phylogenetic analysis: *B. gracillis*, *B. cinerascens*, *B. lanciformis*, *B. albopunctata*, *B. hobbsi*, *B. jimenezi*, *B. tepuiana*, *B. semilineata*, *B. geographica* and *B. diabolica*. Within the *B. punctata* – *B. atlantica* species six OTUs were recovered comprising all major lineages of the species complex (Figure 2).

The lineages present a strong phylogeographic structure with no overlapping in geographic distribution (Figure 2). The clade I includes populations from the lowlands of the

Amazonia: in Loreto and Ucayali departments of Peru, throughout the states of Acre, Amazonas, Pará and Amapá in Brazil, wetlands from Surinam and French Guyana in the Guiana Shield, and the populations of *B. atlantica* from northern São Francisco River in the Atlantic Forest domain. The Clade II contains the *B. punctata* populations from Andean slopes of Colombia and Ecuador. Clade III includes *B. punctata* specimens from a wide wetland area which extends from southwestern Amazonia, in the Madeira and Madre de Dios River basins, through the lowlands of Bolivia in the Beni and Santa Cruz departments, Chacoan region of Argentina, Pantanal and part of Brazilian Cerrado. The Clade IV includes populations from the savannas of the Brazilian Cerrado in the Tocantins and Parnaíba River basins, in the states of Piauí, Maranhão and Tocantins. Clade V contains all specimens of *B. atlantica* from the state of Bahia, in the coastal Atlantic Forest domain, south to the São Francisco River. And the last, Clade VI include specimens from the enclaves of uplands in the Cerrado domain in the state of Mato Grosso do Sul, Brazil.

Genetic distance (uncorrected p-distance) ranges from ~2% to ~5% between the candidate species (Table 1). The populations from Colombia and Ecuador represented in clade II had the highest genetic distance values, with about 5% (range of variation 4.7-5.4%) of distance to all other clades. The divergence between Clade III and Clade VI was 2.2% and represent the minor value of p-distance recovered between the clades. Within each clade, genetic distance was above 2%, except in the Clade II, which presents average 4.2% of divergence.

Morphology

Morphometric measures from 447 specimens are presented in Table 2. The morphometric analysis showed a subtle, but significant variation of SVL among the candidate species (ANOVA: $F_{5,441}=16.57$, $P<0.001$). Through the Tukey's test we reveal that specimens

from Clade IV are smaller than those of Clade I, II, III and V (Table S2). In contrast, Clade V showed a larger body size than all other clades, except for the Clade II (Figure 3A; Table 2). Furthermore, we found differences between the body shape of specimens of Clade IV to the remaining groups (ANOVA: $F_{5,441}=6.646$, $P < 0.001$; Tukey's test: Table S3). The first two axis of PCA of sized corrected morphometric variables explained 62.84% of total variance (PC1 = 55.35%, PC2 = 7.49%; Table S4), however, the clusters recovered overlap in the morphometric space, not allowing us to clearly discriminate between them (Figure 2B). Through the DFA the morphometric data could correctly allocated 51.9% of total specimens to their prior phylogenetic groups (Table 3). Head length, tympanum diameter, tibia length and foot length were the variables that most contributed to this classification, explaining 67.21% by the first two axes of linear discriminant model (LD1 = 45.32% LD2 = 21.94) (Table S5). A moderate proportion of correct assignment and the low variance in loading values observed in linear discriminant function (LD) show the variables are poorly informative to discriminate the groups got in the species delimitation analysis, which allowed us to observe a subtle separation of Clades IV and V from the remaining groups (Figure 4).

Discrete characters and external morphology also showed a low delimitation capacity through the species complex (Figure 4). All specimens exhibited: smooth to finely shagreened skin on dorsum, and granular skin in the ventral surface of chest, belly, and thighs; dorsal surface of head, dorsum, arms, shanks, and thighs covered by chromatophores; a dorsolateral fold extending from upper eyelid to the groin; head slightly broader than longer; a subgular and single vocal sac; mental gland present; small, and distinct tympanum with supratympanic fold, which reach to the dorsolateral fold; subarticular tubercles single and rounded; supernumerary tubercles absent; palmar tubercles absent; thenar tubercle present; enlarged prepollex with prepollical spines not protruding out of skin; fingers basally webbed, toes fully webbed. Variation was observed within the clades regarding the canthus rostralis (indistinct to

well defined), choana shape (rounded to elliptical), short and long rows of vomerine teeth. Despite those overall similarities, we found subtle differences in the snout shape in the lateral view of specimens from Clade I, who presented a slightly rounded snout against the truncate profile observed in the remaining specimens.

Bioacoustic variation

We identified in all populations representing the major clades delimited in bGYMC, a vocal repertoire including the two types of advertisement call (SAC and LAC following Brunetti et al. 2015), which differ from each other by the number of notes and call duration. The advertisement call structure is similar in all genetic group and consists in a multinote call, composed by a series of pulsatile notes, with similar amplitude, resulting in a simple-pulse train of quickly repeated notes (Table 4). The most common vocalizations are composed by a series of SAC, separated by a variable silent interval. However, we often could observe call series that alternate SAC and LAC. Those vocalizations are composed by a series of short calls followed by a sequence of long calls, and often the LAC emitted by one specimen overlap the LAC of nearby calling male. In addition, we identified vocalizations in some populations that fits with the courtship call and territorial call as defined by Brunetti et al. (2015) (Table 4).

Systematic and Taxonomic account

Our result highlighted the cryptic diversity within *B. punctata* complex and confirmed the assumptions of the presence of putative unknown species under the *B. punctata* name. Differences in the morphological, genetic distance and acoustic traits as well as the

phylogeographic pattern found here allow us to untangle some taxonomic issues of this species complex.

First our results support the monophyly of *B. atlantica* as a valid species (Clade V), but with a narrow geographic distribution limited to the state of Bahia populations, including samples close to its type locality, 13 km distance from Itabúna, Bahia (Caramaschi & Velosa 1996). Therefore, the *B. cf. atlantica* from the northern part of Atlantic Forest, which was recovered in the Clade I, should be considered related to the *B. punctata* populations from northern state of Maranhão and the remaining lineage from Amazonia.

Based in our findings, Clade I harbour the highest variation regarding the morphological and acoustic traits. Moreover, it covers the wider area of occurrence with populations from the headwaters of Amazon basin to the lowlands of coastal Maranhão. In the Guiana Shield, this clade includes lineages from French Guiana and Suriname, which allows us to interpretate that represents the *Boana punctata sensu stricto*, described from the coastal swamps of Suriname. Such wide distribution also includes samples/specimens from municipality of Tefé in the Solimões River, and from the “upper Amazon”, type localities of *Hyla variolosa* and *Hyla rhodoporus*. Thus, both remain as synonym of *B. punctata*.

Among the lineages delimited in our analysis, two can be classified as a confirmed candidate species (CCS): Clade II, presents molecular, morphologic, host the lineages from the Andean slopes of Colombia and Ecuador. One of our samples is from the Meta department, same province where *Hyla rubeola* was described by Cochran & Goin (1970). However, since we were not able to analyse the type series of it's the species to compare with our examined specimens from Colombia, we chose not to make any taxonomic decisions now. Furthermore, our results along with those from Guarnizo et al. (2015) and Caminer & Ron (2020) reveals two highly supported clades within this lineage, one in Ecuador and other in

Colombia. For the clade IV, we also assume a conservative approach keep it as a CCS, due the differences found in morphology and acoustic traits.

Regarding the lineage delimited as Clade VI, we refrain to propose any kind of taxonomic debate, due the low number of samples in our analysis and the small number of specimens analysed. Also, the low genetic distance from clade III ($< 2\%$), and the biogeographical pattern makes more possible that bGMYC recovered a structured genetic population. Therefore, we consider it as a UCS.

At last, our results support the hypothesis that recognize the populations from Clade III as the taxa described by Lutz (1951). Our decision is based on our genetic evidence, which encompass samples from near the type locality of *Hyla punctata rubro-lineata*, along with differences in morphology (discrete characters), acoustic and tadpoles' morphology previously documented by Kolenc et al. (2008) and Schülze et al. (2015). Following the interpretation of De Queiroz (2020) on subspecies concept, here we propose to revalidate and elevate it to a full species status, under the name *Boana rubrolineata* (Lutz, 1951).

Species Account

***Boana rubrolineata* (B. Lutz, 1951) (stat. nov.)**

Cresonyms: *Hyla punctata rubro-lineata* – B. Lutz, 1951

Hyla punctata – Duellman, 1974; Caramaschi & Velosa 1996;

Hyla punctata rubrolineata – Barrio 1965; Cei, 1980; Baldo, Krauczuz & Suárez 2002.

Hypsiboas punctatus – Faivovich et al., 2005, 2013; Brunetti et al. 2014, 2015; Silva Jr. et al. 2009; Souza et al. 2010; Pyron, & Wiens 2010; Jansen et al. 2011; Cacciali et al. 2015; Schülze et al. 2015; Duellman et al. 2016

Hypsiboas punctatus rubrolineatus – Brusquetti et al., 2006; Motte et al. 2009; Kolenc et al. 2008; Jansen et al. 2011.

Boana punctata – Dubois, 2017; Taboada et al. 2017; Pinheiro et al. 2018; Lima et al. 2019; Frost 2021; Koroiva et al. 2020; Sturaro et al. 2020;

Lectotype: The species was described based on two syntypes deposited in the Adolpho Lutz Collection, currently housed at the Museu Nacional, Rio de Janeiro (AL-MNRJ 4698–4699), from Buena Vista, Santa Cruz, Bolivia. We here designate the specimen AL-MNRJ 4698 as the lectotype for *Hyla punctata rubro-lineata* B. Lutz, 1951, currently in the combination *Boana rubrolineata* (B. Lutz, 1951). By implication, the specimen AL-MNRJ 4699 is the paralectotype of the species.

Diagnosis

Boana rubrolineata can be characterized by: 1) its medium size body (26-42 mm), 2) semicircular head in dorsal view, broader than larger; 3) truncate to slightly rounded snout in lateral view; 4) cantus rostralis indistinct, 5) eyes and tympanum dorsa-laterally positioned, 6) two slightly separated, patchy and short vomerine teeth row, (7) choana medium size, elliptical, 8) presence of mental gland, 9) single, large and subgular vocal sac, 10) presence of dorsolateral folds (with a dorsolateral stripe in most specimens), 11) presence of dorsal spots formed by iridophores, 12) subarticular tubercles simple, 13) slightly protruding pre-polex with non-evident spine (Figure 6). 14) In life, shows a lime-green dorsal coloration in daytime that changes to a yellowish green pattern with a reddish coloration in dorsal surface of body, head, shanks, and arms, 15) it also shows a yellow dorsolateral stripe with a fine red line above it. 16) the eyes presented a white to silvery iris with some fine black reticulations. During the nighttime, chromatophores expanded near the pupil, and it became brown/light brown in this area.

Morphological variation

Morphological traits are very conservative between the populations of *B. rubrolineata*. Most of variation are subtle and related to discrete characters. We observed slightly differences in snout shape, where some specimens show a rounded snout instead a truncate, observed more often. The head shape in dorsal view was also different between some specimens, presenting more pointed snout, turning the head shape almost rounded. However, such differences did not show geographic or populational assemblance. Regarding the body size, the specimens from Manicoré municipality, AM-Brazil, were bigger than the other populations (SVL = 37 – 44mm), but the lower number of examined individuals, hamper us to further discussions.

In life, the populations are also very variable. The colour patterns of dorsum varying from almost totally green without the reddish or yellowish spots, to a very spotted dorsum. In addition, this spotted pattern also varying from diminutive spots to very large ones, almost blotches (Figure 7).

Vocal repertoire

The vocal repertoire of *Boana rubrolineata* was described by Brunetti et al. (2015) (under the name *Hypsiboas punctatus*) and includes seven types of vocalizations with different behavioural context. It includes two types of advertisement call: short advertisement call (SAC) and long advertisement call (LAC), a territorial call; a courtship call, a release call; an aggressive call and a fighting call.

Advertisements calls are different between each other (SAC and LAC) by the duration and number of notes, but they share some overall acoustic structure. Both calls consist in a train of repetitive notes (multinote call) with variable silent intervals. For further details of the acoustic traits and biological context of these call see Brunetti et al. (2015).

Tadpole

The larval morphology described as *B. punctata* tadpoles from Chacoan region in northern Argentina (Kolenc et al., 2008) and from lowlands of Bolivia (Schulze et al., 2015) can be associated to *B. rubrolineata*. The tadpole was characterized by having a depressed body in lateral view and ovoid shape in dorsal view. Snout rounded in dorsal view and rounded to slightly tapered in lateral view. Oral disc anteroventrally positioned, with lateral folds, marginal papillae single, and a moderate dorsal gap on the upper labium. Also, some few submarginal papillae are present laterally. Labial tooth row formula 2(2)/4(1) was described for Argentina population's and 2(1,2)/3(1) for Bolivian ones. The position of the eyes was described as laterally positioned by Schulze et al. (2015), or dorsally positioned by Kolenc et al. (2008); in both cases, with dorsolateral direction. Nostrils positioned and directed anterodorsally, placed near to the eyes, with small operculum on the inner margin. Spiracle single, lateral, and sinistral. Vent tube medial. Dorsal fin originated at tail-body junction, rising gradually with a convex contour. Ventral fin with an almost flat contour, uniformly low. Tail musculature visible. Colouration in life is brown to olive-green with few dark speckles on dorsum. Transparent in ventral view. Dorsal and ventral fins translucent with light speckles.

Comparisons with its congeners in the *Boana punctata* species group

As part of *B. punctata* species complex, here we nested *B. rubrolineata* in the *B. punctata* group. Data from all other species of the group are provided in parenthesis. In live specimens *B. rubrolineata* can be easily differentiated by reddish (at night-time) or yellowish green/green (at daytime) dorsum with yellow or red scattered irregular spots and a yellowish dorsolateral stripe with a fine reddish line below that became in the canthus region and extended over eyelid reaching the groin, from all other congeners species, *B. cinerascens*

(yellowish green with light yellow spots), *B. gracilis* (yellowish green with light yellow blotches), *B. sibleszi* (green speckled by white or brown, with a cream dorsolateral stripe, and often a transversal bar between the eyes), *B. alemani* (unknown character). By exhibiting a white to silvery iris, with a fine black reticulation, and brown/dark brown area concentrated near to the pupil, and a black ring around the eyeball *B. rubrolineata* differentiated from *B. atlantica* (uniform silvery), *B. cinerascens* (light orange to reddish orange), *B. gracilis* (silver to light brown without reticulation) and *B. sibleszi* (yellowish bronze with black flecks). By its subtle prepollex, it differs from *B. sibleszi* (enlarged prepollex with large protruding prepollical spine in breeding males). *B. rubrolineata* can be differentiate from *B. punctata* and *B. atlantica* by a semi-circular head in dorsal view, *B. punctata* (pointed-rounded), *B. atlantica* (rounded); presence of an elliptical shape choana, *B. punctata* (oval), *B. atlantica* (circular) larger size up to 44 mm (up to 40 in *B. punctata* and *B. atlantica*), indistinct canthus rostralis (well defined in *B. punctata* and *B. atlantica*). In preservative specimens such characters related to colour patterns vanish, which makes the differentiation from *B. cinerascens* and *B. gracilis* difficult, however, it can be differentiate by the semicircular head shape in dorsal view and the absence of small yellow spots scattered on the dorsal surface of backs, arms and shanks (head truncate in dorsal view, much wider than long, with plenty of small yellow spots on dorsum).

Geographic distribution

Boana rubrolineata inhabits the lowlands, swamps and wetlands of the southern Amazon rainforest in the Madre de Dios province of Peru, and in the states of Amazonas and Rondônia in Brazil (associated to the upper Madeira River). It also occurs in savannas, tropical swamps, and wetlands of Bolivia. In central Brazil it inhabits open areas and wetlands of Cerrado domain, as well as the Pantanal – one the most species rich wetlands of South America. Besides, occurs in the Paraná Rivers basin and in the seasonal dry-wet Chacos of

Paraguay and Argentina. As its related species (*B. punctata* and *B. atlantica*), *B. rubrolineata* can be found in lakes and swamps generally associated to floating meadows or wet grasslands.

Discussion

Hidden diversity in *B. punctata* – *B. atlantica* complex has been suggested based on molecular (Almeida et al., 2021; Caminer & Ron, 2020; Guarnizo et al., 2015; Jansen et al., 2011; Lima et al., 2019; Sturaro et al., 2020), and acoustic data (Napoli & Cruz, 2005), however based on reduced samples covering small areas of its total geographic distribution. Our study integrates molecular data, morphology, and bioacoustics covering most of *B. punctata* complex distribution, revealing a high diversity of lineages using molecular species discovery methods. Based on bGMYC results, we found six OTUs, which two could be assigned to the nominal species, *B. punctata sensu stricto* and *B. atlantica*, one of them correspond to *B. rubrolineata* (stat. nov.) and two lineages representing putative species. These results indicate a higher diversity than previous assumptions of cryptic diversity in the *B. punctata* species complex (Caminer & Ron, 2020; Guarnizo et al., 2015; Lima et al., 2019). In widespread species with conservative external morphology, such as *B. punctata*, several unknown lineages are often uncovered, revealing species complexes which with integrative approach could solve taxonomic issues, delimit properly the species boundaries and increase overall species richness (Fouquet et al., 2021; Gehara et al., 2014; Seger et al., 2021; Sturaro et al., 2020).

Species delimitation methods utilizing coalescent-based approaches with single-locus data has played a role in the studies of diversification of neotropical frogs (Fouquet et al., 2014; Moraes et al., 2022; Réjaud et al., 2020; Vacher et al., 2017a), however, taxonomic decisions based on those results should be carefully analysed, since the accuracy of this

models can be affected by speciation rates and sequence lengths (Chan et al., 2022; Reid & Carstens, 2012) and could misidentify population structure with species boundaries (Sukumaran & Knowles, 2017). The taxonomic units (Clades) delimited in our analysis correspond to well supported lineages with genetic distance (16Sr RNA gene) ranging from 2,2% to 5,4%. Values of genetic divergence (p-values) higher than 3% are considered deep and are often related to interspecific variation (Fouquet et al., 2007), although lower values of 16S divergence are found in interspecific variations in hylid species (e.g. Seger et al., 2021). Our findings confirmed the high values of genetic distance reported between the putative species within *B. punctata* (Caminer & Ron, 2020; Guarnizo et al., 2015; Jansen et al., 2011).

Regarding the phenotypic traits, morphology and bioacoustics are very conservative through the lineages of the species complex. However, we found significant differences between body size, coloration and discrete characters that separates *Boana punctata*, *B. atlantica* and *B. rubrolineata*. We also found body shape differences in specimens from Clade IV and V to the remaining groups, but the lack of more samples, including advertisement call, tadpoles and chromosomes are still need to make taxonomic decision regarding these lineages.

The Clades recovered in our analysis showed allopatric distribution through the major ecoregions and wetlands of South America. Overall, we found two widespread lineages: Clade I here assigned as *B. punctata* (**sensu stricto**), and Clade III, here assigned to *B. rubrolineata* (stat. nov.). Moreover, we found other four lineages with more restricted distribution: Clade II, that encompass the populations of eastern slopes of Cordillera Oriental of Colombia and Andean slopes of Ecuador; Clade IV from northern Tocantins River-basin and upper-medium Parnaíba River-basin in northern Cerrado; Clade V here assigned to *B. atlantica* (**sensu stricto**), which encompass the populations from Atlantic Forest of state of Bahia in northeastern Brazil and latter Clade VI, with a small population from northeastern of

the state of Mato Grosso do Sul, in central Brazil. Widely distributed cryptic lineages are quite common in neotropical treefrogs with continental geographic distribution. Such pattern are found in *Dendropsophus minutus* (Gehara et al., 2014), *Dendropsophus nanus* – *Dendropsophus walfordi* species complex (Seger et al., 2021) and *Boana raniceps* (Camurugi et al., 2021), all species that occurs in open and wetland areas through South America such as *B. punctata*.

Moreover, the phylogeographic pattern found here enlighten evidence of a role of geological events that shaped the South American landscape, often related to the diversification of amphibian species (e.g. Camurugi et al., 2020; Fouquet et al., 2012; Fouquet, Marinho, et al., 2021; Gehara et al., 2014; Moraes et al., 2022; Oliveira et al., 2018; Réjaud et al., 2020), as the main drivers of *B. punctata* complex diversification. However, due to limited power of our single-locus approach and the lower support in the relationship of the OTUs in our phylogenetic topology, we refrain to discuss about the biogeography and geological events that shape the *B. punctata* species complex diversity.

The taxonomic changes proposed here consider the evidence found in our study and those previously documented. We assigned to Clade I the taxonomic status of *B. punctata sensu stricto*, because it encompasses samples from the savannas and lowlands of French Guyana and Surinam. Although our samples were not topotypical, populations sampled from the coastal mosaic of swamps and savannas of French Guyana, Suriname, state of Amapá and savannas of Sipaliwini – Suriname, should be conspecific with the populations from the type locality (“Surinam”) (Fouquet, Vacher, Kadosoe, Jairam, & Ouboter, 2015). Regarding the taxonomic status of *Hyla rhodoporus* and *Hyla variolosa*, both remains as synonyms of *B. punctata*, since the geographic distribution of clade I encompass the type localities and samples that could be associated to these names (Günther, 1872; Hoogmoed & Gruber, 1983). Nevertheless, our taxonomic arrangement implies in a species with a much wider distribution

through the Amazon basin, northern coastal of Maranhão and coastal areas of northern Atlantic Forest in the state of Pernambuco and Alagoas in Brazil. This biogeographical pattern is well known, due to an Amazon-Atlantic Forest corridors, a Northeast dispersal route, during the Pliocene-Pleistocene (Ledo & Colli, 2017). Leading to a fauna and flora in the northern Atlantic Forest (Pernambuco Center) more related to the Amazon than to the south Atlantic Forest (Canedo & Haddad, 2012; Santos et al., 2007), which seems the case for *Boana punctata* and *B. atlantica*.

Meanwhile, the inclusion of genomic data could reveal more cryptic lineages, and clarify the species boundaries of this widespread taxa, as has been observed in *Dendropsophus leucophyllatus* (Pirani et al., 2020), a sympatric species from Amazon lowlands. In the case of *Boana punctata sensu stricto* clade, there has been evidence of cytogenetic variation for the populations of northern Atlantic Forest, karyotype formulae (8M, 8SM, 8ST) with NOR bearing chromosomes site in pair 10 (Lima et al., 2019), while in populations from Pará the karyotype formulae is 8M, 12SM, 2ST, with variation in the 11 pair with one chromosome metacentric and other submetacentric, with the presence of a NOR chromosome is this pair (Ferro et al., 2018). Tadpole variation, especially regarding the labial tooth row formulae is also found along this widespread lineage. In populations from Suriname were found a LTRF of 2(1,2)/3(1), same as northern Atlantic Forest (Hoogmoed, 1979; Nascimento et al., 2009), but different from the populations of Iquitos 2(2)/3 (Rodriguez & Duellman, 1994). The inclusion of genomic data could help to elucidate possible intraspecific variation related to different habitats (see Candiotti & Altig, 2010) or hidden diversity within this lineage.

For a long time, the taxonomic status of *B. atlantica* was questioned, due the overlap of diagnosis characters and overall similarities of external morphology between this species and *B. punctata*. Our results along with those from Lima et al. (2019), Napoli & Cruz (2005)

and Camurugi & Juncá (2013), made undoubtful the recognition of *B. atlantica* as a valid species of *B. punctata* species complex, with narrow occurrence in the ecoregion of coastal forest of Bahia (see Vasconcelos, Prado, Da Silva, & Haddad, 2014).

Finally, we reevaluate the taxonomic status of *Hyla punctata rubro-lineata*, elevating it to a full species status under the name *Boana rubrolineata*, assigning it to the Clade III of our bGMYC analysis. Since the description of the former subspecies *Hyla punctata rubro-lineata* by B. Lutz (1951), several authors has pointed out subtle differences in morphology of adults (Barrio, 1965; Cei, 1980), tadpoles (Kolenc et al., 2008; Schulze et al., 2015), and advertisement call (Brunetti et al., 2014, 2015; Napoli & Cruz, 2005), advocating maintenance of subspecies status or even elevation of it to a full species, in disagreement with the synonymy proposed by Duellman, (1974). However, lack of a large-scale analysis, including different sources of evidence hampered the authors from proposing this taxonomic rearrangement, until the present study. Herein we presented sounder evidence based on molecular, discrete characters of morphology, including the analysis of the lectotypes, and acoustic traits that supports our proposition. Furthermore, we consider noteworthy the presence of a lineage of *B. rubrolineata* extending through the Madre de Dios – Madeira River system into the Amazon domain, throughout the Paraguay – Paraná River basins. This pattern is well-known for aquatic vertebrates (Garda & Cannatella, 2007; Gravena, da Silva, da Silva, Farias, & Hrbek, 2015; Muniz et al., 2019; Roberto et al., 2020). Several events related to Andean orogeny and different river capture events are known to contribute for the connection of the Bolivia-Paraguay river basins (Tagliacollo et al., 2015), and can be the drivers of the phylogenetic structure found in *B. rubrolineata* populations.

Regarding the taxonomic status of the two CCS lineages delimited in our study, Clade II and IV, we kept a conservative approach and refrain to propose new taxonomic status for theses putative species. Nevertheless, the lineages from Colombia (Clade II), includes

samples from Meta province, from where *Hyla rubeola* was described (Cochran & Goin, 1970). Herein we did not access the type of material of *H. rubeola*, and due lack of the analysis of the type series we understand as a premature decision to revalidate this synonym. On the other hand, for the lineages of northern Cerrado at the river-basin of Tocantins- River and Parnaíba River (Clade IV), there is not such taxonomic issues to be evaluated, and it probably could be an unknown species. However, we consider that the lack of clarity between the limits of distribution between Clade I and Clade IV, as well as the number of samples that do not adequately cover the distribution of the species in this region, make this decision premature.

Our findings redefined the geographic distributions of nominal species *B. punctata* and *B. atlantica* and proposed the elevation of *B. rubrolineata* to a full species status. Also, we delimitate some putative new species with narrower distributions. Uncovering cryptic species has a significant impact on the criteria used to assess the conservation status of amphibian species (Angulo & Icochea, 2010; Bickford et al., 2007). Although the species of *B. punctata* species complex have been remarkably considered as widespread, tolerant and adapted to anthropic landscape with no major threats to its populations (Carnaval & Pimenta, 2004; La Marca et al., 2010; Torres, Boeris, Insaurrealde, Baldo, & Brunetti, 2021), our results reveal that this context does not apply to *B. atlantica*, *B. rubrolineata* and the remain putative species from Cerrado domain, although, in last years, the wetlands of Pantanal, and the seasonal wet-dry ecosystems have been facing a unprecedented loss of their vertebrate community by wildfire caused by human activity, land use, and a subsequently climate change (Garcia et al., 2021; Hofmann et al., 2021; Tomas et al., 2021). In addition, public policies did not properly support the fire prevention programs or supervise the illegal logging, becoming a chimaera of troubles which, we cannot measure how it will impact the biodiversity. We

believe that those issues should be taken in account in further amphibian conservation evaluation to properly understand the impact of these threats to these overlooked species.

Acknowledgments

We thank, Fabiano Waldez, Wesley Valteran, Drausio Morais, João Paulo, Nilton Slobodizian, Moises Barbosa, Ivan Junqueira for help assistance in our fieldwork. For the loan of tissues samples we acknowledge, Célio F.B. Haddad (UNESP-Rio Claro/SP) and Felipe Curcio (UFMT). This study was supported by the French/Brazilian GUYAMAZON programme (Institut de Recherche pour le Développement-IRD, Centre national de la recherche scientifique-CNRS, Collectivité territoriale de Guyane-CTG, Centre de coopération internationale en recherche agronomique pour le développement-CIRAD and Fundação de Amparo à Pesquisa do Estado do Amazonas-FAPEAM; #062.00962/2018), co-coordinated by A.F. and F.P.W.A.P.A. thank PhD fellowship from FAPEAM – (POSGRAD-UFAM 2016); VTC received fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES/PNPD; U.C acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the fellowship; and FPW thanks financial support from CNPq (productivity fellowship #305535/2017-0), Fundação de Amparo à Pesquisa do Estado do Amazonas-FAPEAM (#062.00962/2018 and 062.01110/2017), #and L’Oréal-Unesco For Women in Science Program (Brazil 2016 and IRT 2017). For the outstanding photos of living specimens of *B. rubrolineata* we thank Matheus Mororti, Kevin K. Caldwell, Pedro F. Dias, and Guilherme Figueiredo.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- Almeida, A. P. de, Moraes, L. J. C. L., Rojas, R. R., Roberto, I. J., Carvalho, V. T. de, Ávila, R. W., ... Gordo, M. (2021). Phylogenetic relationships of the poorly known treefrog *Boana hobbsi* (Cochran & Goin, 1970) (Anura: Hylidae), systematic implications and remarks on morphological variations and geographic distribution. *Zootaxa*, 4933(3), 301–323. <https://doi.org/10.11646/zootaxa.4933.3.1>
- Angulo, A., & Icochea, J. (2010). Cryptic species complexes, widespread species and conservation: Lessons from Amazonian frogs of the *Leptodactylus marmoratus* group (Anura: Leptodactylidae). *Systematics and Biodiversity*, 8(3), 357–370. <https://doi.org/10.1080/14772000.2010.507264>
- Araujo-vieira, K., Pombal Jr., J. P., Caramaschi, U., Novaes-e-Fagundes, G., Orrico, V. G. D., & Faivovich, J. (2020). A neotype for *Hyla x-signata* Spix, 1824 (Amphibia, Anura, Hylidae). *Papéis Avulsos de Zoologia*, 60, e20206056. <https://doi.org/http://doi.org/10.11606/1807-0205/2020.60.56>
- Barrio-Amorós, C. L., Rojas-Runjaic, F. J. M., & Señaris, J. C. (2019). Catalogue of the amphibians of Venezuela: Illustrated and annotated species list, distribution, and conservation. *Amphibian & Reptile Conservation*, 13(1), 1–198.
- Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., ... Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, 22(3), 148–155. <https://doi.org/10.1016/j.tree.2006.11.004>
- Brunetti, A. E., Taboada, C., & Faivovich, J. (2014). The reproductive biology of *Hypsiboas punctatus* (Anura: Hylidae): male territoriality and the possible role of different signals during female choice. *Salamandra*, 50(4), 215–224.
- Brunetti, A. E., Taboada, C., & Faivovich, J. (2015). Extended Vocal Repertoire in *Hypsiboas punctatus* (Anura: Hylidae). *Journal of Herpetology*, 49(1), 46–52.

<https://doi.org/10.1670/13-206>

- Brusquetti, F., Jansen, M., Barrio-Amorós, C., Segalla, M., & Haddad, C. F. B. (2014). Taxonomic review of *Scinax fuscomarginatus* (Lutz, 1925) and related species (Anura; Hylidae). *Zoological Journal of the Linnean Society*, 171(4), 783–821. <https://doi.org/10.1111/zoj.12148>
- Brusquetti, F., & Lavilla, E. (2006). Lista comentada de los anfibios de Paraguay. *Cuadernos de Herpetología*, 20(2), 3–79.
- Caminer, M. A., & Ron, S. R. (2020). Systematics of the *Boana semilineata* species group (Anura : Hylidae), with a description of two new species from Amazonian Ecuador. *Zoological Journal of the Linnean Society*, (January), 1–32. <https://doi.org/10.1093/zoolinnea/zlaa002/5810752>
- Camurugi, F., Gehara, M., Fonseca, E. M., Zamudio, K. R., Haddad, C. F. B., Colli, G. R., ... Garda, A. A. (2021). Isolation by environment and recurrent gene flow shaped the evolutionary history of a continentally distributed Neotropical treefrog. *Journal of Biogeography*, 48(4), 760–772. <https://doi.org/10.1111/jbi.14035>
- Camurugi, F., & Juncá, F. (2013). Reproductive biology of *Hypsiboas atlanticus* (Anura: Hylidae). *Herpetology Notes*, 6(1), 489–495.
- Caramaschi, U., Velosa, A. (1996). Nova especie de *Hyla laurenti*, 1768 do leste brasileiro (Amphibia, Anura, Hylidae). *Boletim Di Museu Nacional*.
- Cei, J. M. (1980). Amphibians of Argentina. *Italian Journal of Zoology*.
- Chan, K. O., Hertwig, S. T., Neokleous, D. N., Flury, J. M., & Brown, R. M. (2022). Widely used, short 16S rRNA mitochondrial gene fragments yield poor and erratic results in phylogenetic estimation and species delimitation of amphibians. *BMC Ecology and Evolution*, 22(1), 1–9. <https://doi.org/10.1186/s12862-022-01994-y>
- Cochran, D. M., & Goin, C. J. (1970). Frogs of Colombia. In *Bulletin of the United States*

Museum.

- De Queiroz, K. (2007). Species concepts and species delimitation. *Systematic Biology*, 56(6), 879–886. <https://doi.org/10.1080/10635150701701083>
- Duellman, William E. (1978). The Biology of an Equatorial Herpetofauna in Amazonian Ecuador. *Miscellaneous Publications, University of Kansas, Museum of Natural History*, 65, 372. <https://doi.org/10.2307/1443434>
- Duellman, William E. (1970). The Hylid Frogs of Middle America. In *Monograph Museum of Natural History* (Vol. 2). <https://doi.org/10.2307/1442868>
- Duellman, William E. (1974). A Reassessment of the taxonomic status of some Neotropical hylid frogs. *Occasional Papers of The Museum of Natural History*, (27), 1–27.
- 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), 1. [https://doi.org/10.1206/0003-0090\(2005\)294\[0001:srotff\]2.0.co;2](https://doi.org/10.1206/0003-0090(2005)294[0001:srotff]2.0.co;2)
- Fouquet, A., Gilles, A., Vences, M., Marty, C., Blanc, M., & Gemmell, N. J. (2007). Underestimation of species richness in neotropical frogs revealed by mtDNA analyses. *PLoS ONE*, 2(10). <https://doi.org/10.1371/journal.pone.0001109>
- Fouquet, A., Loebmann, D., Castroviejo-Fisher, S., Padial, J. M., Orrico, V. G. D., Lyra, M. L., ... Rodrigues, M. T. (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(2), 547–561. <https://doi.org/10.1016/j.ympev.2012.07.012>
- Fouquet, A., Marinho, P., Réjaud, A., Carvalho, T. R., Caminer, M. A., Jansen, M., ... Ron, S. (2021). Systematics and biogeography of the *Boana albopunctata* species group (Anura,

Hylidae), with the description of two new species from Amazonia. *Systematics and Biodiversity*, 19(4), 375–399.

<https://doi.org/https://doi.org/10.1080/14772000.2021.1873869>

Fouquet, A., Santana Cassini, C., Fernando Baptista Haddad, C., Pech, N., & Trefaut Rodrigues, M. (2014). Species delimitation, patterns of diversification and historical biogeography of the Neotropical frog genus *Adenomera* (Anura, Leptodactylidae). *Journal of Biogeography*, 41(5), 855–870. <https://doi.org/10.1111/jbi.12250>

Fouquet, A., Vacher, J. P., Kadosoe, V., Jairam, R., & Ouboter, P. (2015). Checklist of the amphibians of the Sipaliwini area, suriname. *Herpetology Notes*, 8(March), 63–68.

Garcia, L. C., Szabo, J. K., Roque, F. D. O., Matos, A. De, Pereira, M., Nunes, C., ... Ribeiro, D. B. (2021). Record-breaking wildfires in the world ’ s largest continuous tropical wetland: Integrative fire management is urgently needed for both biodiversity and humans. *Journal of Environmental Management*, 293(April), 112870.

Gehara, M., Crawford, A. J., Orrico, V. G. D., Rodríguez, A., Lötters, S., Fouquet, A., ... Köhler, J. (2014). High Levels of Diversity Uncovered in a Widespread Nominal Taxon: Continental Phylogeography of the Neotropical Tree Frog *Dendropsophus minutus*. *PLoS ONE*, 9(9), e103958. <https://doi.org/10.1371/journal.pone.0103958>

Gravena, W., da Silva, V. M. F., da Silva, M. N. F., Farias, I. P., & Hrbek, T. (2015). Living between rapids : genetic structure and hybridization in botos (Cetacea : Iniidae : Inia spp .) of the Madeira River , Brazil. *Biological Journal of the Linnean Society*, 114(4), 764–777. <https://doi.org/https://doi.org/10.1111/bij.12463>

Guarnizo, C. E., Paz, A., Muñoz-Ortiz, A., Flechas, S. V., Méndez-Narváez, J., & Crawford, A. J. (2015). DNA Barcoding Survey of Anurans across the Eastern Cordillera of Colombia and the Impact of the Andes on Cryptic Diversity. *PLoS ONE*, 10(5), 1–20. <https://doi.org/10.1371/journal.pone.0127312>

- Guerra, V., Jardim, L., Llusia, D., Márquez, R., & Bastos, R. P. (2020). Knowledge status and trends in description of amphibian species in Brazil. *Ecological Indicators*, 118(July), 106754. <https://doi.org/10.1016/j.ecolind.2020.106754>
- Günther, A. (1872). Note on *Hyla punctata* and *Hyla rhodoporus*. *Proceedings of the Zoological Society of London*, 1872, 662–663. <https://doi.org/10.1111/j.1469-7998.1833.tb06418.x>
- Heyer, W. R., Rand, A. S., Cruz, C. A. G. da, Peixoto, O. L., & Nelson, C. E. (1990). Frogs of Boraceia. *Arquivos de Zoologia - Museu de Zoologia Da Universidade de São Paulo*, 31(4), 231–410.
- Hillis, D. M. (2019). Species Delimitation in Herpetology. *Journal of Herpetology*, 53(1), 3. <https://doi.org/10.1670/18-123>
- Hofmann, G. S., Cardoso, M. F., Alves, R. J. V, Weber, E. J., Barbosa, A. A., Toledo, P. M. De, ... Oliveira, L. F. B. De. (2021). The Brazilian Cerrado is becoming hotter and drier. *Global Change Biology*, 27(17), 4060–4073. <https://doi.org/10.1111/gcb.15712>
- Hoogmoed, M. S. (1979). Resurrection of *Hyla ornatissima* Noble 1923 (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.
- 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*, 9, 319–415.
- Jansen, M., Bloch, R., Schulze, A., & Pfenninger, M. (2011). Integrative inventory of Bolivia's lowland anurans reveals hidden diversity. *Zoologica Scripta*, 40(6), 567–583. <https://doi.org/10.1111/j.1463-6409.2011.00498.x>
- Kawaguchi, A., Miya, M., & Nishida, M. (2001). Complete mitochondrial DNA sequence of *Aulopus japonicus* (Teleostei: Aulopiformes), a basal Eurypterygii: Longer DNA

- sequences and higher-level relationships. *Ichthyological Research*, 48(3), 213–223. <https://doi.org/10.1007/s10228-001-8139-0>
- Köhler, J., Jansen, M., Rodríguez, A., Kok, P. J. R., Toledo, L. F., Emmrich, M., ... Vences, M. (2017). The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. *Zootaxa*, 4251(1), 1. <https://doi.org/10.11646/zootaxa.4251.1.1>
- Kolenc, F., Borteiro, C., Alcalde, L., Baldo, D., Cardozo, D., & Faivovich, J. (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, 66. <https://doi.org/10.11646/zootaxa.1927.1.1>
- Lima, L. R., Dubeux, M. J. M., do Nascimento, F. A. C., Bruschi, D. P., & Mott, T. (2019). Uncovering Neotropical treefrog diversity: integrative taxonomy reveal paraphyly in *Boana atlantica* (Amphibia, Anura, Hylidae). *Amphibia-Reptilia*, (July), 1–11. <https://doi.org/10.1163/15685381-20191109>
- Mângia, S., Oliveira, E. F. de, Santana, D. J., Koroiva, R., Paiva, F., & Garda, A. A. (2020). Revising the taxonomy of *Proceratophrys* Miranda-Ribeiro, 1920 (Anura: Odontophrynidae) from the Brazilian semiarid Caatinga: Morphology, calls and molecules support a single widespread species. *Journal of Zoological Systematics and Evolutionary Research*, (November 2019), 1–22. <https://doi.org/10.1111/jzs.12365>
- Melin, D. (1941). Contributions to the knowledge of the Amphibia of South America. *Göteborgs Kungl. Vetenskaps-Och Vitterhets-Samhälles. Handlingar. Serien B, Matematiska Och Naturvetenskapliga Skrifter*, 1, 1–71.
- Meseguer, A. S., Antoine, P., Fouquet, A., Delsuc, F., & Condamine, F. L. (2020). The role of the Neotropics as a source of world tetrapod biodiversity. *Global Ecology and Biogeography*, 29(9), 1565–1578. <https://doi.org/10.1111/geb.13141>

- Miya, M., & Nishida, M. (2000). Use of mitogenomic information in Teleostean molecular phylogenetics: A tree-based exploration under the maximum-parsimony optimality criterion. *Molecular Phylogenetics and Evolution*, 17(3), 437–455. <https://doi.org/10.1006/mpev.2000.0839>
- Moraes, L. J. C. L., Werneck, F. P., Réjaud, A., Rodrigues, M. T., Prates, I., Glaw, F., ... Fouquet, A. (2022). Diversification of tiny toads (Bufonidae: Amazophrynella) sheds light on ancient landscape dynamism in Amazonia. *Biological Journal of the Linnean Society*, 136(1), 75–91. <https://doi.org/10.1093/biolinnean/blac006>
- Motta, J., Menin, M., Almeida, A. P., Hrbek, T., & Pires Farias, I. (2018). When the unknown lives next door: A study of central Amazonian anurofauna. *Zootaxa*, 4438(1), 79–104. <https://doi.org/10.11646/zootaxa.4438.1.3>
- Moura, M. R., & Jetz, W. (2021). Shortfalls and opportunities in terrestrial vertebrate species discovery. *Nature Ecology and Evolution*, 5(5), 631–639. <https://doi.org/10.1038/s41559-021-01411-5>
- Muniz, F. L., Ximenes, A. M., Bittencourt, P. S., Hernández-Rangel, S. M., Campos, Z., Hrbek, T., & Farias, I. P. (2019). Detecting population structure of *Paleosuchus trigonatus* (Alligatoridae: Caimaninae) through microsatellites markers developed by next generation sequencing. *Molecular Biology Reports*, 0(0), 0. <https://doi.org/10.1007/s11033-019-04709-7>
- Myers, C. W., & Duellman, W. E. (1982). *Hyla graceae* New-Species From Cerro-Colorado and Other Tree Frog Records and Geographical Notes From Western Panama. *American Museum Novitates*, (2752), 1–32. Retrieved from <http://search.ebscohost.com/login.aspx?direct=true&db=boh&AN=BACD198375070596&site=ehost-live>
- Napoli, M., & Cruz, I. (2005). The advertisement call of *Hyla atlantica* Caramaschi & Velosa,

- 1996, with considerations on its taxonomic status (Amphibia, Anura, Hylidae). *Arquivos Do Museu Nacional*, 63(2), 283–288.
- Oliveira, E. F., Gehara, M., São-Pedro, V. A., Costa, G. C., Burbrink, F. T., Colli, G. R., ... Garda, A. A. (2018). Phylogeography of Muller's termite frog suggests the vicariant role of the Central Brazilian Plateau. *Journal of Biogeography*, 45(11), 2508–2519. <https://doi.org/10.1111/jbi.13427>
- Orrico, V. G. D., Nunes, I., Mattedi, C., Fouquet, A., Lemos, A. W., Rivera-Correa, M., ... Haddad, C. F. B. (2017). Integrative taxonomy supports the existence of two distinct species within *Hypsiboas crepitans* (Anura: Hylidae). *Salamandra*, 53(1), 99–113.
- Padial, J. M., Miralles, A., De La Riva, I., & Vences, M. (2010). The integrative future of taxonomy. *Frontiers in Zoology*, 7, 16. Retrieved from <http://www.frontiersinzoology.com/content/7/1/16>
- Pirani, R. M., Peloso, P. L. V., Prado, J. R., Polo, É. M., Knowles, L. L., Ron, S. R., ... Werneck, F. P. (2020). Diversification history of clown tree frogs in Neotropical rainforests (Anura, Hylidae, *Dendropsophus leucophyllatus* group). *Molecular Phylogenetics and Evolution*, 150(November 2019), 106877. <https://doi.org/10.1016/j.ympev.2020.106877>
- Reid, N. M., & Carstens, B. C. (2012). Phylogenetic estimation error can decrease the accuracy of species delimitation : a Bayesian implementation of the general mixed Yule-coalescent model. *BMC Evolutionary Biology*, 12(196), 1–11. Retrieved from <http://www.biomedcentral.com/1471-2148/12/196>
- Réjaud, A., Rodrigues, M. T., Crawford, A. J., Castroviejo-Fisher, S., Jaramillo, A. F., Chaparro, J. C., ... Fouquet, A. (2020). Historical biogeography identifies a possible role of Miocene wetlands in the diversification of the Amazonian rocket frogs (Aromobatidae: Allobates). *Journal of Biogeography*, (May), 1–11.

<https://doi.org/10.1111/jbi.13937>

- Riveiro, J. A. (1961). Salientia of Venezuela. *Bulletin of The Museum of Comparative Zoology*, 126(1), 1–614.
- Rivero, J. A. (1971). Notas sobre los anfibios de Venezuela. I. Sobre los Hilidos de la Guayana Venezolana. *Caribbean Journal of Science*, 11(3–4), 181–193.
- Roberto, I. J., Bittencourt, P. S., Muniz, F. L., Hernández-Rangel, S. M., Nóbrega, Y. C., Ávila, R. W., ... Hrbek, T. (2020). Unexpected but unsurprising lineage diversity within the most widespread Neotropical crocodilian genus *Caiman* (Crocodylia, Alligatoridae) . *Systematics and Biodiversity*, 18(4), 377–395.
<https://doi.org/10.1080/14772000.2020.1769222>
- Rojas, R. R., Fouquet, A., Ron, S. R., Hernández-Ruz, E. J., Melo-Sampaio, P. R., Chaparro, J. C., ... Hrbek, T. (2018). A Pan-Amazonian species delimitation: high species diversity within the genus *Amazophrynella* (Anura: Bufonidae). *PeerJ*, 6, e4941.
<https://doi.org/10.7717/peerj.4941>
- Savage, J. M., & Heyer, W. R. (1967). Variation and distribution in the tree-frog genus *Phyllomedusa* in Costa Rica , central America. *Beitrage Zur Neotropischen Fauna*, 5(2), 111–131.
- Schneider, J. G. (1799). *Historia Amphibiorum Naturalis et Literariae. Fasciculus Primus. Continens Ranas, Calamitas, Bufones, Salamandras et Hydrosin genera et species descriptos Notisque Suis Distinctos. In Jena Frederici Frommani.*
- Schulze, A., Jansen, M., & Köhler, G. (2015). Tadpole diversity of Bolivia's lowland anuran communities: molecular identification, morphological characterisation, and ecological assignment. *Zootaxa*, 4016(1), 1–111. <https://doi.org/10.11646/zootaxa.4016.1.1>
- Seba, A. (1734). *Locupletissimi Rerum Naturalium Thesauri Accurata Descriptio. 4 Vols. fo.*
- Seger, K. R., da Veiga Teixeira, B. F., Annibale, F. S., Rossa-Feres, D. de C., Lima, A. P.,

- Andrade, G. V., ... Lourenço, L. B. (2021). Five Independent Lineages Revealed by Integrative Taxonomy in the *Dendropsophus nanus*–*Dendropsophus walfordi* Species Complex. *Diversity*, 13(11), 522. <https://doi.org/10.3390/d13110522>
- Spix, J. B. von. (1824). *Animalia Nova Sive Species Novae Testudinum et Ranarum, quas in Itinere per Brasiliam annis 1817 - 1820*.
- Sturaro, M. J., Costa, J. C. L., Maciel, A. O., Lima-Filho, G. R., Rojas-Runjaic, F. J. M., Mejia, D. P., ... Peloso, P. L. V. (2020). Resolving the taxonomic puzzle of *Boana cinerascens* (Spix, 1824), with resurrection of *Hyla granosa gracilis* Melin, 1941 (Anura: Hylidae). *Zootaxa*, 4750(1), 1–30. <https://doi.org/https://doi.org/10.11646/zootaxa.4750.1.1>
- Sukumaran, J., & Knowles, L. L. (2017). Multispecies coalescent delimits structure , not species. *PNAS*, 114(7), 1607–1612. <https://doi.org/https://doi.org/10.1073/pnas.1607921114>
- Taboada, C., Brunetti, A. E., Alexandre, C., Lagorio, M. G., & Faivovich, J. (2017). Fluorescent Frogs: A Herpetological Perspective. *South American Journal of Herpetology*, 12(1), 1–13. <https://doi.org/10.2994/sajh-d-17-00029.1>
- Taboada, C., Brunetti, A. E., Pedron, F. N., Carnevale Neto, F., Estrin, D. A., Bari, S. E., ... Faivovich, J. (2017). Naturally occurring fluorescence in frogs. *Proceedings of the National Academy of Sciences*, 114(14), 3672–3677. <https://doi.org/10.1073/pnas.1701053114>
- Thom, G., Amaral, F. R. Do, Hickerson, M. J., Aleixo, A., Araujo-Silva, L. E., Ribas, C. C., ... Miyaki, C. Y. (2018). Phenotypic and genetic structure support gene flow generating gene tree discordances in an Amazonian floodplain endemic species. *Systematic Biology*, 67(4), 700–718. <https://doi.org/10.1093/sysbio/syy004>
- Tomas, W. M., Berlinck, C. N., Chiaravalloti, R. M., Faggioni, G. P., Strüssmann, C.,

- Libonati, R., ... Morato, R. (2021). Distance sampling surveys reveal 17 million vertebrates directly killed by the 2020's wildfires in the Pantanal, Brazil. *Scientific Reports*, 11(1), 1–9. <https://doi.org/10.1038/s41598-021-02844-5>
- Torres, P. J., Boeris, J. M., Insaurralde, J. A., Baldo, D., & Brunetti, A. E. (2021). Potential effects of dams in the geographic range expansion of hylid frogs associated with aquatic macrophytes. *Herpetological Conservation and Biology*, 16(2), 259–270. <https://doi.org/10.15468/dl.5ffqhq>
- Vacher, J. P., Kok, P. J. R., Rodrigues, M. T., Lima, J. D., Lorenzini, A., Martinez, Q., ... Fouquet, A. (2017). Cryptic diversity in Amazonian frogs: Integrative taxonomy of the genus *Anomaloglossus* (Amphibia: Anura: Aromobatidae) reveals a unique case of diversification within the Guiana Shield. *Molecular Phylogenetics and Evolution*, 112, 158–173. <https://doi.org/10.1016/j.ympev.2017.04.017>
- Vasconcelos, T. S., Prado, V. H. M., Da Silva, F. R., & Haddad, C. F. B. (2014). Biogeographic distribution patterns and their correlates in the diverse frog fauna of the atlantic forest hotspot. *PLoS ONE*, 9(8), 1–9. <https://doi.org/10.1371/journal.pone.0104130>
- von May, R., Catenazzi, A., Santa-Cruz, R., Gutierrez, A. S., Moritz, C., & Rabosky, D. L. (2019). Thermal physiological traits in tropical lowland amphibians: Vulnerability to climate warming and cooling. *PLoS ONE*, 14(8). <https://doi.org/10.1371/journal.pone.0219759>
- Wake, D. B., & Koo, M. S. (2018). Amphibians. *Current Biology*, 28(21), R1237–R1241. <https://doi.org/10.1016/j.cub.2018.09.028>
- Watters, J. L., Cummings, S. T., Flanagan, R. L., & Siler, C. D. (2016). Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. *Zootaxa*, 4072(4), 477–495.

<https://doi.org/10.11646/zootaxa.4072.4.6>

Wells, K. D. (1977). The social behaviour of anuran amphibians. *Animal Behaviour*, 25(PART 3), 666–693. [https://doi.org/10.1016/0003-3472\(77\)90118-X](https://doi.org/10.1016/0003-3472(77)90118-X)

Wells, K. D., & Schwartz, J. J. (2006). The Behavioral Ecology of Anuran Communication. *Hearing and Sound Communication in Amphibians*, 44–86. https://doi.org/10.1007/978-0-387-47796-1_3

LIST OF TABLE

Table 1. Genetic distances between and within (values in parenthesis) the major lineages (Clades) recovered in the phylogenetic analysis and bGYMC species delimitation.

	Clade I	Clade II	Clade III	Clade IV	Clade V	Clade VI
Clade I	(0.016)					
Clade II	0.051	(0.042)				
Clade III	0.035	0.047	(0.013)			
Clade IV	0.039	0.050	0.042	(0.017)		
Clade V	0.043	0.054	0.031	0.041	(0.011)	
Clade VI	0.035	0.047	0.022	0.036	0.028	(0.009)

Table 2. Measures of morphometric variables of *B. punctata* species complex 447 specimens. Columns indicates the group delimited in the bGYMC analysis and the number os speciemnns examined per clade. Values presented are mean values $\pm$ standard deviation.

	Clade I (n=153)	Clade II (n=48)	Clade III (n=160)	Clade IV (n=51)	Clade V (n=26)	Clade VI (n=9)
SVL	33.59 $\pm$ 3.07	34.31 $\pm$ 2.17	32.77 $\pm$ 3.11	31.01 $\pm$ 1.61	36.37 $\pm$ 1.91	31.39 $\pm$ 2.06
HL	10.86 $\pm$ 1.06	11.24 $\pm$ 0.86	10.32 $\pm$ 1.02	10.14 $\pm$ 0.61	11.39 $\pm$ 0.73	9.84 $\pm$ 0.60
HW	12.01 $\pm$ 1.04	12.46 $\pm$ 1.20	11.61 $\pm$ 1.10	11.24 $\pm$ 0.62	13.07 $\pm$ 0.93	11.38 $\pm$ 0.90
END	3.31 $\pm$ 0.36	3.35 $\pm$ 0.41	3.14 $\pm$ 0.35	3.19 $\pm$ 0.22	3.84 $\pm$ 0.40	2.82 $\pm$ 0.32
SL	4.83 $\pm$ 0.48	5.01 $\pm$ 0.49	4.53 $\pm$ 0.46	4.56 $\pm$ 0.27	5.39 $\pm$ 0.34	4.59 $\pm$ 1.26
ED	3.57 $\pm$ 0.35	3.47 $\pm$ 0.28	3.28 $\pm$ 0.33	3.36 $\pm$ 0.25	3.89 $\pm$ 0.36	3.16 $\pm$ 0.21
TD	2.20 $\pm$ 0.30	2.21 $\pm$ 0.29	2.08 $\pm$ 0.35	2.12 $\pm$ 0.24	2.34 $\pm$ 0.29	2.04 $\pm$ 0.26
THL	17.73 $\pm$ 1.50	18.11 $\pm$ 1.18	17.17 $\pm$ 2.12	16.45 $\pm$ 2.40	19.63 $\pm$ 0.93	16.75 $\pm$ 0.83
TIL	16.81 $\pm$ 1.56	17.60 $\pm$ 1.17	16.07 $\pm$ 1.59	15.45 $\pm$ 0.86	18.48 $\pm$ 0.91	15.31 $\pm$ 1.15
TAL	9.68 $\pm$ 0.88	10.28 $\pm$ 0.90	9.36 $\pm$ 1.05	9.06 $\pm$ 0.66	11.09 $\pm$ 0.96	9.19 $\pm$ 0.78
FL	13.63 $\pm$ 1.52	13.69 $\pm$ 1.03	13.34 $\pm$ 1.66	12.88 $\pm$ 0.87	14.62 $\pm$ 1.09	12.93 $\pm$ 1.57
FAL	6.19 $\pm$ 0.66	6.50 $\pm$ 0.65	5.97 $\pm$ 0.68	5.96 $\pm$ 0.51	7.00 $\pm$ 0.59	5.78 $\pm$ 0.68

Table 3. Discriminant Function classification for 447 specimens of *B. punctata* complex for the groups previously designed by bGYMC species delimitation. Bold values represent the total of correctly assignment specimens and the proportion of accuracy of the predictive variables.

	Delimited groups from bGYMC analysis					
Prediction	Clade I	Clade II	Clade III	Clade IV	Clade V	Clade VI
Clade I	81	20	42	13	11	1
Clade II	12	15	4	0	0	0
Clade III	44	10	105	18	5	7
Clade IV	9	0	4	20	0	0
Clade V	4	3	5	0	10	0
Clade VI	3	0	0	0	0	1
Total N	153	48	160	51	26	9
Proportion	52.9%	31.2%	65.6%	39.2%	38.5%	11.1%

Table 4. Morphometric measurements comparisons between the three nominal species of the *B. punctata* complex. Values presented are the characters variation (mean $\pm$ SD) and in parenthesis the minimum and maximum values.

	<i>Boana punctata</i> (stricto sensu) (n = 147)	<i>Boana rubrolineata</i> (n = 160)	<i>Boana atlantica</i> (n = 26)
SVL	33.3 $\pm$ 3.4 (24.7-40.6)	32.5 $\pm$ 3.3 (25.9-44.2)	36.4 $\pm$ 1.9 (31.7-39.3)
HL	10.7 $\pm$ 1.1 (7.8-13.4)	10.8 $\pm$ 7.8 (7.6-10.8)	11.4 $\pm$ 0.7 (9.8-12.7)
HW	11.9 $\pm$ 1.1 (8.3-14.1)	11.5 $\pm$ 1.2 (8.7-14.7)	13.1 $\pm$ 0.9 (11.6-15)
END	3.3 $\pm$ 0.4 (2.3-4.4)	3.1 $\pm$ 0.4 (2.2-4.2)	3.8 $\pm$ 0.4 (3.1-4.8)
SL	4.8 $\pm$ 0.5 (3.2-6.3)	4.5 $\pm$ 0.5 (2.2-5.6)	5.4 $\pm$ 0.3 (4.8-6)
IOD	3.5 $\pm$ 0.4 (2.6-5.0)	3.6 $\pm$ 0.4 (2.6-4.6)	4.3 $\pm$ 0.4 (3.5-5)
ED	3.6 $\pm$ 0.4 (2.7-4.5)	3.3 $\pm$ 0.3 (2.4-4)	3.9 $\pm$ 0.4 (3.3-4.6)
TD	2.2 $\pm$ 0.3 (1.4-2.9)	2.0 $\pm$ 0.4 (1.1-3.1)	2.3 $\pm$ 0.3 (1.8-2.9)
THL	17.7 $\pm$ 1.6 (13.6-21.6)	17.0 $\pm$ 2.2 (11.4-23.8)	19.6 $\pm$ 0.9 (17.9-20.9)
TIL	16.7 $\pm$ 1.7 (9.4-21.1)	16.0 $\pm$ 1.7 (13-21)	18.5 $\pm$ 0.9 (9.9-13.4)
TAL	9.6 $\pm$ 1.0 (6.7-11.5)	9.3 $\pm$ 1.2 (6.6-12.1)	11.1 $\pm$ 1.0 (9.9-13.4)
FL	13.6 $\pm$ 1.7 (10-17.8)	13.2 $\pm$ 1.7 (8.2-18.2)	14.6 $\pm$ 1.1 (12.8-16.9)
FAL	6.2 $\pm$ 0.7 (4.3-8)	5.9 $\pm$ 0.7 (4.2-8)	7.0 $\pm$ 0.6 (5.3-8.4)
HAL	10.3 $\pm$ 1.1 (7.3-12.9)	10.0 $\pm$ 1.2 (7.5-13.7)	11.3 $\pm$ 0.8 (9.8-12.8)

LIST OF FIGURES

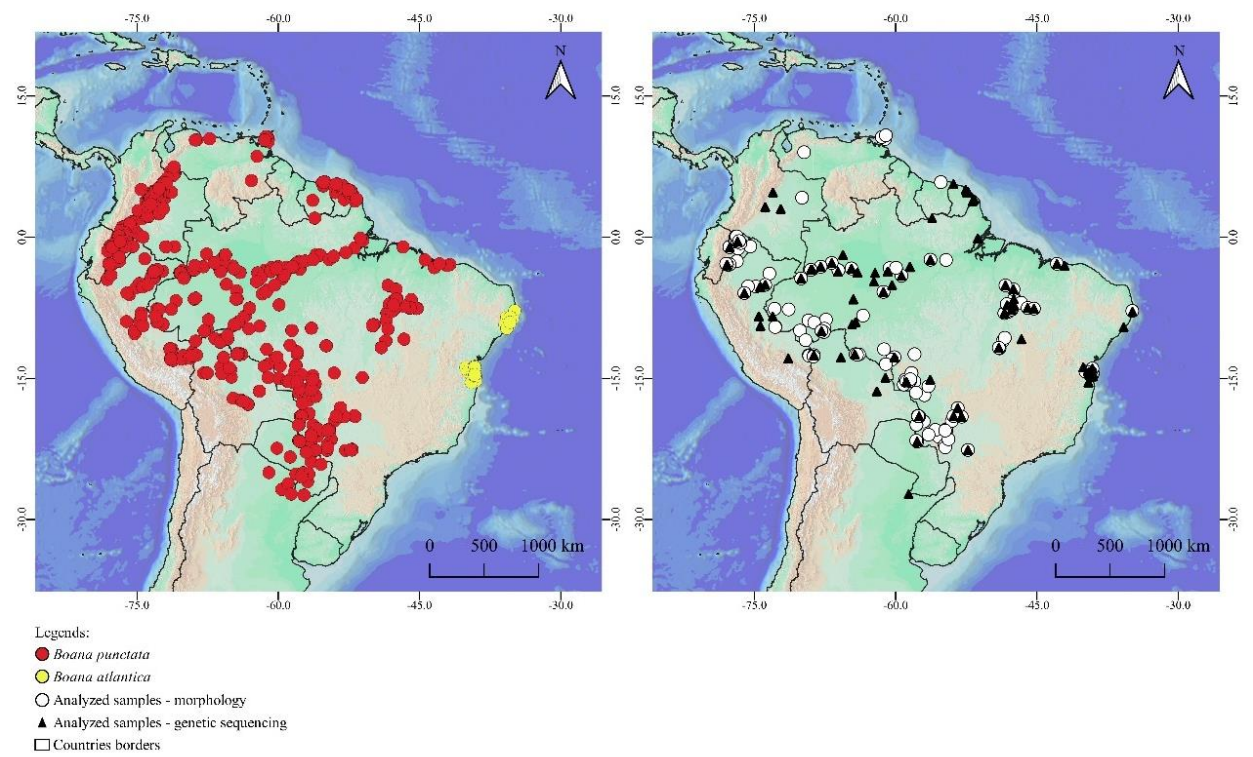


Figure 1. Known geographic distribution of the nominal *Boana punctata* (red circles) and *Boana atlantica* (yellow circles) (left) and sampling effort of molecular (black triangle) and morphologic data (white circles) (right).

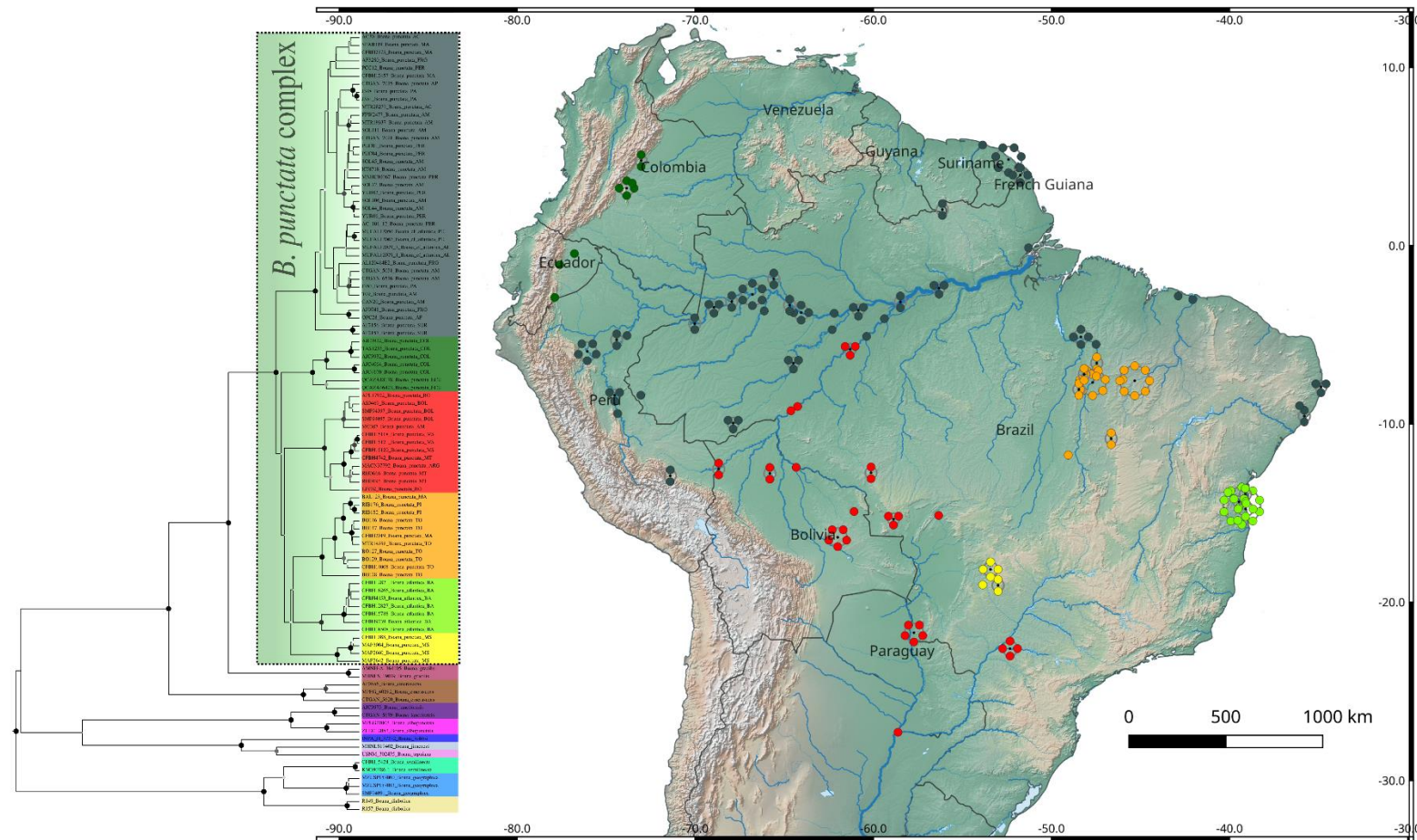


Figure 2. The 16S ultrametric tree inferred by Bayesian analysis in BEAST and bGYMC species delimitation result in vertical bars. Black dots in the nodes represent posterior probability >0.95 , clear nodes represent low supported relationships. The *B. punctata* complex are highlighted and comprises the well supported clades from the top to the bottom as the following scheme: Clade I (gray bars) *B. punctata* sensu stricto; Clade II (dark green bars); Clade III (red bars) *B. rubrolineata* (stat. nov.); Clade IV (orange bars); Clade V (lime-green bars) *B. atlantica*; Clade VI (yellow bars). Remaining groups correspond to the congener's species of *B. punctata* group, *B. albopunctata* group, *B. semilineata* group and *B. benitezi* group respectively, used as outgroup in our analysis

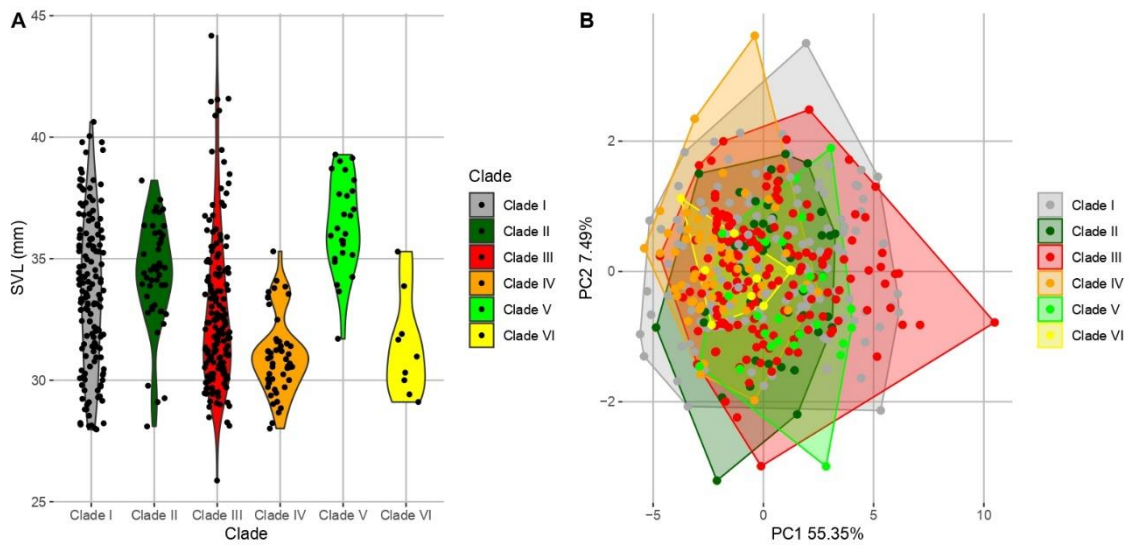


Figure 3. Morphological analysis of *B. punctata* complex. A) Violin Plot of body size (SVL) differences between the clades delimited in species delimitation. B) Principal components analysis (PCA) plot of the residuals of morphometric measures (sized-free) from the clades de bGYMC analysis.

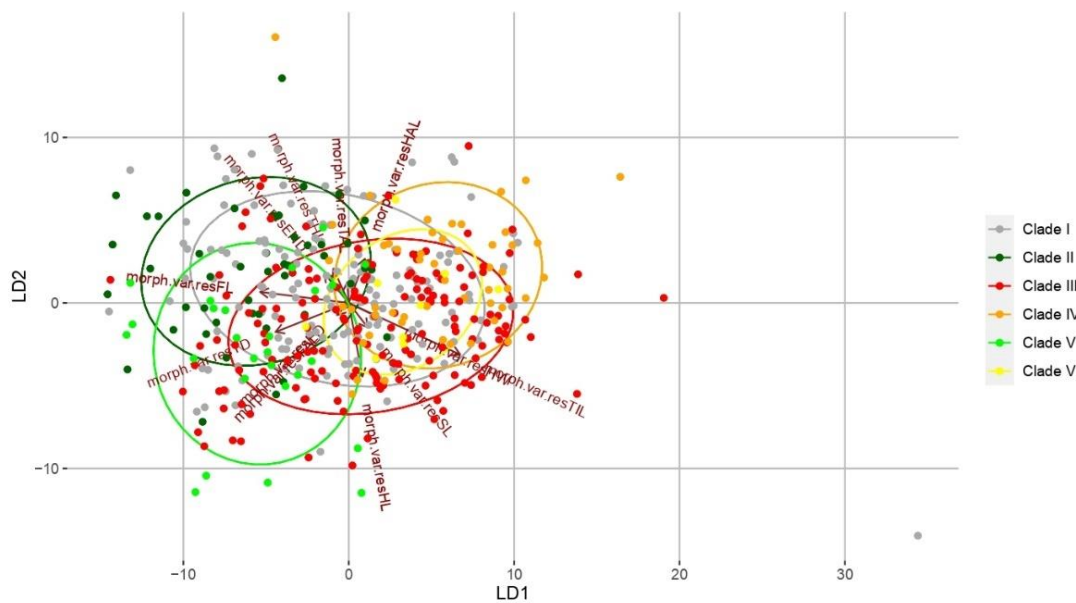


Figure 4. Discriminant Function Analysis (DFA) plot showing the morphometric variable (sized-free) weight in the separation of the groups delimited in the bGYMC analysis.

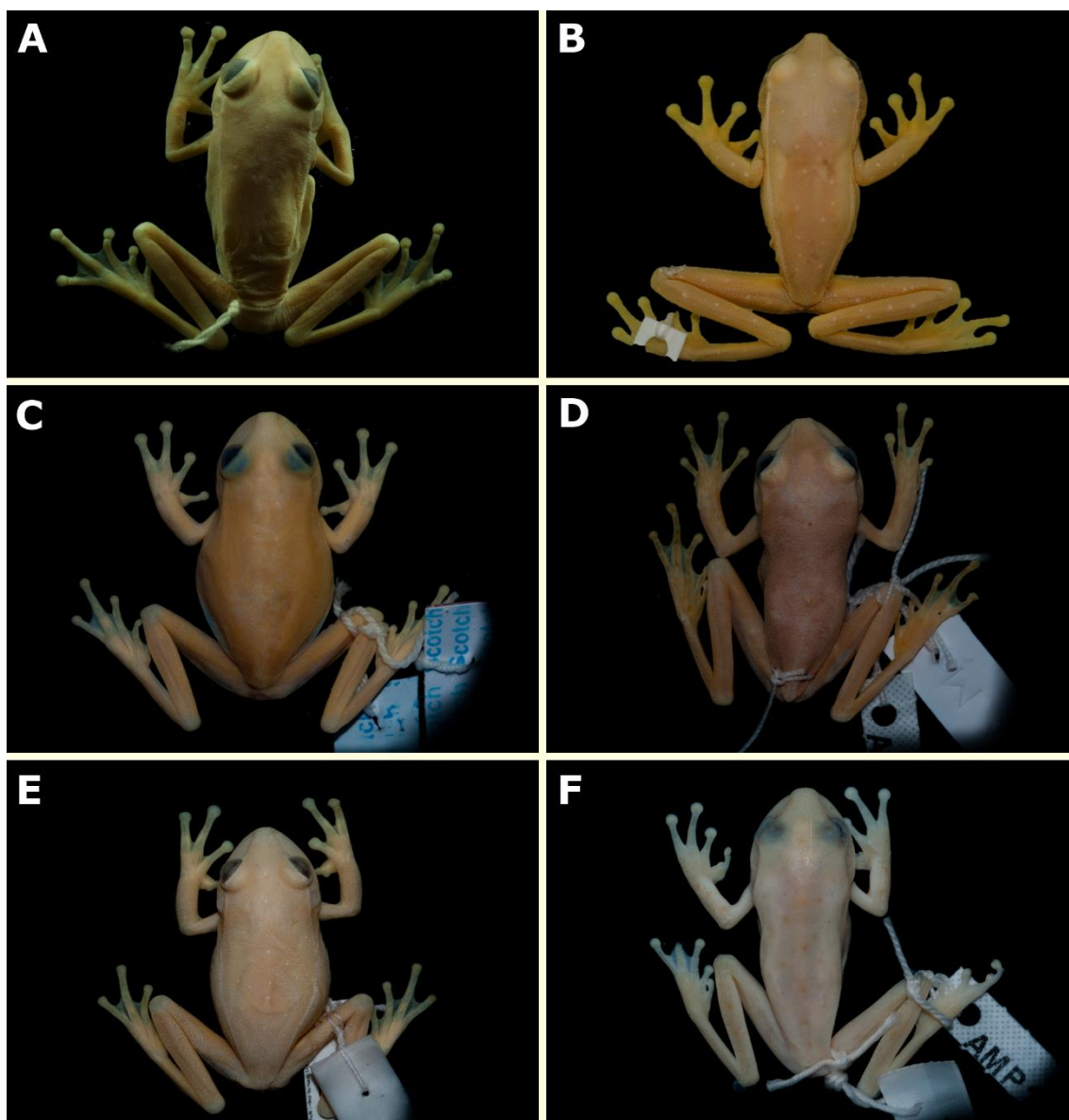


Figure 5. Overall view of the conservative external morphology of preserved specimens *B. punctata* complex in dorsal view. A) Clade I: MTR 36266-São Pedro, Amazonas, Brazil; B) Clade II: QCAZ-A 8029-Limoncocha, Orellana, Ecuador; C) Clade III, UFMT-A 1084-Campo Grande, Mato Grosso do Sul, Brazil D) Clade IV: ZUFMS-AMP 07949, Araguaína, Tocantins, Brazil, E) Clade V: CFBH 32060 – Ilhês, Bahia, Brazil, F) Clade VI: ZUFMS-AMP 11515, Parque Estadual Nascentes do Rio Taquari, Mato Grosso do Sul, Brazil.

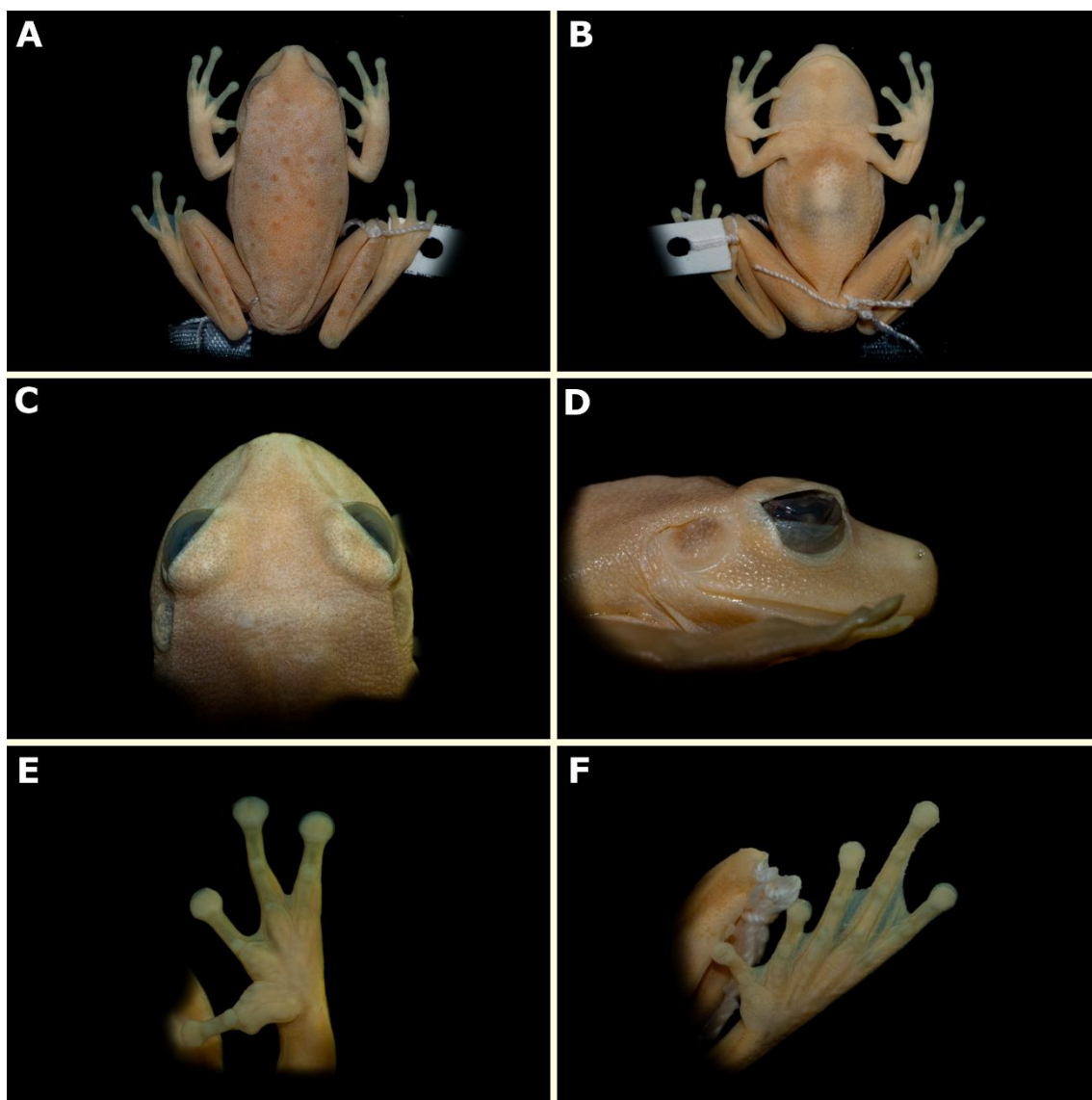


Figure 9. Morphological details of *Boana rubrolineata* (**stat nov.**). Dorsal (A) and Ventral (B) views of adult male (CFBH 41004, SVL = 33.66 mm). Semicircular (C) and truncate shapes (D) of the head in dorsal and lateral view respectively (UFMT-A 10128, HW = 12.36 mm, HL = 11.19). Ventral surfaces of hand (E) and foot (F).

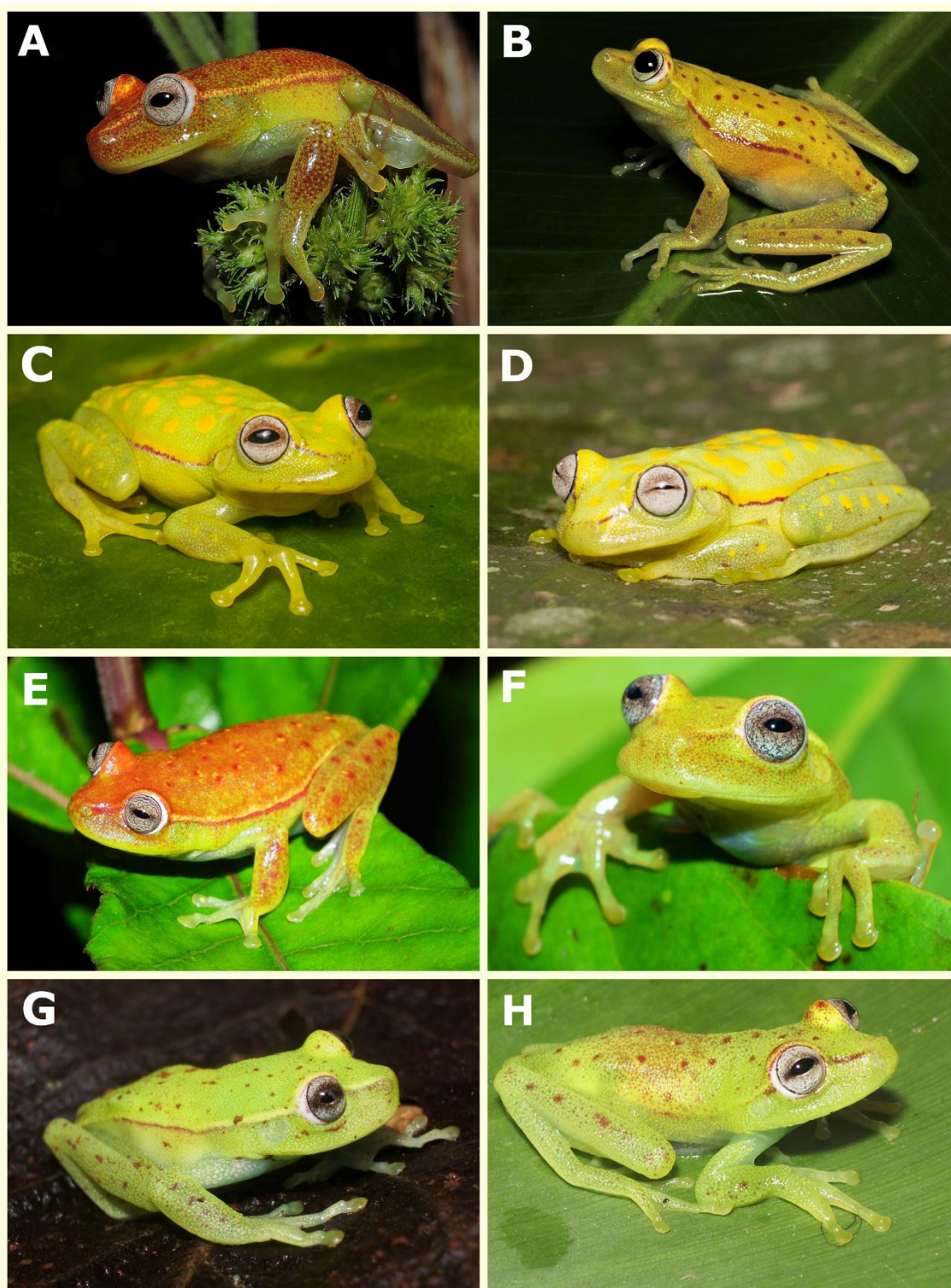


Figure 7. Living specimens of *B. rubrolineata* and morphological variations in dorsal pattern of coloration through its geographic distribution. (A) Campo Grande – MS; (B) PENRT Taquari – MS; (C - D) Madre de Dios, Peru; (E) Rondônia; (F) Centenário do Sul – PR; (G) Jauru – MT; (H) Corumbá – MS. Photos: (A-B): Matheus Mororti; (C-D): Kevin K. Caldwell; (E): Pedro F. Dias; (F): Guilherme Figueiredo; (G-H) Robson W. Ávila.

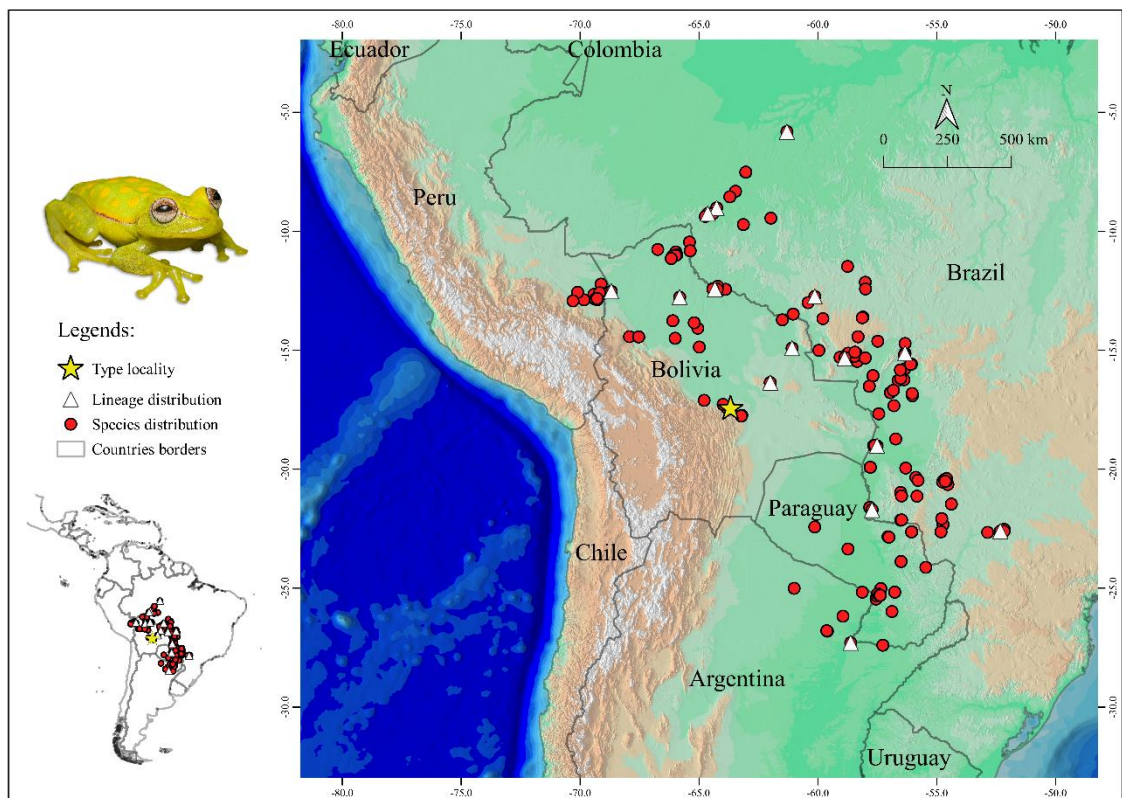


Figure 8. Geographic distribution of *B. rubrolineata* from south-west of Amazon, Madre de Dios – Madeira Rivers basin, lowlands of Bolivia, Pantanal and wetlands of Paraguay, Argentina, in the Paraguay and Paraná basin. Red circles indicate distribution data from GBIF repository (as *B. punctata*) and white triangles indicates genetic samples used in our phylogenetic analysis. The yellow star indicates the type locality of *Hyla punctata rubrolineata* Lutz, (1951)

Appendix I

Specimens examined

Boana atlantica (N=26): **BRAZIL: Bahia:** Aurelino Leal, CFBH 18724 – 18726; Camamu, CFBH 27814; Ilhéus, CFBH 32060, 34086, 35716, 39425, 42513 – 42517; Itagiba, CFBH 27690; Serra do Condurú, MTR 35125; Uruçuca, CFBH 23379, 32365, 32366, 13262, 32462, 34050, 13256, 13259, 13260, 13258, 37964.

Boana cf. atlantica (N=2): **BRAZIL: Pernambuco:** Igarussú: CFBH 2514, 2483.

Boana punctata: (N=246): **VENEZUELA: Guanare-Portuguesa,** UF 108635. **COLOMBIA: Orinoquia:** Vichada, UF 8517, 8519, 8518-1 – 8518-6. **TRINIDAD & TOBAGO: Arouca,** Tunapuna-Piarco Road: UF 3637; **Sangre-Grande:** Biche, UF 10988; Grande Riviere, UF 7300-1 – 7300-8; **San Juan:** Laventille, UF 7727-1, 7727-2. **ECUADOR: Pastaza:** Bataburo Lodge, QCAZ-A 39441; Kapawi Lodge, QCAZ-A 9021. 25487; **Orellana:** QCAZ-A 62207; El Desacanso, QCAZ-A 43790, 43792; Huino, QCAZ-A 30939; Pozo Coca, QCAZ-A 52374; Puerto Francisco de Orellana, QCAZ-A 489; Yasuni, QCAZ-A 22753, 51622, 54676, 55358; **Sucumbios:** Lago Agrio, QCAZ-A 6470; Limoncocha, QCAZ-A 8029, QCAZ-43105, 43117 – 43119, 43122, 61770, 63544; Santa Cecilia, QCAZ-A 177; **Napo:** Misahualli, QCAZ-A 406; Reserva Jatun Sacha, QCAZ-A 2874, 18178; San Pedro, QCAZ-A 57222; **Morona Santiago:** QCAZ-A 54450, 54452 – 54454; Namipias, QCAZ-A 46401 – 46402; Pankints, QCAZ-A 46424 – 46425. **PERU: Loreto:** Iquitos, MUBI 8821, 8842 – 8849; Requena, MUBI 9029 – 9030, 9098 – 9099, 9101, 9103 – 9105, 9107 – 9110, 9113 – 9114, 9116; Lagunas, MUBI 12715; Pacaya-Samiria, MUBI 10066 – 10067; Yurimaguas, YUR 01 – 02, YUR 05, YUR 11 – 12; **Ucayalli:** Yurua, MUBI 15179, 15181 – 15183. **SURINAME: Paramaribo:** UF 108636, 108640 – 108642, 108644 – 108646, 108648, 108649, 10655. **BRAZIL: Amazonas:** Benjamin Constant, RSOL 44, 45; Estação Ecológica (ESEC) Baixo Juruá, INPA-H 28533, 28534, 28536 – 28540; Iranduba – Lago do Catalão, CZPB-AA 505, 620, 654,969 – 971, INPA-H 1823 – 1827; Jutai, RSOL 99 – 106; Manacapuru, MANAC 381, 411, 413; Puruzinho, CTGA-N 6516; RESX Rio Gregório, INPA-H 30197; Santo Antônio do Içá, RSOL 79, 82, 83, 92, 97; São Pedro – Rio Içá, MTR 36266, 35750, 35799, 36706; Tefé, RSOL 100, 111; Terra Vermelha – Rio Purus, MTR 19338. **Acre:** Assis Brasil, UFAC 4066; Boca do Acre, UFAC 5296, 5297; Fazenda Catuaba, UFAC 537; Cruzeiro do Sul – Rio Moa/Igarapé Pentecoste, UFAC 6196, 6197; Manuel Urbano, UFAC 3343 – 3345; PAREST Chandless, UFAC 5429; Rio Branco, UFAC 138, 142, 144, AC 27; Sena Madureira, UFAC 286, 287, 292, 293, 452, 4778, 5530; Parque Zoobotânico-UFAC, UFAC 27, 30, 32, 42, 43, 207, 221, 228, 234, 3039. **Maranhão:** Balsas, BAL 123, CFBH 14014, UFMT-A 11220; Barreirinha, CFBH 26616; Estreito, CFBH 9160; Feira Nova do Maranhão, MAP 2652; Imperatriz, CFBH 8121; Porto Franco, CFBH 25935; Riachão, MAP 2650, 2651; São Pedro de Água Branca, SPAB 114, 117 – 119; **Tocantins:** Araguaína, CFBH 11425, 11435; MAP 2666, 5347; ZUFMS-AMP 7949, 7950; Babaçulândia – Wanderlândia road, CFBH 8168, 28515; Barra do Ouro, BO 125 – 130, 146 – 148; Brejinho de Nazaré, CFBH 28918; Colinas do Tocantins, CFBH 19886, 19887, 23494; Gurupi, CFBH 23509, 28926; Palmeirante, CFBH 28441, 28439; Porto Nacional, CFBH 33368 – 33370. **Pará:** Juruti, J346 – J351; Santarém, SANTAREM09, SANTAREM11. **Piauí:** URCA 2225, 2325, 2326, 2328, RIB, 161, 162, 175 – 182.

Boana rubrolineata (stat. nov.) (N= 169): **PERU: Madre de Dios,** Puerto Maldonado, MUBI 8153; Puerto Maldonado – El Prado, MUBI 8059, 8060; Puerto Maldonado – La cachuela, MUBI 8073; Pampas del Health, MUBI 11085, 11086, 128752, 12876. **BRAZIL: Amazonas:** Manicoré, MG 270 – 274, 306 – 308. **Mato Grosso:** Araputanga, UFMT-A 5891, 5898, 5941, 5994, 6001, 6660, 11734; Brasnorte, UFMT-A 10126 – 10131; Cáceres, UFMT-A 1083, 1084, 1092; Jauru, UFMT-A 1214, 5944, 10836 – 10840, 10842 – 10846, 10849; Lambari D'Oeste, CFBH 21786; Nossa Senhora do Livramento, UFMT-A 4350, 5777, 5778; Poconé, UFMT-A

53, 279, 436, 507; Pontes Lacerda, UFMT-A 15304, 15452, 16258; Reserva do Cabaçal, UFMT-A 2221, 2245, 2249, 2255, 2258; Tangará da Serra, UFMT-A 2240, 2252, 2271, 2289, 2296; Vale de São Domingos, UFMT-A 1915, 14717. **Mato Grosso do Sul:** Aquidauana, ZUFMS-AMP 1419, 1843, 1843(1); Bonito, ZUFMS-AMP 3521, 3522; Camapuã, MAP 203, ZUFMS-AMP 11172, 11718, 11719; Campo Grande, MAP 2613, 2614, ZUFMS-AMP 1076, 1079, 1417, 1619, 3309, 3326, 3530, 3532, 3535, 7951, 7955, 9697, 9699, 9700, 10177, 10178, 10180 – 10186, 10872; Corumbá, CFBH 4360, 4361, 40991 – 40993, 41003, 41004, 41008, 41010 – 41013, ZUFMS-AMP 1332, 1739, 11667, 11669, 11671, 11673; Costa Rica, ZUFMS-AMP 10854; Dourados, ZUFMS-AMP 10991; Itaporã, ZUFMS-AMP 478, 2333; Ladario, ZUFMS-AMP 1882, Nova Alvorada do Sul, ZUFMS-AMP 045, 467, 2302; Paraíso das Águas, ZUFMS-AMP 10855; PENRT-Taquari, ZUFMS-AMP 11511 – 11515; Porto Murtinho, ZUFMS-AMP 11891 – 11898; Sidrolândia-Niaoque, CFBH 14276. **Rondônia:** Costa Marques, INPA-H 6479, 6480, 6531, 6546; ESEC-Cuniã, Porto Velho, INPA-H 6667, 6669, 6696, 6697, 6699; Pimenta Bueno, UFMT-A 19314; Pimenteiras do Oeste, INPA-H 6744, 6746, 6747; Vilhena, INPA-H, 7167, UFMT-A 13737, 13780. **São Paulo:** Teodoro Sampaio - Parque Morro do Diabo, CFBH 12014, 12016 – 12020.

Supplemental Material

Table S1. List of species included in our phylogenetic and species discovery analysis, along with their identification of Voucher or Field Number, GenBank accession, locality information and sequence references. Newly collected samples and generated sequences are highlighted in bold.

Species	Voucher/Field Number	GenBank Accession number	Locality	References
<i>Boana albopunctata</i>	MPEG21003	MN781650	Querência/MT-Brazil	Stutato et al. (2020)
<i>Boana albopunctata</i>	ZUEC12053	AY549317	Campinas/ SP-Brazil	Faivovich et al. (2005)
<i>Boana atlantica</i>	CFBH12821	MK348482	Camamu/BA-Brazil	Lima et al. (2019)
<i>Boana atlantica</i>	CFBH16266	MK348489	Uruçuca/BA-Brazil	Lima et al. (2019)
<i>Boana atlantica</i>	CFBH4153	MK348497	Uruçuca/BA-Brazil	Lima et al. (2019)
<i>Boana atlantica</i>	CFBH12827	To be submitted	Camumu/BA-Brazil	This study
<i>Boana atlantica</i>	CFBH15788	To be submitted	Ilheus/BA- Brazil	This study
<i>Boana atlantica</i>	CFBH9239	MK348498	Aurelino Leal/BA-Brazil	Lima et al. (2019)
<i>Boana atlantica</i>	CFBH18508	MK348493	Ilheus/BA- Brazil	Lima et al. (2019)
<i>Boana cf. atlantica</i>	MUFAL13050	MK348505	Paulista/ PE- Brazil	Lima et al. (2019)
<i>Boana cf. atlantica</i>	MUFAL13067	MK348506	Paulista/ PE- Brazil	Lima et al. (2019)
<i>Boana cf. atlantica</i>	MUFAL12079_3	MK348503	Maceió/ AL- Brazil	Lima et al. (2019)
<i>Boana cf. atlantica</i>	MUFAL12079_1	MK348502	Maceió/ AL- Brazil	Lima et al. (2019)
<i>Boana cinerascens</i>	AF0556	KR811175	Savane Virginie-French Guiana	Fouquet et al. (2015)
<i>Boana cinerascens</i>	MPEG_40282	MN781653	Tefé/AM- Brazil	Stutato et al. (2020)
<i>Boana cinerascens</i>	CTGAN_5620	MG806013	Manaus/AM-Brasil	Motta et al. (2018)
<i>Boana diabolica</i>	R149	KU168871	Oiapoque/AP-Brazil	Fouquet et al. (2016)
<i>Boana diabolica</i>	R157	KU168874	Nouragues- French Guiana	Fouquet et al. (2016)
<i>Boana geographica</i>	MZUSP157060	KU168903	Tefé/AM-Brazil	Fouquet et al. (2016)
<i>Boana</i>	MZUSP157083	KU168897	Lago Chaviana,	Fouquet et

<i>geographica</i>			Rio Purus	al. (2016)
<i>Boana geographica</i>	SMF94091	KF723059	Concepcion/Bolivia	Fouquet et al. (2016)
<i>Boana gracilis</i>	AMNH_A_164105	AY549336	Iwokrama, Muri Scrub camp- Guyana	Faivovich et al. (2004)
<i>Boana gracilis</i>	MNHLS_19039	MN781660	San José de Chipiro- Venezuela (alto Orinoco)	Sturaro et al. (2020)
<i>Boana hobbsi</i>	INPA_H_37272	MW423781	Amazonas, rio Japurá, Japurá, near Villa Bittencourt	Almeida et al. (2021)
<i>Boana jimenezi</i>	MHNLS19402	MN688742	Bolívar, Gran Sabana- Venezuela	Sturaro et al. (2020)
<i>Boana lanciformis</i>	AJC3973	KP149465	Casañare, Sabanalarga- Colombia	Guarnizo et al. (2015)
<i>Boana lanciformis</i>	CTGAN_5679	MG806025	Manaus/AM- Brazil	Motta et al. (2018)
<i>Boana punctata</i>	AC30	To be submitted	Rio Branco/AC- Brazil	This study
<i>Boana punctata</i>	SPAB119	To be submitted	São Pedro de Água Branca / MA- Brazil	This study
<i>Boana punctata</i>	CFBH2175	To be submitted	Imperatriz/MA- Brazil	This study
<i>Boana punctata</i>	AF3280	To be submitted	Taparabo/AP- Brasil	This study
<i>Boana punctata</i>	PCC12	To be submitted	Pilcopata/Peru- Brazil	This study
<i>Boana punctata</i>	CFBH12457	To be submitted	Barreirinha/MA- Brasil	This study
<i>Boana punctata</i>	CTGAN_7015	To be submitted	Furo do Mazagão, Mazagão/AP- Brazil	This study
<i>Boana punctata</i>	J348	To be submitted	Juruti/PA- Brazil	This study
<i>Boana punctata</i>	J351	To be submitted	Juruti/PA- Brazil	This study
<i>Boana punctata</i>	MTR28279	To be submitted	Serra do Divisor, AC - Brazil	This study
<i>Boana punctata</i>	FPW2477	To be submitted	RESEX Baixo Juruá/ AM- Brazil	This study
<i>Boana punctata</i>	MTR19337	To be submitted	Terra Vermelha,Rio Purus/ AM- Brazil	This study
<i>Boana punctata</i>	SOL111	To be submitted	Jutaí/ AM- Brasil	This study
<i>Boana</i>	CTGAN_7021	To be	Itacoatiara/AM-	This study

<i>punctata</i>		submitted	Brazil	
<i>Boana punctata</i>	PUC01	To be submitted	Pucallpa-Peru	This study
<i>Boana punctata</i>	PUC04	To be submitted	Pucallpa-Peru	This study
<i>Boana punctata</i>	SOL45	To be submitted	Benjamin Constant/AM-Brazil	This study
<i>Boana punctata</i>	HT8718	To be submitted	Paraná dos Mundurucus/AM-Brazil	This study
<i>Boana punctata</i>	MNHC10067	To be submitted	Pacaya Samiria_Loreto-Peru	This study
<i>Boana punctata</i>	SOL72	To be submitted	São Paulo de Olivença/ AM-Brazil	This study
<i>Boana punctata</i>	YUR02	To be submitted	Yurimaguas-Peru	This study
<i>Boana punctata</i>	SOL106	To be submitted	Jutaí/AM- Brazil	This study
<i>Boana punctata</i>	SOL44	To be submitted	Benjamin Constant/AM-Brazil	This study
<i>Boana punctata</i>	YUR06	To be submitted	Yurimaguas-Peru	This study
<i>Boana punctata</i>	AC_101_12	MN172521	Pilcopata, Cusco-Peru	von May et al. (2019)
<i>Boana punctata</i>	AL120414E2	KDQF01001677	Roura-French Guiana	Vacher et al. 2020
<i>Boana punctata</i>	CTGAN_5031	To be submitted	Novo Aripuanã/AM-Brazil	This study
<i>Boana punctata</i>	CTGAN_6516	To be submitted	Comunidade Puruzinho/AM-Brazil	This study
<i>Boana punctata</i>	J350	To be submitted	Juruti/PA- Brazil	This study
<i>Boana punctata</i>	T09	To be submitted	Tefe/AM- Brazil	This study
<i>Boana punctata</i>	CAN20	To be submitted	Canutama/AM-Brazil	This study
<i>Boana punctata</i>	AF0748	KDQF01000234	St Georges, savane- French Guiana	Vacher et al. 2020
<i>Boana punctata</i>	OPC28	KDQF01003802	Oiapoque/ AP-Brazil	Vacher et al. 2020
<i>Boana</i>	AF2156	KDQF01000866	Sipaliwini-	Vacher et

<i>punctata</i>			Suriname	al. 2020
<i>Boana punctata</i>	AF2157	KDQF01000867	Sipaliwini-Suriname	Vacher et al. 2020
<i>Boana punctata</i>	AJC3922	KP149390	Caserio Miraflores-Colombia	Guarnizo et al. (2015)
<i>Boana punctata</i>	TAS1233	To be submitted	Meta, San Martín, Palmasol-Colômbia	This study
<i>Boana punctata</i>	AJC3932	KP149476	Caserio Miraflores-Colombia	Guarnizo et al. (2015)
<i>Boana punctata</i>	AJC4064	KP149397	Casañare, Sabanalarga-Colômbia	Guarnizo et al. (2015)
<i>Boana punctata</i>	AJC4100	KP149307	Casanare, Sabanalarga-Colômbia	Guarnizo et al. (2015)
<i>Boana punctata</i>	QCAZA18178	MN921759	Estacion Biologica Jatun Sacha/Napo-Ecuador	Caminer & Ron (2020)
<i>Boana punctata</i>	QCAZA46423	MN921809	Morona Santiago-Pankints- Ecuador	Caminer & Ron (2020)
<i>Boana punctata</i>	APL17922	MK348508	Rondônia-Brazil	Lima et al. (2019)
<i>Boana punctata</i>	AS0469	JF790126	Beni, Yucuma Los Lagos- Bolívia	Jansen et al. (2011)
<i>Boana punctata</i>	SMF94397	KF723063	Yucuma, Los Lagos- Bolivia	Jansen et al. (2011)
<i>Boana punctata</i>	SMF94095	KF723062	San Sebastian-Bolivia	Jansen et al. (2011)
<i>Boana punctata</i>	MACN37792	AY549353	Chaco, Resistencia-Argentina	Faivovich et al. (2013)
<i>Boana punctata</i>	BAL123	To be submitted	Balsas / MA-Brazil	This study
<i>Boana punctata</i>	RIB176	To be submitted	Ribeiro Gonçalves / PI- Brazil	This study
<i>Boana punctata</i>	RIB182	To be submitted	Ribeiro Gonçalves / PI- Brazil	This study
<i>Boana punctata</i>	BO146	To be submitted	Barra do Ouro/ TO-Brazil	This study
<i>Boana punctata</i>	BO147	To be submitted	Barra do Ouro/ TO-Brazil	This study
<i>Boana punctata</i>	CFBH2019	To be submitted	Estreito/MA-Brazil	This study
<i>Boana punctata</i>	MTR14588	To be submitted	EESGT (Estação Ecológica Serra Geral)/ TO- Brazil	This study
<i>Boana</i>	BO127	To be	Barra do Ouro/ TO-	This study

<i>punctata</i>		submitted	Brazil	
<i>Boana punctata</i>	BO129	To be submitted	Barra do Ouro/ TO-Brazil	This study
<i>Boana punctata</i>	CFBH14008	To be submitted	Colinas do Tocantins/TO	This study
<i>Boana punctata</i>	BO128	To be submitted	Barra do Ouro/ TO-Brazil	This study
<i>Boana punctata</i>	CFBH1088	To be submitted	Paraíso/MS- Brazil	This study
<i>Boana punctata</i>	MAP3004	To be submitted	Alcinópolis/MS- Brazil	This study
<i>Boana punctata</i>	MAP2660	To be submitted	Camapuã/MS- Brazil	This study
<i>Boana punctata</i>	MAP2642	To be submitted	Alcinópolis/MS- Brazil	This study
<i>Boana rubrolineata</i>	MG307	To be submitted	Manicoré/AM- Brazil	This study
<i>Boana rubrolineata</i>	CFBH15118	To be submitted	Porto Murtinho/MS- Brazil	This study
<i>Boana rubrolineata</i>	CFBH15121	To be submitted	Porto Murtinho/MS- Brazil	This study
<i>Boana rubrolineata</i>	CFBH4742	To be submitted	Acorizal/MT-Brazil	This study
<i>Boana rubrolineata</i>	RHO666	To be submitted	Jauru/MT-Brazil	This study
<i>Boana rubrolineata</i>	RHO193	To be submitted	Jauru/MT-Brazil	This study
<i>Boana rubrolineata</i>	LJV02	To be submitted	Vilhena/RO-Brazil	This study
<i>Boana semilineata</i>	CFBH_5424	AY843779	Rio de Janeiro, Duque de Caxias- Brazil	Faivovich et al. (2005)
<i>Boana semilineata</i>	KM390786	KM390786	Rio de Janeiro- Brazil	Chaves et al unpublished
<i>Boana tepuiana</i>	USNM302435	AY843606	Venezuela: Brazil, Roraima, Villa Pacaraima, border marker BV8 (Brazil)	Faivovich et al. (2005)

Table S2. Summary statistics of Tukey's test performed to body size variable (Snout-vent length – SVL) between the groups delimited in the bGMYC analysis. Asterisk indicates the significant ($p < 0.05$) differences in body-size between the assigned groups.

	diff	lwr	upr	p adj
Clade I-Clade II	0.7120588	-0.6116800	2.035797678	0.6387354
Clade I-Clade III	-0.8225662	-1.7273339	0.082201515	0.0988296
Clade I-Clade IV	-2.5807843	-3.8745478	-1.287020791	0.0000003*
Clade I-Clade V	2.7816742	1.0843502	4.478998192	0.0000532*
Clade I-Clade VI	-2.2007190	-4.9452058	0.543767926	0.1981562
Clade II-Clade III	-1.5346250	-2.8514306	-0.217819356	0.0118107
Clade II-Clade IV	-3.2928431	-4.9019412	-1.683745073	0.0000001*
Clade II-Clade V	2.0696154	0.1212106	4.018020177	0.0299368
Clade II-Clade VI	-2.9127778	-5.8192500	-0.006305558	0.0491442*
Clade III-Clade IV	-1.7582181	-3.0448869	-0.471549337	0.0014790*
Clade III-Clade V	3.6042404	1.9123180	5.296162740	0.0000000*
Clade III-Clade VI	-1.3781528	-4.1193023	1.362996760	0.7030805
Clade IV-Clade V	5.3624585	3.4342934	7.290623643	0.0000000*
Clade IV-Clade VI	0.3800654	-2.5128778	3.273008543	0.9990189
Clade V-Clade VI	-4.9823932	-8.0769375	-1.887848783	0.0000776*

Table S3. Summary statistics of Tukey's test performed to the residuals of morphometric variables PCA performed between the groups delimited in the bGMYC analysis. Asterisk indicates the significant ($p < 0.05$) differences in body-size between the assigned groups. PCA - Tukey test

	diff	lwr	upr	p adj
Clade II-Clade I	-0.05280786	-1.3138565	1.2082408	0.9999965
Clade III-Clade I	0.01082208	-0.8510973	0.8727414	1.0000000
Clade IV-Clade I	-2.18494793	-3.4174409	-0.9524550	0.0000085*
Clade V-Clade I	0.49736933	-1.1195721	2.1143107	0.9510718
Clade VI-Clade I	-1.18642753	-3.8009399	1.4280848	0.7856459
Clade III-Clade II	0.06362993	-1.1908139	1.3180738	0.9999910
Clade IV-Clade II	-2.13214007	-3.6650338	-0.5992463	0.0011233*
Clade V-Clade II	0.55017718	-1.3059543	2.4063086	0.9581320
Clade VI-Clade II	-1.13361967	-3.9024460	1.6352066	0.8500765
Clade IV-Clade III	-2.19577000	-3.4215042	-0.9700358	0.0000065*
Clade V-Clade III	0.48654725	-1.1252484	2.0983429	0.9547822
Clade VI-Clade III	-1.19724960	-3.8085827	1.4140835	0.7781939
Clade V-Clade IV	2.68231725	0.8454670	4.5191675	0.0005015*
Clade VI-Clade IV	0.99852040	-1.7574176	3.7544584	0.9052229
Clade VI-Clade V	-1.68379685	-4.6317885	1.2641948	0.5758354

Table S4. Character loadings, and eigenvalues of variance explained for two principal components of PCA for morphometric variables.

	PC1	PC2
HL	0.2529972	-0.43543336
HW	0.2597056	-0.50323109
END	0.2917198	-0.12445191
SL	0.3192809	-0.16018515
ED	0.3242911	-0.04031619
TD	0.3018289	-0.03075332
THL	0.2842596	0.31114792
TIL	0.2772356	0.43812367
TAL	0.2769701	0.38337642
FL	0.2802424	-0.03721447
FAL	0.3012145	0.25349133
HAL	0.2856533	-0.10978117

Table S5. Loadings of variance explained by the discriminant axes of DFA performed for morphometric variables.

	LD1	LD2
HL	0.061966451427429	-0.482876289864274
HW	0.141533076853756	-0.0996984427073323
END	-0.111996802147437	0.193798348333043
SL	0.0858088530222968	-0.159709565418518
ED	-0.0265267225025234	-0.0357801911445681
TD	-0.334497433495478	-0.190738012212031
THL	-0.0699761157473926	0.225019089601552
TIL	0.50857521581434	-0.338307873569401
TAL	-0.00653534547488894	0.136745784649025
FL	-0.404864692354334	0.0720977330959317
FAL	-0.0536715688811007	-0.0799398364855758
HAL	0.0735299334317965	0.288752492266638

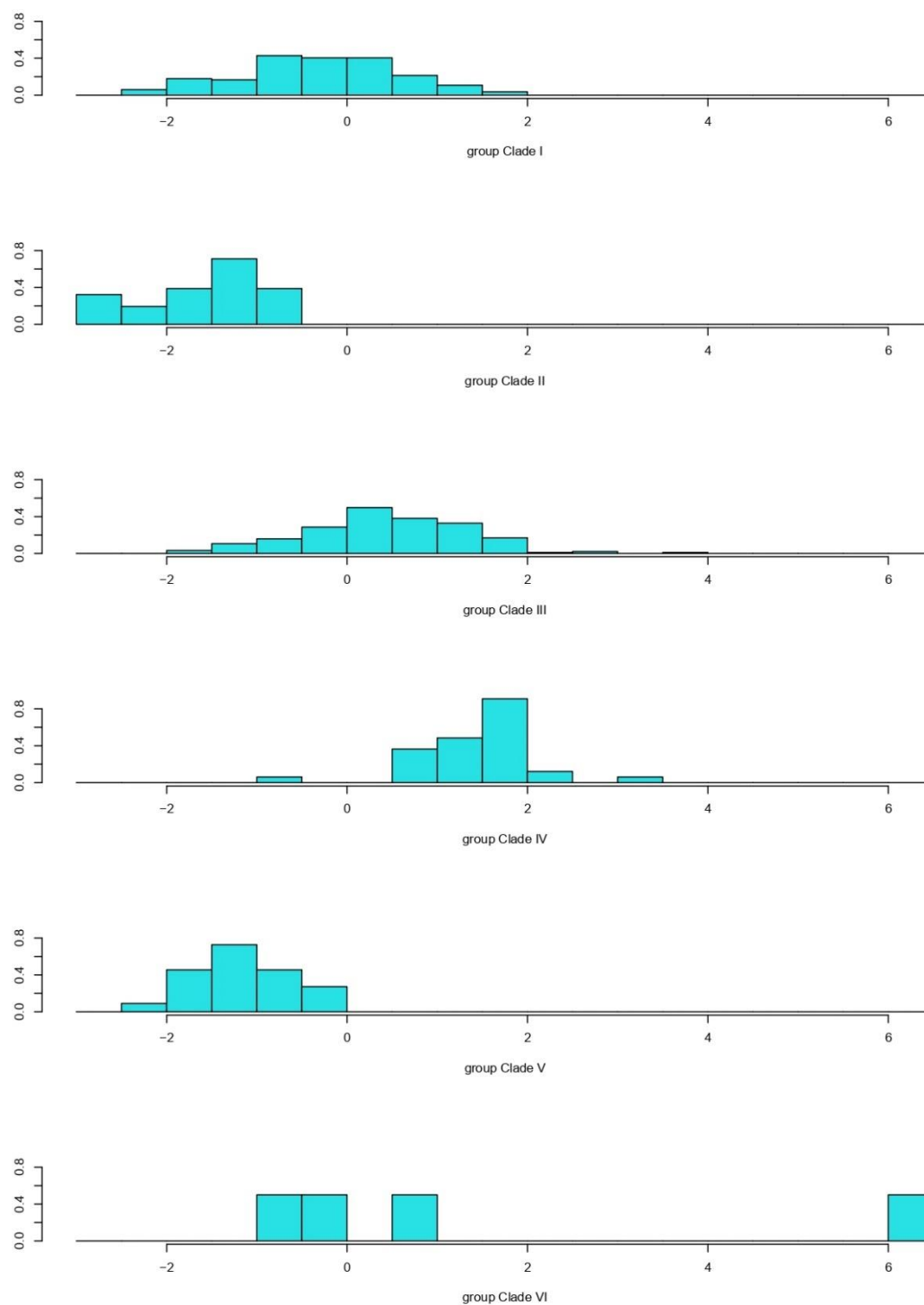


Figure S1. Linear discriminant histogram of DFA for morphometric variables of *B. punctata* complex specimens, among the groups delimited in bGMYC analysis.

*Boana punctata*

CAPÍTULO 3

Diversification and phylogeography of the continental *Boana punctata* species complex (Anura: Hylidae) Taxonomy and evolutionary history.

Alexandre P. Almeida; Erico Polo; Rommel R. Rojas; Igor J. Roberto; Sandra R. Hernandez; Vinicius T. de Carvalho; Antoine Fouquet; Miguel Trefaut Rodrigues; Juan Carlos Chaparro; Diego J. Santana; Teddy Angarita; Luis F. Marin da Fonte; Leandro Moraes; Robson W. Avila; Fernanda Werneck; Tomas Hrbek; Izeni Pires Farias; Marcelo Gordo. Manuscrito em fase de preparação para ser submetido no periódico Zoological Journal of The Linnean Society.

Diversification and phylogeography of the continental *Boana punctata* species complex (Anura: Hylidae): systematics and evolutionary history

^{1,2*}Alexandre P. Almeida; ² Erico Polo; ³ Rommel R. Rojas; ⁴ Igor J. Roberto; ² Sandra R. Hernandez; ^{2,5} Vinicius T. de Carvalho; ⁶ Antoine Fouquet; ⁷ Miguel Trefaut Rodrigues; ⁸ Juan Carlos Chaparro; ⁹ Diego J. Santana; ¹⁰ Teddy Angarita; ¹¹ Luis F. Marin da Fonte; ¹² Leandro Moraes; ¹³ Robson W. Avila; ¹⁴ Fernanda Werneck; ² Tomas Hrbek; ² Izeni Pires Farias; ^{1,15} Marcelo Gordo.

1. Programa de Pós-graduação em Zoologia, Instituto de Ciências Biológicas, Universidade Federal do Amazonas, Manaus, AM, Brazil.
2. Laboratório de Evolução e Genética Animal, Departamento de Genética, Universidade Federal do Amazonas, Manaus, AM, Brazil.
3. Universidad Nacional de la Amazonía Peruana, Iquitos, Loreto, Peru.
4. Laboratório de Biologia e Ecologia de Animais Silvestres, Universidade Federal do Cariri Brejo Santo, CE, Brazil.
5. Instituto de Biociências, Cidade Universitária, Universidade Federal do Mato Grosso do Sul, Campo Grande, MS, Brazil.
6. Laboratoire Evolution et Diversité Biologique, UMR 5174, CNRS, IRD, Université Paul Sabatier, Toulouse, France.
7. Universidade de São Paulo, Instituto de Biociências, Departamento de Zoologia, São Paulo, SP, Brazil.
8. Departament of Herpetology, Museo de Biodiversidad del Perú (MUBI), Cusco, Peru.
9. Instituto de Biociências, Cidade Universitária, Universidade Federal do Mato Grosso do Sul, Campo Grande, MS, Brazil.
10. Universidad Nacional de Colombia, sede La Paz, Cesar, Colombia.
11. Amphibian Survival Alliance (ASA)
12. Universidade de São Paulo, Instituto de Biociências, Departamento de Zoologia, São Paulo, SP, Brazil
13. Departamento de Biologia, Centro de Ciências, Campus do Pici, Universidade Federal do Ceará, Fortaleza, CE, Brazil.
14. Coordenação de Biodiversidade, Programa Coleções Científicas Biológicas, Instituto Nacional de Pesquisas da Amazonia (INPA), Manaus, AM, Brazil.
15. Instituto de Ciências Biológicas, Departamento de Biologia, Coordenação de Aperfeiçoamento Pessoal de Nível Superior – CAPES, Universidade Federal do Amazonas, Manaus, AM, Brazil

*Corresponding author - alexandre.dealmeida@hotmail.com

KEYWORDS

Ancient Amazon landscape, Biogeography; Cryptic diversity; Open habitats; Riverine environments; South America

ABSTRACT

Uncovering the high levels of cryptic diversity in the neotropical amphibians has changed our perception of species boundaries with directed effect in our comprehension of taxonomy, conservation, and biogeography of these taxa. In addition, the advances for the usage of wide genomic data and integrative approach considering coalescent models have improved our accuracy in delimit species and its diversification. Here we estimated the phylogenetic diversity of the widespread *Boana punctata* complex, by the usage of next generation sequencing (NGS) data to delimit putative species and perform a time-calibrated tree analysis to infer about the events related to the species complex diversification. Our results reveal the existence of at least seven structured lineages, which three represent the nominal *Boana punctata*, *Boana atlantica* and *Boana rubrolineata*, and four putative species candidates. Our time calibrated tree analysis reveals a diversification starting about late Miocene followed by subsequent events along the Plio-Pleistocene. Such findings indicate the role of demising of Pebas system and the subsequent landscape changes, with the spread of riverine wetlands that promotes the dispersion and diversification of *B. punctata* complex in most of the open habitat through major river basin in north and central of South America.

INTRODUCTION

The enormous species richness, as well as the high rates of endemism on vertebrate biodiversity, have made the Neotropics a priority area of research interest for biologists and conservationists over the world (Jenkins, Pimm, & Joppa, 2013). In addition, the region is home to a high diversity of landscapes that reflect a dynamic geological history that generated intricate patterns of distribution of richness (Antonelli, et al., 2018a). For amphibians, this region is particularly special because it host something about 43% of all known species (Jenkins, Pimm, & Joppa, 2013; Meseguer et al., 2020; Segalla et al., 2021). However, it's astonishing diversity contrast with the ongoing populational decline caused mainly by a fungal pandemic disease and habitat losses in consequence of human activity, increasing the number of endangered species (Ortega-Andrade et al., 2021; Scheele et al., 2019).

Although the discovery of new species has dramatically increased in the recent decade, the knowledge on the amphibia diversity remain vastly unknown in the Neotropics (Guerra et al., 2020; Vacher et al., 2020). The uncertainty about amphibia diversity is affect mainly by the two knowledge gap: first, several geographic regions remain unexplored due remoteness or difficult logistics, and second the high levels of cryptic diversity found in this taxa (Mayer, Marin, & Lötters, 2019; Vacher et al., 2020). Those issues not only result in taxonomic deficiencies but also hampers our understanding on the biogeographic patterns, blurring the species distribution boundaries and the drivers of the diversification of these organisms. So, in face of the ongoing unprecedented biodiversity crisis, overcome it became an important task to achieve conservation goals (Bickford et al., 2007; Trontelj & Fier, 2009).

Uncovering cryptic lineages, and the usage of species delimitation methods in addition to the acquisition of robust genomic data (e.g., RAD-seq) to delimit taxonomic units has improved our knowledge about the species boundaries, and the processes that underlying their evolutionary history. This approach increases the accuracy of biogeographic models and

improves our interpretation of the period and pathways of diversification, thus creating a macroevolutionary picture about neotropical amphibians history (Antonelli et al., 2018a; Fouquet et al., 2019; Hillis, 2019; Werneck, 2011).

Several hypotheses have been suggested to understand the processes that have defined the high diversity of the Neotropics. Most of them tried to reveal how actual and historical riverine configuration, or climatic and landscape changes were related to the patterns of distribution of vertebrates' groups (Wallace, 1852; Haffer, 1969; Azevedo-Ramos & Galatti, 2002). However, nowadays became clear that the riverine-barrier hypothesis cannot be broadly applied for all taxonomic groups (Naka & Brumfield, 2018; Naka & Pil, 2020), and for amphibians it's probably worked as a second barrier of dispersion instead as vicariant force for diversification (Godinho & Da Silva, 2018; Pirani et al., 2019). In addition, newly dated phylogenetic reconstruction has shown an older diversification in most of the taxonomic group, making the refuge hypothesis inaccurate regarding divergence periods. With the advance of techniques for obtaining molecular data and analysis, and consequently the construction of more accurate paleogeographic models, became clear that a mosaic of historical events, and changes in climate and landscape, created in the neotropics a favourable condition for species diversification through the forested biomes (e.g. Amazonia, Atlantic Forest), highlands (e.g. Tepuis) and open vegetation biomes (e.g. Pantanal, Cerrado and Chaco) (Bicudo et al., 2019; Brusquetti et al., 2019; Hoorn et al., 2010, 2022; Kok et al., 2017; Werneck, 2011).

For amphibians, most studies have focused on species that inhabit forested areas/biomes, such as Amazonia and Atlantic Forest (e.g. Fouquet et al., 2012; Rojas et al., 2018a), making biases on the perception of the role of open habitats in the species evolutionary history. More recently, attention on the so-called Dry Diagonal biomes (Caatinga-Cerrado-Chaco) has increased, providing more clues about the patterns of divergence and the events related to the

phylogeography and biogeography of its endemic biota (Brusquetti et al., 2018, 2019; Oliveira et al., 2018; Thomé, Martins, & Brasileiro, 2021; Thomé et al., 2016). However, to understand the historical interchanges of biota in those different kinds of habitat (forested x open vegetation biomes) remains intriguing. In this case, widespread taxa, occurring in most biomes of the continent can be a good model to investigate broader patterns of diversity and how the pathways of diversification are related to the landscape history (e.g. Gehara et al., 2014) in South America. Although these species are widely known and generally easy to find, they are overlooked regarding their taxonomy, phylogeography and biogeography, as in the case of species in the *Boana punctata* complex.

This species complex has a wide distribution in South America, occurring from the Island of Trinidad (Trinidad & Tobago) to the Chacoan region of northern Argentina. Through this wide distribution, its occurrence is remarkable associated to wetlands with open vegetation, such as the *várzea* of Amazon Basin, Pantanal, Llanos and seasonal swamps and lakes of Cerrado. In addition, the species also occur in swamps and seasonal flooded savannas in coastal areas of Guyana Shield and Atlantic Forest. This wide distribution, and the environmental differences in habitat occupancy of this species, was debated for a long time as argument for propose taxonomic arrangements.

CURRENT STATUS OF SYSTEMATICS AND DIVERSITY OF *BOANA PUNCTATA*

SPECIES COMPLEX

The *Boana punctata* complex is part of the homonym *B. punctata* group proposed by Faivovich et al. (2005). Since the original composition, systematic changes were proposed based in molecular data and morphologic characters, rendering new taxonomic arrangement for this group (Almeida et al., 2021; Caminer & Ron, 2020; Faivovich, McDiarmid, & Myers, 2013; Pinheiro et al., 2018). Presently, *B. punctata* group comprises: *Boana punctata* (Schneider, 1799), *Boana atlantica* (Caramaschi & Velosa, 1996), *Boana alemani* (Rivero,

1964), *Boana cinerascens* (Spix, 1824), *Boana gracilis* (Melin, 1941) and *Boana sibleszi* (Rivero, 1971). Even so, uncertainties regarding the taxonomic status of *B. alemani* and phylogenetic relationship of *B. sibleszi* remain unsolved (Almeida et al., 2021; Barrio-Amorós, Rojas-Runjaic, & Señaris, 2019; Pinheiro et al., 2018). Moreover, high levels of cryptic diversity in *B. cinerascens* and *B. punctata* could render the inclusion of more taxa in this taxonomic arrangement (Guarnizo et al., 2015; Jansen et al., 2011; Lima et al., 2019; Motta et al., 2018; Sturaro et al., 2020).

The discovery of hidden diversity within the nominal *B. cinerascens* and *B. punctatus* with taxonomic implications put both species with the status of species complex (hereafter *B. cinerascens* complex and *B. punctata* complex). Both species were considered unique taxonomic unities, with a wide distribution. In a recent review of taxonomic status of some *B. cinerascens* populations, Sturaro et al. (2020) resurrected *B. gracilis* based on molecular data and phenotypic traits, putting it as part of the *B. cinerascens* complex, which probably encompass more unknown species (Caminer & Ron, 2020; Motta et al., 2018). Meanwhile, the taxonomic status of the putative lineages discovered within *B. punctata* distribution remains unclear, and for the moment, only *B. atlantica* is recognized as full species (Lima et al., 2019). In the most comprehensive study of *B. punctata* complex' diversity, Almeida et al. (unpublished data) revealed at least six putative species, and proposed to elevate *Boana rubrolineata* (B. Lutz, 1951) to a full species status. Although the authors have provided important addresses on the phylogenetic diversity and taxonomic implications of the species complex, lack of more informative genetic data prevented them from properly access the phylogenetic relationship, and subsequently diversification and biogeography of this group. Thus, in this study we aim to achieve these goals using genome-wide Single Nucleotide Polymorphism (SNP) to perform a sounder analysis of coalescent-based species delimitation,

and subsequently investigated the divergence timing evaluating the correspondence with the known events related to the neotropical amphibian's diversification.

MATERIAL AND METHODS

SAMPLING AND MOLECULAR DATA GENERATION

Aiming to cover the phylogenetic diversity recently described, we assessed 30 tissue samples of *B. punctata* complex used in the study of Almeida et al. (unpublished data). The samples selected encompass all nominal species of the complex and all lineages delimited by their study covering most of the geographic distribution of the species complex. In addition, we accessed three samples of the sister taxa, *B. cinerascens* complex, used as outgroup and calibration point for our divergence time estimation analysis. Details about locality and samples identification are presented in Supplemental Material (Table S1). We extracted genomic DNA from the tissue samples using the phenol-chloroform (Sambrook & Russel, 2001) or CTAB 2% (Doyle & Doyle, 1987) protocols. DNA extracts were used to generate a genomic library and subsequently extraction of single nucleotide polymorphisms (SNP).

To obtain a representative sample of the genome, we constructed reduced libraries following the ddRADseq (double digest RAD sequencing) protocol proposed by Peterson et al. (2012) with modifications. We used an adapted protocol, available on GitHub (<https://github.com/legalLab>), that allows the use of the Ion Torrent platform (Thermo Fisher) and reduces the preparation time of genomic libraries using two restriction enzymes (SdaI and Csp6I) to digest 200 ng of genomic DNA of each individual, and two Ion Torrent adapters (A and P) to be linked to the digested fragments, all in the same step. Adapter A has a molecular barcode that allows the identification of each individual and the subsequent demultiplexing of the sequenced fragments. Only the ddRAD fragments that contain an adapter A and adapter P on each side are enriched through PCR, and subsequently, all individuals are mixed equimolarly. Finally, the fragments between 320 and 400 are selected in the Pippin Prep (Sage

Science). The final library is diluted and placed in the Ion Chef™ system, where the template preparation and loading of the Ion 318™ chip is performed; the chip is subsequently sequenced in the Ion PGM™ system, all using the Ion PGM™ HiQ™ View Chef kit.

DATA PROCESSING

The FASTQ file with raw reads was fully processed using PyRAD v3.0.66 (Eaton 2014). The reads were demultiplexed and barcodes (individual tags) trimmed. Only trimmed reads with a maximum of eight bases below a quality score of 25 were included. Clusterization and alignment for loci distinction were made with VSEARCH (Rognes et al 2016) and MUSCLE (Edgar 2004), first within each individual reads and then, after heterozygosity estimates and creation of consensus sequences, between individuals. Only reads with a minimum 0.88 identity were allowed to be in the same cluster and only clusters with at least five reads were included. Finally, only loci with at least four individuals were kept in the final overall dataset.

PHYLOGENETICS AND POPULATIONAL STRUCTURE

To assess population structure, we adopted an approach suited for make use of all information available in RAD sequences, fineRADstructure (Malinsky et al, 2018). It works by first generating a coancestry matrix based on all available SNPs in each locus and then searching for the optimal number of clusters and individual assignments through MCMC. Only the *B. punctata* complex samples were used. The MCMC was run for 1 million steps, being sampled every 1000th step, after another 1 million steps burn-in. Convergence was checked visually by plotting the likelihood trace

The Multispecies Coalescent (MSC) framework (Rannala & Yang, 2003) takes advantage of the prior knowledge about individual assignments to lineages, allowing the intra and inter group coalescent events to tell apart from each other, making it possible

to deal with incomplete lineage sorting (ILS) when estimating species trees. we decided to first explore the species' history with an approach that could reconstruct a species tree using individual information.

ASTRAL (Mirarab et al., 2014) is a method that estimates species trees by maximizing compatibility among quartets in pre-estimated gene trees and that is consistent under the coalescent, therefore being also capable of dealing with ILS, without a prior assignment of individuals to lineages. We estimated gene trees with RAxML v8.2.11 (Stamatakis, 2014) by finding the best tree between 10 independently calculated ones and adding support values after 100 bootstraps, for each locus. In order to dismiss extreme unlikely relations between individuals (Mirarab, 2019), clades with support values below 10 were collapsed into polytomies using Newick Utilities (Junier e Zdobnov, 2010). Finally, we used the resulting gene trees to estimate the supertree with ASTRAL-III (Zhang, Sayyari & Mirarab, 2017)

MORPHOLOGIC VARIABLES AND DATA TREATMENT

To build a morphologic matrix for our dataset, we assessed standard morphometric measures from 441 specimens from *B. punctata* complex. The specimens represent most of the species complex geographic distribution, including regions that we did not access molecular data. We used the morphologic matrix of linear measures from 14 morphometric characters: Snout-vent length (SVL); Head length (HL); Head width (HW); Snout length (SL); Eye-nostril distance (END); Interorbital distance (IOD); Eye diameter (ED); Tympanum diameter (TD) Thigh length (ThL); Tibia length (TiL); Tarsus length (TaL); Foot length (FL); Forearm length (FaL) and Hand length (HaL). Terminologies and acronyms follow Watters, Cummings, Flanagan, & Siler (2016). As well as the samples used in our phylogenetic analysis, this morphologic matrix was

obtained by Almeida et al. (unpublished data). Those measures were taken following standard procedures as described by the authors.

SPECIES DELIMITATION METHODS

To perform lineage delimitation, considering both genomic and morphometric data, we used iBPP (Solís-Lemus et al., 2015). This method is an adaptation of BPP (Yang & Rannala 2010) capable of incorporating numerical characters of continuous distribution in the analysis. We used a subset of the ddRAD loci that contained at least 50% of the samples and the complete matrix of morphometric characters. The groups defined *a priori* followed the delimitation of populations previously obtained with fineRADstructure. As the number of samples available with morphometric data is much larger than that with ddRAD, a geographical association criterion was adopted between the groups defined in fineRADstructure and the morphometric data resulting in the use of most of the data (Table S2). The iBPP runs were performed with the aid of iBPPwrapper (available at (<https://github.com/ericopolo/iBPPwrapper>), which performs multiple runs with gradually increasing chain size and burn-in values, until stabilization (estimated by ESS values), drawing at each run subsets of the genetic data (when used) and different parameters of the delimitation algorithms offered by iBPP, drawing the values of its parameters among those suggested in the iBPP manual. To evaluate separately the influence of genetic markers and morphometry, besides the delimitation including both matrices, the process was repeated twice, using only each one of them. In the end, only the races whose lowest ESS value among the MCMC parameters was higher than 300 were considered. The probability reported for each hypothetical divergence event is the average of the probability among all runs used.

TIME DIVERGENCE ANALYSIS

To achieve the diversification timeframe of *B. punctata* complex we inferred a time-calibrated tree using StarBeast2 v0.15.5 (Ogilvie et al., 2017) running under BEAST2 v2.6.1 (Bouckaert et al., 2014) with a dataset of 51 loci that comprises at least 25 of the totals of 31 samples (*B. punctata* complex + *B. cinerascens* outgroup). For this approach we constrained the groups delimited in fineRADstructure, allowing us to infer the divergence between the lineages besides the groups delimited in our species discovery analysis. For time calibration we used the point of divergence between *B. punctata* complex and its sister *B. cinerascens* complex estimated by Fouquet et al. (2021a). The authors proposed this time of diversification based in the divergences proposed in a broader phylogenomic analysis (Feng et al., 2017). Our reconstructed time -calibrated tree was performed under a Yule-calibrated tree prior, and the analysis were undertaken using a Strict clock model of distribution of the rate between branch lengths and evolutionary time (Ferreira & Suchard, 2008). The Markov chain Monte Carlo (MCMC) was run for 5 billion iterations with sampling every 5.000 generations. The convergence of our priors was checked in Tracer v1.7.1 (Rambaut et al., 2018) and our maximum clade credibility tree were extracted using Tree annotator 2.6.

RESULTS

PHYLOGENETICS AND POPULATIONAL STRUCTURE

The fineRADstructure analysis has identified eight structured populations with low evidence of gene flow between them congruent with the lineages of our phylogenetic analysis, suggesting these lineages are phylogenetic entities evolving individually. The supertree estimated in ASTRAL has resulted in a well resolved phylogeny with highly supported lineages (>0.95) representing the major clades within the monophyletic *B. punctata* complex.

About the current taxonomic arrangement, the tree topology has recovered a paraphyletic *B. punctata* due the phylogenetic relationship of Colombian lineage. Moreover, the analysis supported the monophyly of *B. atlantica* as a sister taxon of the putative *B. rubrolineata*. On the other hand, the analysis recovers *B. rubrolineata* monophyletic, but with two lineages of structured populations.

SPECIES DELIMITATION, PHYLOGEOGRAPHIC PATTERNS AND DIVERGENCE TIME ESTIMATION

To perform our coalescence species delimitation, filtering ddRAD loci containing at least 50% of individuals resulted in a matrix of 737 loci. To summarize the probabilities of hypothetical divergences we have performed 21 runs retained for analysis using genomic data only, 27 using morphometric data only and 26 using both data matrices. Runs using genomic data alone recognized the greatest number of divergences, with only one having a joint support smaller than 0.9 (Figure 1), resulting in eight independent evolutionary lineages recognized. Morphometry alone, on the other hand, provides no support for two of the divergences, thus recognizing only six lineages. When used together, the genomic and morphometric matrices result in a pattern similar to that of the genomic matrix, but with not full support for the most recent divergence

events, making the most likely number of seven independent lineages (Figure 1). The groups delimited in iBPP have presented a structured geographic pattern of distribution through the South American ecoregions as described above (Figure 1): Group 1) a lineage from the Andean slopes and Llanos in the Colombian Amazon; Group 2) along the Amazon basin, extending from the headwaters in Peru highlands to the low-wetlands in the Amazon river; Group 3) from the coastal region of northern Atlantic Forest in Pernambuco state along with Parnaíba river basin in Piauí state and Tocantins river at Tocantins state; Group 4) also from the Tocantins river basin along with the northern coastal Maranhão state; Group 5) from the wetlands of Madre de Dios – Madeira river system in southern Amazon domain; Group 6) the group encompass the structured lineage from upper Taquari river basin, the lineage with populations of Paraná basin and the Cerrado + Patanal domain; and latter Group 7) a lineage from the coastal Atlantic Forest in the Bahia state.

The well supported lineages delimited in our iBPP analysis recovered a concise phylogeographic pattern described for the major clades in our topology (Figure 1). The *B. punctata* complex diverge from the most recent common ancestor (MRCA) with its sister species, *Boana cinerascens*, about ~10.16 Mya (12.59–7.88) in the late Miocene. The first coalescence date within the species complex occurs about ~4.36 Mya (5.68–2.88) in early Pliocene, diverging the lineage of Eastern Cordillera foothills of Colombia from the remaining lineages which occurs in open areas and wetlands of South America. Subsequently an event of diversification at ~2.58 Mya (3.5–1.8) in the Pliocene–Pleistocene transition have separated the clade of Amazon basin + Northeastern and Tocantins lineages from the clade of Madre de Dios – Madeira rivers system + Cerrado–Pantanal–Paraná basin and Atlantic Forest lineages. Further in the early and mid-Pleistocene, the events of diversification dated a coalescence of *B.*

atlantica about ~1.8 Mya (1.21–2.55) in the early Pleistocene, and a split between the lineages from the southern Amazon to the lineages from the dry seasonal wetlands at mid-Pleistocene at ~1.14 Mya (0.7–1.63). In northern clade, the mid Pleistocene events also promote diversification in separate the lineage from Amazon River to those of northern Cerrado + Northeastern Brazil about ~0.95 (0.57 – 1.36) and 0.42 (0.19 – 0.7) Mya respectively.

SYSTEMATIC AND TAXONOMIC IMPLICATIONS

Our results along with the findings of Almeida et al. (unpublished data), confirms the *B. punctata* complex as a monophyletic group of cryptic species with subtle but significant variations on morphology. Phylogenetic and phylogeographic analysis clarify the relationships between these lineages and putative taxa, resulted of diversification that starts with the split between two major clades that includes populations from northern and southern part of the species complex distribution, followed by subsequently diversification eastward. A major southern clade includes the nominal *B. atlantica* and *B. rubrolineata* as sister taxon. The northern clade includes the lineage of the populations spread through the amazon basin and the lineage from northern Cerrado + Coastal northeastern. Both major clades are diverged from an older lineage that isolated the populations from northwestern South America. Despite a taxonomic account of the delimited groups are beyond our goals, is noteworthy to highlight the evidence of unknown species as well as a putative taxonomic reassessment. The most divergent lineage (Group 1), encompass samples of the Colombian population from Meta department, a population that could be associated to the synonym *Hyla rubeola* Cochran & Goin (1970) described from the Serrania de La Macarena in the same province, which should be reevaluated. Another intriguing finding regards the taxonomic status of the lineages of northeastern Brazil, which we assume as unknown species, but as a one

taxonomic unit including both lineages (groups 3 and 4) due the lack of morphologic evidence and a very recent coalescence.

DISCUSSION

HIDDEN DIVERSITY AND PHYLOGENETIC RELATIONSHIPS

Our study reassesses the phylogeography of *B. punctata* complex through genomic and morphologic data, revealing that the current taxonomic arrangement may not represent the real diversity within this group. Our findings reveal an even higher diversity than the suggested in earlier studies that found maximum of six lineages within it (e.g., Almeida et al., unpublished data; Caminer & Ron, 2020; Lima, Dubeux et al., 2019; Sturaro et al., 2020). Here we presented eight well supported lineages, that composes a species complex with three recognized species (*B. punctata*, *B. rubrolineata* and *B. atlantica*) and at least four putative with molecular and morphologic evidence confirmed by a sounder species discovery analysis. Moreover, lack of samples from the populations of the Guyana Shield, Venezuela, and Bolivia may underestimate the diversity in this complex, as it has been observed in other widespread hylid frog complexes (Fouquet et al., 2021a; Jansen et al., 2011, 2019; Pirani et al., 2020).

Cryptic diversity is not a novelty for Neotropical anurans (e.g. Carvalho et al., 2022; Ribeiro-Júnior et al., 2020; Roberto et al., 2020). Unveiling it has remarkable effect in the rate of newly discovered species in the recent decades (Guerra et al., 2020) and has been the keystone to untangle taxonomic issues and access the process underneath the species diversification (e.g. Carvalho et al., 2019; Fouquet et al., 2021b; Mângia et al., 2020; Moraes et al., 2022; Rojas et al., 2018a, 2018b). In the *B. punctata* complex, karyotype, genetic and phenotypic evidences revealed hidden diversity within it, however the discussion on systematics and inner species boundaries are shallowly debated, mostly due the absence of geographic representativity in the sampling, or by the lack of robust genetic data (Almeida et al., 2021; Caminer & Ron, 2020; Jansen et al., 2011; Lima et al., 2019; Napoli & Cruz, 2005; Sturaro et al., 2020). Recently

Almeida et al. (unpublished data) provided a comprehensive analysis of the diversity within *B. punctata*, recovering similar groups of delimited lineages, but with low phylogenetic support for their relationship. Our SNPs-based data and coalescent model of species discovery provide a reliable phylogenetic systematics that supports the presence of one highly divergent lineage from the Eastern Cordillera+Llanos of Colombia, in the inner nodes a northern clade with lineages that occurs in the wetlands of Amazon River and lowlands of northern Cerrado and Atlantic Forest, besides closer relationship between *B. atlantica* with *B. rubrolineata* in the southern wetlands and savannas of central South America. Such geographic structured phylogeny gives us clues about the events that drove the *B. punctata* complex diversification.

PHYLOGEOGRAPHY AND BIOGEOGRAPHY THROUGH A CONTINENTAL HISTORY

We found most recent common ancestor between *B. punctata* complex and *B. cinerascens* complex diverged about 10 Mya, in late Miocene, like a similar time frame proposed by Duellman, Marion, & Hedges (2016) and Fouquet et al. (2021a). During the late Miocene the demising of the Pebas System in western Amazonia has been elected as one the most important events to the biota diversification, where a complex scenario of swamps and riverine systems was gradually transitioned to the modern river basin (Albert, Val, & Hoorn, 2018; Hoorn et al., 2010; Wesselingh & Salo, 2006). In this landscape ancestors could occupy these different environments and diverged gradually in these two widespread species complex. Despite our assumptions about the region where this diversification happens are speculative, the role of western Amazonia for the evolution of amphibia biodiversity in the neotropics is highly supported (Antonelli et al., 2018b). Moreover, these events have been related to the diversification along several groups of frogs as well as *Allobates* (Réjaud et al., 2020), *Amazophrynella*

(Moraes et al., 2022; Rojas et al., 2018a), *Dendropsophus leucophyllatus* group (Pirani et al., 2020), *Dendropsophus minutus* (Gehara et al., 2014) and *Boana albopunctata* group (Fouquet, et al., 2021a).

The comprehension of how Pebas System and the afterward scenarios in early Pliocene has influenced the diversification of Neotropical biota has been debated for a long time. For a while, the lacustrine system was only seen as a barrier of dispersion promoting diversification along major upland species in Amazon forest (Wesselingh & Salo, 2006). However, recent novelties and more detailed models showed a continuously dynamic landscape with remarkable events of river captures, a complex mosaic of wetlands and floodplains that shaped the modern river basin and its riverine habitats which may contribute with different pathways of diversification in Neotropical taxa (Albert et al., 2021, 2018; Bicudo et al., 2019; Hoorn et al., 2022).

This dynamic mosaic seems more suitable with our timeframe of lineages diversification and indicates a linkage between orogenic and climates changes of the Plio-Pleistocene with the coalescence of the present lineages. Two main cladogenesis that shape the major *B. punctata* complex phylogeographic structure coincide with the capture and establishment of Orinoco and Madre de Dios basins during the Pliocene, as an effect of the gradual uplift of Vaupes and Fitzcarrald archs (Espurt et al., 2010; Hoorn et al., 2017; Mora et al., 2011). Those landscape changes seem to promote a separation and a posterior dispersion of the populations, first in a northern way possible through the Llanos of Orinoco (Group 1) and probably to the coastal of Guyana Shield, and subsequently to the south of Amazon River system through the riverine system of Madre de Dios basin (ancestor of groups 5, 6, 7 and 8). Those events has been proposed as drivers for diversification in aquatic taxa (Dagosta & De Pinna, 2019; Roberto et al.,

2020), and were recently reported for *Dendropsophus leucophyllatus* group, separating the sister taxa *Dendropsophus triangulum* and *Dendropsophus arndti* (Pirani et al., 2020). Afterwards, the speciation events occurred during the Pliocene, where dramatic fluctuation of climate created a cyclical network of vegetation between the Amazon and Atlantic Forest (Ledo & Colli, 2017). Those shift of forested and open vegetation created corridors of dispersion that promote a biotic interchange between those biomes, allowing the colonization of suitable areas and the diversification of species (Dal Vechio et al., 2018; de Sá et al., 2019). This dynamic interchange is clearly related to the coalescence of *B. atlantica*, which splits from their common ancestor with *B. rubrolineata*. At the same time, climatic conditions as well as the establishment of the Pantanal and Paraguay-Parana river basins, and the Cerrado's savannas in the Pleistocene (Assine et al., 2016; Assine & Soares, 2004; Oliveira et al., 2018; Werneck et al., 2012), created suitable areas to dispersion and further diversification of the actual *B. rubrolineata*.

Most of the biogeographic models and the drivers of neotropical anuran' diversification were proposed for forest dwelling species. For these taxa, rivers, and biomes with open vegetation such as, savannas, grasslands and riverine wetlands have played a role as a vicariant barrier for gene flow or dispersion at least once on their evolutionary history (Carvalho et al., 2020; Pirani et al., 2019; Vacher et al., 2017). However, for *B. punctata* complex these habitats seem to work in an opposite way, promoting the dispersion through the open landscapes such as the riverine wetlands of Amazon basin (*várzeas*), Llanos, Pantanal and Cerrado. In fact, these ecological affinity with the wetlands and its vegetation remains conserved in actual lineages, with the floating meadows and river-lake connectivity still working as drivers for dispersion and maintenance of genetic

diversity (Böning et al., 2017; de Matos et al., 2022; Fonte et al., 2021; Ramalho et al., 2016; Ramalho, Machado, & Vieira, 2018; Schiesari et al., 2003; Upton et al., 2014).

ACKNOWLEDGMENT

We are thankful to Celio F.B. Haddad (UNESP-Rio Claro) and Ross MacCulloch (Royal Ontario Museum – ROM) for kindly loan of tissues samples of *B. punctata* and *B. cinerascens*. This study was supported by the French/Brazilian GUYAMAZON programme (Institut de Recherche pour le Développement-IRD, Centre national de la recherche scientifique-CNRS, Collectivité territoriale de Guyane-CTG, Centre de coopération internationale en recherche agronomique pour le développement-CIRAD and Fundação de Amparo à Pesquisa do Estado do Amazonas-FAPEAM; #062.00962/2018), co-coordinated by A.F. and F.P.W. APA thank FAPEAM for PhD fellowship (POSGRAD-UFAM 2016). VTC thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES for fellowship (PNPD), and FPW thanks financial support from CNPq (productivity fellowship #305535/2017-0), Fundação de Amparo à Pesquisa do Estado do Amazonas-FAPEAM (#062.00962/2018 and 062.01110/2017), #and L’Oréal-Unesco For Women in Science Program (Brazil 2016 and IRT 2017).

REFERENCES

- Albert, J. S., Bernt, M. J., Fronk, A. H., Fontenelle, J. P., Kuznar, S. L., & Lovejoy, N. R. (2021). Late Neogene megariver captures and the Great Amazonian Biotic Interchange. *Global and Planetary Change*, 205(July), 103554. <https://doi.org/10.1016/j.gloplacha.2021.103554>
- Albert, J. S., Val, P., & Hoorn, C. (2018). The changing course of the amazon river in the neogene: Center stage for neotropical diversification. *Neotropical Ichthyology*, 16(3), 1–24. <https://doi.org/10.1590/1982-0224-20180033>
- Almeida, A. P. de, Moraes, L. J. C. L., Rojas, R. R., Roberto, I. J., Carvalho, V. T. de, Ávila, R. W., ... Gordo, M. (2021). Phylogenetic relationships of the poorly known treefrog

- Boana hobbsi* (Cochran & Goin, 1970) (Anura: Hylidae), systematic implications and remarks on morphological variations and geographic distribution. *Zootaxa*, 4933(3), 301–323. <https://doi.org/10.11646/zootaxa.4933.3.1>
- Antonelli, A., Ariza, M., Albert, J., Andermann, T., Azevedo, J., Bacon, C., ... Edwards, S. V. (2018a). Conceptual and empirical advances in Neotropical biodiversity research. *PeerJ*, 6, e5644. <https://doi.org/10.7717/peerj.5644>
- Antonelli, A., Zizka, A., Carvalho, F. A., Scharn, R., Bacon, C. D., Silvestro, D., & Condamine, F. L. (2018b). Amazonia is the primary source of Neotropical biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, 115(23), 6034–6039. <https://doi.org/10.1073/pnas.1713819115>
- Assine, Mario L., Merino, E. R., Pupim, F. N., Warren, L. V., Guerreiro, R. L., & McGlue, M. M. (2016). Geology and geomorphology of the pantanal basin. *Handbook of Environmental Chemistry*, 37(June 2018), 23–50. https://doi.org/10.1007/698_2015_349
- Assine, Mario Luis, & Soares, P. C. (2004). Quaternary of the Pantanal, west-central Brazil. *Quaternary International*, 114(1), 23–34. [https://doi.org/10.1016/S1040-6182\(03\)00039-9](https://doi.org/10.1016/S1040-6182(03)00039-9)
- Azevedo-Ramos, C., & Galatti, U. (2002). Patterns of amphibian diversity in Brazilian Amazonia: Conservation implications. *Biological Conservation*, 103(1), 103–111. [https://doi.org/10.1016/S0006-3207\(01\)00129-X](https://doi.org/10.1016/S0006-3207(01)00129-X)
- Barrio-Amorós, C. L., Rojas-Runjaic, F. J. M., & Señaris, J. C. (2019). Catalogue of the amphibians of Venezuela: Illustrated and annotated species list, distribution, and conservation. *Amphibian & Reptile Conservation*, 13(1), 1–198.
- Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., ... Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology*

- and Evolution*, 22(3), 148–155. <https://doi.org/10.1016/j.tree.2006.11.004>
- Bicudo, T. C., Sacek, V., Almeida, R. P. De, Bates, J. M., & Ribas, C. C. (2019). Andean Tectonics and Mantle Dynamics as a Pervasive Influence on Amazonian Ecosystem. *Scientific Reports*, (November), 1–11. <https://doi.org/10.1038/s41598-019-53465-y>
- Böning, P., Wolf, S., Upton, K., Menin, M., Venegas, P. J., & Lötters, S. (2017). Amphibian diversity and its turnover in floating meadows along the amazon river. *Salamandra*, 53(3), 379–388.
- Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C. H., Xie, D., ... & Drummond, A. J. (2014). BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS computational biology*, 10(4), e1003537.
- Brusquetti, F., Netto, F., Baldo, D., & Haddad, C. F. B. (2019). The influence of Pleistocene glaciations on Chacoan fauna: Genetic structure and historical demography of an endemic frog of the South American Gran Chaco. *Biological Journal of the Linnean Society*, 126(3), 404–416. <https://doi.org/10.1093/biolinnean/bly203>
- Brusquetti, F., Netto, F., Baldo, D., & Haddad, C. F. B. (2018). What happened in the South American Gran Chaco? Diversification of the endemic frog genus *Lepidobatrachus* Budgett, 1899 (Anura: Ceratophryidae). *Molecular Phylogenetics and Evolution*, 123(February), 123–136. <https://doi.org/10.1016/j.ympev.2018.02.010>
- Caminer, M. A., & Ron, S. R. (2020). Systematics of the *Boana semilineata* species group (Anura: Hylidae), with a description of two new species from Amazonian Ecuador. *Zoological Journal of the Linnean Society*, (January), 1–32. <https://doi.org/10.1093/zoolinnean/zlaa002/5810752>
- Caramaschi, U., Velosa, A. (1996). Nova especie de *Hyla Laurenti*, 1768 do leste Brasileiro (Amphibia, Anura, Hylidae). *Boletim Di Museu Nacional*.
- Carvalho, V. T. de, Vogt, R. C., Rojas, R. R., Nunes, M. da S., de Fraga, R., Ávila, R. W., ...

- Farias, I. P. (2022). Four in One: Cryptic Diversity in Geoffroy's Side-Necked Turtle *Phrynops geoffroanus* (Schweigger 1812) (Testudines: Pleurodira: Chelidae) in Brazil. *Diversity*, 14(5), 360. <https://doi.org/10.3390/d14050360>
- Carvalho, T. R. D., Moraes, L. J. C. L., Lima, A. P., Fouquet, A., Peloso, P. L. V, Pavan, D., ... Haddad, C. F. B. (2020). Systematics and historical biogeography of Neotropical foam-nesting frogs of the *Adenomera heyeri* clade (Leptodactylidae), with the description of six new Amazonian species. *Zoological Journal of the Linnean Society*, 191(2), 395–433. <https://doi.org/10.1093/zoolinnean/zlaa051>
- Carvalho, T. R., Giaretta, A. A., Maciel, N. M., Barrera, D. A., Aguilar-Puntriano, C., Haddad, C. F. B., ... Angulo, A. (2019). On the Uncertain Taxonomic Identity of *Adenomera hylaedactyla* (Cope, 1868) and the Composite Type Series of *A. andreae* (Müller, 1923) (Anura, Leptodactylidae). *Copeia*, 107(4), 708. <https://doi.org/10.1643/CH-19-237>
- Cochran, D. M., & Goin, C. J. (1970). Frogs of Colombia. In *Bulletin of the United States Museum*.
- Dagosta, F. C. P., & De Pinna, M. (2019). The Fishes Of The Amazon : Distribution And Biogeographical Patterns, With A Comprehensive List Of Species. *Bulletin Of The American Museum Of Natural History*, 431, 163.
- Dal Vechio, F., Prates, I., Grazziotin, F. G., Zaher, H., & Rodrigues, M. T. (2018). Phylogeography and historical demography of the arboreal pit viper *Bothrops bilineatus* (Serpentes, Crotalinae) reveal multiple connections between Amazonian and Atlantic rain forests. *Journal of Biogeography*, 45(10), 2415–2426. <https://doi.org/10.1111/jbi.13421>
- de Matos, L. R. A., Ramalho, W. P., de Arruda, F. V., Ceron, K., Luna, P., Virgílio, L. R., & Vieira, L. J. S. (2022). Environmental Drivers and Network Structure of Hylid Anurans (Amphibia: Hylidae) in Floating Meadows from Amazonian Oxbow Lakes. *Wetlands*,

42(3), 1–13. <https://doi.org/10.1007/s13157-022-01541-x>

- de Sá, R. O., Tonini, J. F. R., van Huss, H., Long, A., Cuddy, T., Forlani, M. C., ... Haddad, C. F. B. (2019). Multiple connections between Amazonia and Atlantic Forest shaped the phylogenetic and morphological diversity of *Chiasmocleis* Mehely, 1904 (Anura: Microhylidae: Gastrophryninae). *Molecular Phylogenetics and Evolution*, 130(October 2018), 198–210. <https://doi.org/10.1016/j.ympev.2018.10.021>
- Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19, 11–15.
- Duellman, W. E., Marion, A. B., & Hedges, S. B. (2016). Phylogenetics, classification, and biogeography of the treefrogs (Amphibia: Anura: Arboranae). *Zootaxa*, 4104(1), 1–109. <https://doi.org/10.11646/zootaxa.4104.1.1>
- Eaton, D.A. (2014). PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics* 30: 1844–1849.
- Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32, 1792–1797.
- Espurt, N., Baby, P., Brusset, S., Roddaz, M., Hermoza, W., & Barbarand, J. (2010). The Nazca Ridge and Uplift of the Fitzcarrald Arch: Implications for Regional Geology in Northern South America. In Carine Hoorn & F. P. Wesselingh (Eds.), *Amazonia, Landscape and Species Evolution: A Look into the Past* (1st ed., pp. 89–100). <https://doi.org/10.1002/9781444306408.ch6>
- 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), 1. [https://doi.org/10.1206/0003-0090\(2005\)294\[0001:srotff\]2.0.co;2](https://doi.org/10.1206/0003-0090(2005)294[0001:srotff]2.0.co;2)

- Faivovich, J., McDiarmid, R. W., & Myers, C. W. (2013). Two new species of *Myersiohyla* (Anura: Hylidae) from Cerro de la Neblina, Venezuela, with comments on other species of the genus. *American Museum Novitates*, (3792), 63.
- Feng, Y.-J., Blackburn, D. C., Liang, D., Hillis, D. M., Wake, D. B., Cannatella, D. C., & Zhang, P. (2017). Phylogenomics reveals rapid, simultaneous diversification of three major clades of Gondwanan frogs at the Cretaceous–Paleogene boundary. *Proceedings of the National Academy of Sciences*, 114(29), E5864–E5870. <https://doi.org/10.1073/pnas.1704632114>
- Ferreira, M. A. R. & Suchard, M. A. (2008) Bayesian analysis of elapsed times in continuous-time Markov chains. *Canadian Journal of Statistics*, 36(3), 355–368.
- Fonte, L. F. M. da, Latombe, G., Gordo, M., Menin, M., de Almeida, A. P., Hui, C., & Lötters, S. (2021). Amphibian diversity in the Amazonian floating meadows: a Hanski core-satellite species system. *Ecography*, 44(9), 1325–1340. <https://doi.org/10.1111/ecog.05610>
- Fouquet, A., Ferrier, B., Salmona, J., Tirera, S., Vacher, J. P., Courtois, E. A., ... de Thoisy, B. (2019). Phenotypic and life-history diversification in Amazonian frogs despite past introgressions. *Molecular Phylogenetics and Evolution*, 130(June 2018), 169–180. <https://doi.org/10.1016/j.ympev.2018.09.010>
- Fouquet, A., Leblanc, K., Framit, M., Réjaud, A., Rodrigues, M. T., Castroviejo-Fisher, S., ... Fabre, A.-C. (2021b). Species diversity and biogeography of an ancient frog clade from the Guiana Shield (Anura: Microhylidae: *Adelastes*, *Otophryne*, *Synapturanus*) exhibiting spectacular phenotypic diversification. *Biological Journal of the Linnean Society*, 1–24.
- Fouquet, A., Loebmann, D., Castroviejo-Fisher, S., Padial, J. M., Orrico, V. G. D., Lyra, M. L., ... Rodrigues, M. T. (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(2), 547–561. <https://doi.org/10.1016/j.ympev.2012.07.012>

Fouquet, A., Marinho, P., Réjaud, A., Carvalho, T. R., Caminer, M. A., Jansen, M., ... Ron, S. (2021a). Systematics and biogeography of the *Boana albopunctata* species group (Anura, Hylidae), with the description of two new species from Amazonia. *Systematics and Biodiversity*, 19(4), 375–399.

<https://doi.org/https://doi.org/10.1080/14772000.2021.1873869>

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: Biological Sciences*, 279(1734), 1806–1814. <https://doi.org/10.1098/rspb.2011.1653>

Gehara, M., Crawford, A. J., Orrico, V. G. D., Rodríguez, A., Lötters, S., Fouquet, A., ... Köhler, J. (2014). High Levels of Diversity Uncovered in a Widespread Nominal Taxon: Continental Phylogeography of the Neotropical Tree Frog *Dendropsophus minutus*. *PLoS ONE*, 9(9), e103958. <https://doi.org/10.1371/journal.pone.0103958>

Godinho, M. B. D. C., & Da Silva, F. R. (2018). The influence of riverine barriers, climate, and topography on the biogeographic regionalization of Amazonian anurans. *Scientific Reports*, 8(1), 1–11. <https://doi.org/10.1038/s41598-018-21879-9>

Guarnizo, C. E., Paz, A., Muñoz-Ortiz, A., Flechas, S. V., Méndez-Narváez, J., & Crawford, A. J. (2015). DNA Barcoding Survey of Anurans across the Eastern Cordillera of Colombia and the Impact of the Andes on Cryptic Diversity. *PLoS ONE*, 10(5), 1–20. <https://doi.org/10.1371/journal.pone.0127312>

Guerra, V., Jardim, L., Llusia, D., Márquez, R., & Bastos, R. P. (2020). Knowledge status and trends in description of amphibian species in Brazil. *Ecological Indicators*, 118(July), 106754. <https://doi.org/10.1016/j.ecolind.2020.106754>

- Haffer, J. "Speciation in Amazonian forest birds". *Science*, 165, p. 131-37, 1969
- Hillis, D. M. (2019). Species Delimitation in Herpetology. *Journal of Herpetology*, 53(1), 3.
<https://doi.org/10.1670/18-123>
- Hoorn, C., Wesselingh, F. P., Ter Steege, H., Bermudez, M. A., Mora, A., Sevink, J., ... Antonelli, A. (2010). Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science*, 330(6006), 927–931.
<https://doi.org/10.1126/science.1194585>
- Hoorn, Carina, Bogotá-A, G. R., Romero-Baez, M., Lammertsma, E. I., Flantua, S. G. A., Dantas, E. L., ... Chemale, F. (2017). The Amazon at sea: Onset and stages of the Amazon River from a marine record, with special reference to Neogene plant turnover in the drainage basin. *Global and Planetary Change*, 153, 51–65.
<https://doi.org/10.1016/j.gloplacha.2017.02.005>
- Hoorn, Carina, Boschman, L. M., Kukla, T., Sciumbata, M., & Val, P. (2022). The Miocene wetland of western Amazonia and its role in Neotropical biogeography. *Botanical Journal of the Linnean Society*, 199(1), 25–35.
<https://doi.org/10.1093/botlinnean/boab098>
- Jansen, M., Bloch, R., Schulze, A., & Pfenninger, M. (2011). Integrative inventory of Bolivia's lowland anurans reveals hidden diversity. *Zoologica Scripta*, 40(6), 567–583.
<https://doi.org/10.1111/j.1463-6409.2011.00498.x>
- Jansen, M., Santana, D. J., Teixeira, B. F. da V., & Köhler, G. (2019). A new striped species of *Dendropsophus* (Anura : Hylidae) with a composite advertisement call and comments on the *D . rubicundulus* group. *Vertebrate Zoology*, 69(3), 227–246.
<https://doi.org/10.26049/VZ69-3-2019-01>
- Jenkins, C. N., Pimm, S. L., & Joppa, L. N. (2013). Global patterns of terrestrial vertebrate diversity and conservation. *Proceedings of the National Academy of Sciences of the*

United States of America, 110(28), E2603–E2610.
<https://doi.org/10.1073/pnas.1302251110>

- Junier, T. and Zdobnov, E.M., 2010. The Newick utilities: high-throughput phylogenetic tree processing in the UNIX shell. *Bioinformatics*, 26(13), pp.1669-1670.
- Kok, P. J. R., Russo, V. G., Ratz, S., Means, D. B., MacCulloch, R. D., Lathrop, A., ... Bossuyt, F. (2017). Evolution in the South American ‘Lost World’: insights from multilocus phylogeography of stefanias (Anura, Hemiphractidae, *Stefania*). *Journal of Biogeography*, 44(1), 170–181. <https://doi.org/10.1111/jbi.12860>
- Ledo, R. M. D., & Colli, G. R. (2017). The historical connections between the Amazon and the Atlantic Forest revisited. *Journal of Biogeography*, 44(11), 2551–2563. <https://doi.org/10.1111/jbi.13049>
- Lima, L. R., Dubeux, M. J. M., do Nascimento, F. A. C., Bruschi, D. P., & Mott, T. (2019). Uncovering Neotropical treefrog diversity: integrative taxonomy reveal paraphyly in *Boana atlantica* (Amphibia, Anura, Hylidae). *Amphibia-Reptilia*, (July), 1–11. <https://doi.org/10.1163/15685381-20191109>
- Lutz, B. (1951). Frogs in the Aldopho Lutz collection at the Instituto Oswaldo Cruz. IV. Species allied to the green *Hylas* from southeast Brazil. *Memórias Do Instituto Oswaldo Cruz*, 47(3–4), 319–335.
- Malinsky, M., Trucchi, E., Lawson, D.J. and Falush, D., 2018. RADpainter and fineRADstructure: population inference from RADseq data. *Molecular biology and evolution*, 35(5), pp.1284-1290.
- Mângia, S., Oliveira, E. F. de, Santana, D. J., Koroiva, R., Paiva, F., & Garda, A. A. (2020). Revising the taxonomy of *Proceratophrys* Miranda-Ribeiro, 1920 (Anura: Odontophrynidae) from the Brazilian semiarid Caatinga: Morphology, calls and molecules support a single widespread species. *Journal of Zoological Systematics and*

- Evolutionary Research*, (November 2019), 1–22. <https://doi.org/10.1111/jzs.12365>
- Mayer, M., Marin, L. F., & Lötters, S. (2019). Mind the gap ! A review of Amazonian anurans in GenBank. *Salamandra*, 55(2), 89–96.
- Melin, D. (1941). Contributions to the knowledge of the Amphibia of South America. *Göteborgs Kungl. Vetenskaps-Och Vitterhets-Samhälles. Handlingar. Serien B, Matematiska Och Naturvetenskapliga Skrifter*, 1, 1–71.
- Meseguer, A. S., Antoine, P., Fouquet, A., Delsuc, F., & Condamine, F. L. (2020). The role of the Neotropics as a source of world tetrapod biodiversity. *Global Ecology and Biogeography*, (April), 1–14. <https://doi.org/10.1111/geb.13141>
- Mirarab S, Reaz R, Bayzid MS, Zimmermann T, Swenson MS, Warnow T. (2014). ASTRAL: genome-scale coalescent-based species tree estimation. *Bioinformatics* 30: i541–i548.
- Mirarab, S., 2019. Species tree estimation using ASTRAL: practical considerations. arXiv preprint arXiv:1904.03826.
- Mora, A., Baby, P., Roddaz, M., Parra, M., Brusset, S., Hermoza, W., & Espurt, N. (2011). Tectonic History of the Andes and Sub-Andean Zones: Implications for the Development of the Amazon Drainage Basin. In *Amazonia: Landscape and Species Evolution* (pp. 38–60). <https://doi.org/10.1002/9781444306408.ch4>
- Moraes, L. J. C. L., Werneck, F. P., Réjaud, A., Rodrigues, M. T., Prates, I., Glaw, F., ... Fouquet, A. (2022). Diversification of tiny toads (Bufonidae: Amazophrynella) sheds light on ancient landscape dynamism in Amazonia. *Biological Journal of the Linnean Society*, 136(1), 75–91. <https://doi.org/https://doi.org/10.1093/biolinnean/blac006>
- Motta, J., Menin, M., Almeida, A. P., Hrbek, T., & Pires Farias, I. (2018). When the unknown lives next door: A study of central Amazonian anurofauna. *Zootaxa*, 4438(1), 79–104. <https://doi.org/10.11646/zootaxa.4438.1.3>
- Naka, L. N., & Brumfield, R. T. (2018). The dual role of Amazonian rivers in the generation

- and maintenance of avian diversity. *Science Advances*, 4(8).
<https://doi.org/10.1126/sciadv.aar8575>
- Naka, L. N., & Pil, M. W. (2020). Moving beyond the riverine barrier vicariant paradigm. *Molecular Ecology*, (March), 1–4. <https://doi.org/10.1111/mec.15465>
- Napoli, M., & Cruz, I. (2005). The advertisement call of *Hyla atlantica* Caramaschi & Velosa, 1996, with considerations on its taxonomic status (Amphibia, Anura, Hylidae). *Arquivos Do Museu Nacional*, 63(2), 283–288.
- Ogilvie, H. A., Bouckaert, R. R., Drummond, A.J. (2017) StarBEAST2 Brings Faster Species Tree Inference and Accurate Estimates of Substitution Rates. *Molecular Biology and Evolution*, 34 (8), 2101–2114. <https://doi.org/10.1093/molbev/msx126>
- Oliveira, A. P. G., Machado, R., Ribeiro, A. A., Miotto, C. L., Júnior, J. M., Saad, A. R., ... Filho, A. C. P. (2018). The expression of neotectonics in the Pantanal da Nhecolândia, State of Mato Grosso do Sul - Brazil. *Anais Da Academia Brasileira de Ciencias*, 90(2), 1293–1308. <https://doi.org/10.1590/0001-3765201820170474>
- Oliveira, E. F., Gehara, M., São-Pedro, V. A., Costa, G. C., Burbrink, F. T., Colli, G. R., ... Garda, A. A. (2018). Phylogeography of Muller’s termite frog suggests the vicariant role of the Central Brazilian Plateau. *Journal of Biogeography*, 45(11), 2508–2519. <https://doi.org/10.1111/jbi.13427>
- Ortega-Andrade, H. M., Rodes Blanco, M., Cisneros-Heredia, D. F., Guerra Arévalo, N., López De Vargas-Machuca, K. G., Sánchez-Nivicela, J. C., ... Muñoz, M. H. Y. (2021). Red List assessment of amphibian species of Ecuador: A multidimensional approach for their conservation. *PLoS ONE*, 16(5 May), 1–28. <https://doi.org/10.1371/journal.pone.0251027>
- Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. (2012) Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-

model species. *Plos One* 7: e37135.

- Pinheiro, P. D. P., Noonan, B. P., Haddad, C. F. B., Kok, P. J. R., Means, D. B., & Faivovich, J. (2018). A new genus of Cophomantini, with comments on the taxonomic status of *Boana liliae* (Anura: Hylidae). *Zoological Journal of the Linnean Society*, 185(1), 226–245. <https://doi.org/10.1093/zoolinnean/zly030>
- Pirani, R. M., Peloso, P. L. V., Prado, J. R., Polo, É. M., Knowles, L. L., Ron, S. R., ... Werneck, F. P. (2020). Diversification history of clown tree frogs in Neotropical rainforests (Anura, Hylidae, *Dendropsophus leucophyllatus* group). *Molecular Phylogenetics and Evolution*, 150(November 2019), 106877. <https://doi.org/10.1016/j.ympev.2020.106877>
- Pirani, R. M., Werneck, F. P., Thomaz, A. T., Kenney, M. L., Sturaro, M. J., Ávila, T. C. S., ... Knowles, L. L. (2019). Testing main Amazonian rivers as barriers across time and space within widespread taxa. *Journal of Biogeography*, 1–13. <https://doi.org/10.1111/jbi.13676>
- Ramalho, W. P., Andrade, M. S., Matos, L. R. A. de, & Vieira, L. J. S. (2016). Amphibians of varzea environments and floating meadows of the oxbow lakes of the Middle Purus River, Amazonas, Brazil. *Biota Neotropica*, 16(1), 1–15. <https://doi.org/10.1590/1676-0611-bn 2015-0093>
- Ramalho, W. P., Machado, I. F., & Vieira, L. J. S. (2018). Do flood pulses structure amphibian communities in floodplain environments? *Biotropica*, 50(2), 338–345. <https://doi.org/10.1111/btp.12523>
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic biology* 67: 901.
- Rannala B, Yang Z. (2003). Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics* 164: 1645–1656.

- Réjaud, A., Rodrigues, M. T., Crawford, A. J., Castroviejo-Fisher, S., Jaramillo, A. F., Chaparro, J. C., ... Fouquet, A. (2020). Historical biogeography identifies a possible role of Miocene wetlands in the diversification of the Amazonian rocket frogs (Aromobatidae: Allobates). *Journal of Biogeography*, (May), 1–11. <https://doi.org/10.1111/jbi.13937>
- Ribeiro-Júnior, M. A., Choueri, E., Lobos, S., Venegas, P., Torres-Carvajal, O., & Werneck, F. (2020). Eight in one: morphological and molecular analyses reveal cryptic diversity in Amazonian alopoglossid lizards (Squamata: Gymnophthalmoidea). *Zoological Journal of the Linnean Society*, (1984), 1–44. <https://doi.org/10.1093/zoolinlean/zlz155>
- Rivero, J. A. (1964). Salientios (Amphibia) en la Colección de la Sociedad de Ciencias Naturales la Salle de Venezuela. *Caribbean Journal of Science*, 4(1), 297–305.
- Rivero, J. A. (1971). Notas sobre los anfibios de Venezuela. I. Sobre los Hilidos de la Guayana Venezolana. *Caribbean Journal of Science*, 11(3–4), 181–193.
- Roberto, I. J., Bittencourt, P. S., Muniz, F. L., Hernández-Rangel, S. M., Nóbrega, Y. C., Ávila, R. W., ... Hrbek, T. (2020). Unexpected but unsurprising lineage diversity within the most widespread Neotropical crocodilian genus *Caiman* (Crocodylia, Alligatoridae). *Systematics and Biodiversity*, 18(4), 377–395. <https://doi.org/10.1080/14772000.2020.1769222>
- Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ*. 18;4:e2584. doi: 10.7717/peerj.2584.
- Rojas, R. R., Fouquet, A., De Carvalho, V. T., Ron, S., Chaparro, J. C., Vogt, R. C., ... Hrbek, T. (2018b). Redescription of the Amazonian tiny tree toad *Amazophrynella minuta* (Melin, 1941) (Anura: Bufonidae) from its type locality. *Zootaxa*, 4482(3), 511–526. <https://doi.org/10.11646/zootaxa.4482.3.4>
- Rojas, R. R., Fouquet, A., Ron, S. R., Hernández-Ruz, E. J., Melo-Sampaio, P. R., Chaparro,

- J. C., ... Hrbek, T. (2018a). A Pan-Amazonian species delimitation: high species diversity within the genus *Amazophrynella* (Anura: Bufonidae). *PeerJ*, 6, e4941. <https://doi.org/10.7717/peerj.4941>
- Sambrook, J. & Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual*. 3rd Edition, Vol. 1, Cold Spring Harbor Laboratory Press, New York.
- Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W., ... Canessa, S. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science*, 363(6434), 1459–1463. <https://doi.org/10.1126/science.aav0379>
- Schiesari, L., Zuanon, J., Azevedo-Ramos, C., Garcia, M., Gordo, M., Messias, M., & Vieira, E. M. (2003). Macrophyte rafts as dispersal vectors for fishes and amphibians in the Lower Solimões River, Central Amazon. *Journal of Tropical Ecology*, 19(3), 333–336. <https://doi.org/10.1017/S0266467403003365>
- Schneider, J. G. (1799). *Historia Amphibiorum Naturalis et Literariae. Fasciculus Primus. Continens Ranas, Calamitas, Bufones, Salamandras et Hydrosin genera et species descriptos Notisque Suis Distinctos*. In *Jena Frederici Frommani*.
- Segalla, M., Berneck, B., Canedo, C., Caramaschi, U., Cruz, C. A. G., Garcia, P. C. A., ... Langone, J. A. (2021). List of Brazilian Amphibians. *Herpetologia Brasileira*, 10(1), 121–216. <https://doi.org/10.5281/zenodo.4716176>
- Solis-Lemus C, Knowles LL, Ané C. (2014). Bayesian species delimitation combining multiple genes and traits in a unified framework. *Evolution* 69: 492–507.
- Stamatakis A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- Spix, J. B. von. (1824). *Animalia Nova Sive Species Novae Testudinum et Ranarum, quas in Itinere per Braziliam annis 1817 - 1820*.
- Sturaro, M. J., Costa, J. C. L., Maciel, A. O., Lima-Filho, G. R., Rojas-Runjaic, F. J. M.,

- Mejia, D. P., ... Peloso, P. L. V. (2020). Resolving the taxonomic puzzle of *Boana cinerascens* (Spic, 1824), with resurrection of *Hyla granosa gracilis* Melin, 1941 (Anura: Hylidae). *Zootaxa*, 4750(1), 1–30. <https://doi.org/https://doi.org/10.11646/zootaxa.4750.1.1>
- Thomé, M. T. C., Martins, M., & Brasileiro, C. A. (2021). Higher Diversity in Open Cerrado Supports the Role of Regional Processes in Shaping an Anuran Assemblage in Southeastern Brazil. *South American Journal of Herpetology*, 19(1), 1–7. <https://doi.org/10.2994/SAJH-D-18-00039.1>
- Thomé, M. T. C., Sequeira, F., Brusquetti, F., Carstens, B., Haddad, C. F. B., Rodrigues, M. T., & Alexandrino, J. (2016). Recurrent connections between Amazon and Atlantic forests shaped diversity in Caatinga four-eyed frogs. *Journal of Biogeography*, 43(5), 1045–1056. <https://doi.org/10.1111/jbi.12685>
- Trontelj, P., & Fier, C. (2009). Cryptic species diversity should not be trivialised. *Systematics and Biodiversity*, 7(1), 1–3. <https://doi.org/10.1017/S1477200008002909>
- Upton, K., Warren-Thomas, E., Rogers, I., & Docherty, E. (2014). Amphibian Diversity on Floating Meadows in Flooded Forests of the Peruvian Amazon. *Herpetological Review*, 45(2), 209–212.
- Vacher, J., Chave, J., Ficetola, F. G., Sommeria-Klein, G., Tao, S., Thébaud, C., ... Fouquet, A. (2020). Large-scale DNA-based survey of frogs in Amazonia suggests a vast underestimation of species richness and endemism. *Journal of Biogeography*, 47(8), 1781–1791. <https://doi.org/10.1111/jbi.13847>
- Vacher, J. P., Kok, P. J. R., Rodrigues, M. T., Lima, J. D., Lorenzini, A., Martinez, Q., ... Fouquet, A. (2017). Cryptic diversity in Amazonian frogs: Integrative taxonomy of the genus *Anomaloglossus* (Amphibia: Anura: Aromobatidae) reveals a unique case of diversification within the Guiana Shield. *Molecular Phylogenetics and Evolution*, 112,

158–173. <https://doi.org/10.1016/j.ympev.2017.04.017>

- Wallace, A. R. (1876). The geographical distribution of animals; with a study of the relations of living and extinct faunas as elucidating the past changes of the Earth's surface. New York: Harper & Brothers. Volume 1.
- Watters, J. L., Cummings, S. T., Flanagan, R. L., & Siler, C. D. (2016). Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. *Zootaxa*, 4072(4), 477–495. <https://doi.org/10.11646/zootaxa.4072.4.6>
- Werneck, F. P. (2011). The diversification of eastern South American open vegetation biomes: Historical biogeography and perspectives. *Quaternary Science Reviews*, 30(13–14), 1630–1648. <https://doi.org/10.1016/j.quascirev.2011.03.009>
- Werneck, F. P., Nogueira, C., Colli, G. R., Sites, J. W., & Costa, G. C. (2012). Climatic stability in the Brazilian Cerrado: Implications for biogeographical connections of South American savannas, species richness and conservation in a biodiversity hotspot. *Journal of Biogeography*, 39(9), 1695–1706. <https://doi.org/10.1111/j.1365-2699.2012.02715.x>
- Wesselingh, F. P., & Salo, J. A. (2006). A Miocene perspective on the evolution of the Amazonian biota. *Scripta Geologica*, (133), 439–458.
- Yang, Z., & Rannala, B. (2010). Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences*, 107(20), 9264–9269.
- Zhang C, Sayyari E, Mirarab S. (2017). ASTRAL-III: increased scalability and impacts of contracting low support branches. In RECOMB international workshop on comparative genomics, pp. 53–75. Springer, Cham

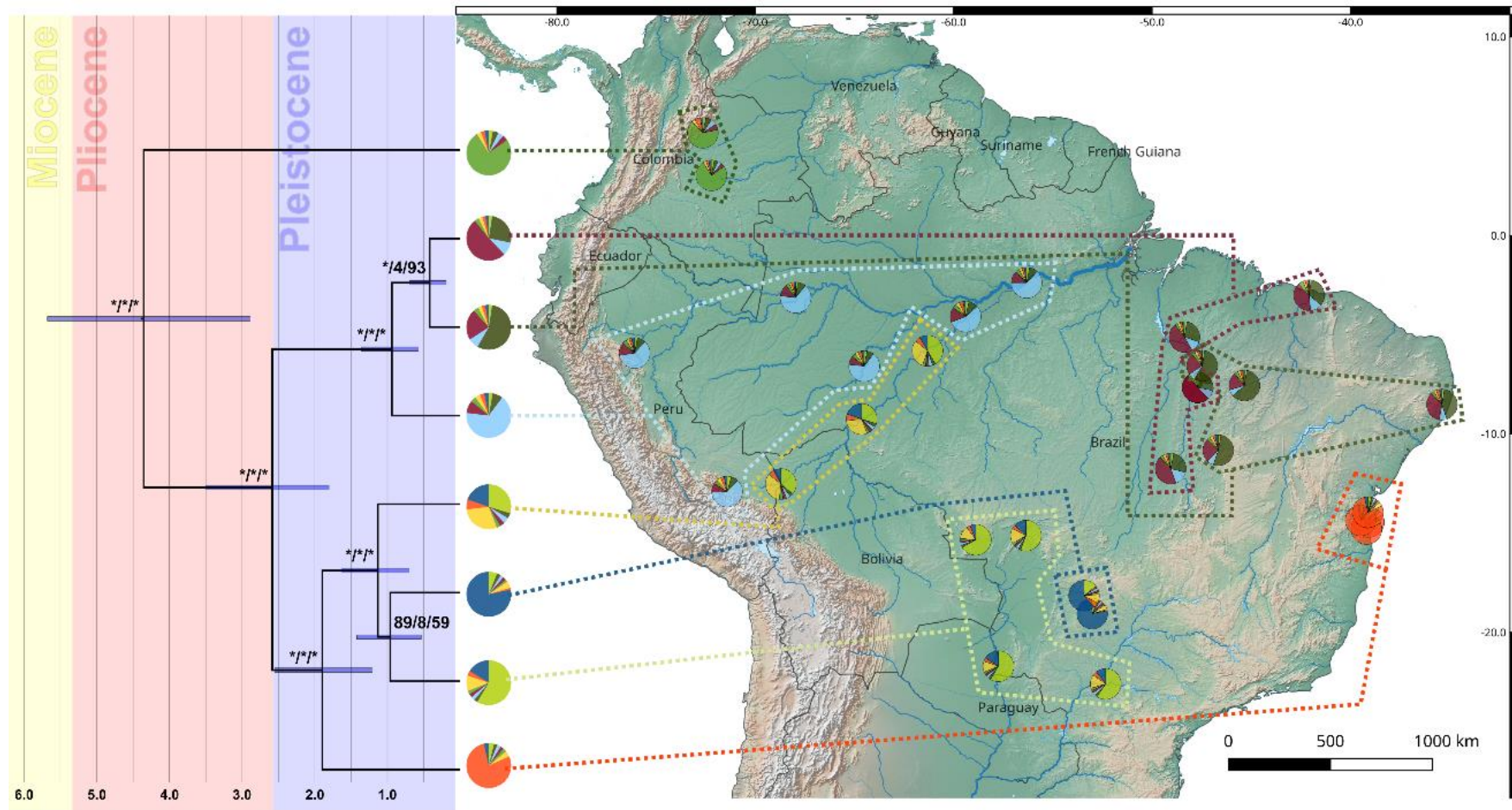


Figure 1. Time-calibrated tree obtained by the “supertree” in ASTRAL from 30 samples of *B. punctata* complex. Nodes values indicates the support of iBPP species discovery analysis with genetic data only/morphological data only/both data combined respectively Pie charts in the tip of the terminal indicate the populational structure obtained in fineRADstructure, for the delimited lineages. Pie charts and dashed lines indicates the geographic distribution and structure of the lineages, numbered from 1 to 8 (from the top to the bottom). The 95% confidence intervals for delimited lineages nodes are highlighted by horizontal blue bars.

Appendix I

Specimens examined

Boana atlantica (N=26): **BRAZIL: Bahia:** Aurelino Leal, CFBH 18724 – 18726; Camamu, CFBH 27814; Ilhéus, CFBH 32060, 34086, 35716, 39425, 42513 – 42517; Itagiba, CFBH 27690; Serra do Condurú, MTR 35125; Uruçuca, CFBH 23379, 32365, 32366, 13262, 32462, 34050, 13256, 13259, 13260, 13258, 37964.

Boana cf. atlantica (N=2): **BRAZIL: Pernambuco:** Igarussú: CFBH 2514, 2483.

Boana punctata: (N=246): **VENEZUELA: Guanare-Portuguesa,** UF 108635. **COLOMBIA: Orinoquia:** Vichada, UF 8517, 8519, 8518-1 – 8518-6. **TRINIDAD & TOBAGO: Arouca,** Tunapuna-Piarco Road: UF 3637; **Sangre-Grande:** Biche, UF 10988; Grande Riviere, UF 7300-1 – 7300-8; **San Juan:** Laventille, UF 7727-1, 7727-2. **ECUADOR: Pastaza:** Bataburo Lodge, QCAZ-A 39441; Kapawi Lodge, QCAZ-A 9021. 25487; **Orellana:** QCAZ-A 62207; El Desacanso, QCAZ-A 43790, 43792; Huino, QCAZ-A 30939; Pozo Coca, QCAZ-A 52374; Puerto Francisco de Orellana, QCAZ-A 489; Yasuni, QCAZ-A 22753, 51622, 54676, 55358; **Sucumbios:** Lago Agrio, QCAZ-A 6470; Limoncocha, QCAZ-A 8029, QCAZ-43105, 43117 – 43119, 43122, 61770, 63544; Santa Cecilia, QCAZ-A 177; **Napo:** Misahualli, QCAZ-A 406; Reserva Jatun Sacha, QCAZ-A 2874, 18178; San Pedro, QCAZ-A 57222; **Morona Santiago:** QCAZ-A 54450, 54452 – 54454; Namipias, QCAZ-A 46401 – 46402; Pankints, QCAZ-A 46424 – 46425. **PERU: Loreto:** Iquitos, MUBI 8821, 8842 – 8849; Requena, MUBI 9029 – 9030, 9098 – 9099, 9101, 9103 – 9105, 9107 – 9110, 9113 – 9114, 9116; Lagunas, MUBI 12715; Pacaya-Samiria, MUBI 10066 – 10067; Yurimaguas, YUR 01 – 02, YUR 05, YUR 11 – 12; **Ucayalli:** Yurua, MUBI 15179, 15181 – 15183. **SURINAME: Paramaribo:** UF 108636, 108640 – 108642, 108644 – 108646, 108648, 108649, 10655. **BRAZIL: Amazonas:** Benjamin Constant, RSOL 44, 45; Estação Ecológica (ESEC) Baixo Juruá, INPA-H 28533, 28534, 28536 – 28540; Iranduba – Lago do Catalão, CZPB-AA 505, 620, 654,969 – 971, INPA-H 1823 – 1827; Jutai, RSOL 99 – 106; Manacapuru, MANAC 381, 411, 413; Puruzinho, CTGA-N 6516; RESX Rio Gregório, INPA-H 30197; Santo Antônio do Içá, RSOL 79, 82, 83, 92, 97; São Pedro – Rio Içá, MTR 36266, 35750, 35799, 36706; Tefé, RSOL 100, 111; Terra Vermelha – Rio Purus, MTR 19338. **Acre:** Assis Brasil, UFAC 4066; Boca do Acre, UFAC 5296, 5297; Fazenda Catuaba, UFAC 537; Cruzeiro do Sul – Rio Moa/Igarapé Pentecoste, UFAC 6196, 6197; Manuel Urbano, UFAC 3343 – 3345; PAREST Chandless, UFAC 5429; Rio Branco, UFAC 138, 142, 144, AC 27; Sena Madureira, UFAC 286, 287, 292, 293, 452, 4778, 5530; Parque Zoobotanico-UFAC, UFAC 27, 30, 32, 42, 43, 207, 221, 228, 234, 3039. **Maranhão:** Balsas, BAL 123, CFBH 14014, UFMT-A 11220; Barreirinha, CFBH 26616; Estreito, CFBH 9160; Feira Nova do Maranhão, MAP 2652; Imperatriz, CFBH 8121; Porto Franco, CFBH 25935; Riachão, MAP 2650, 2651; São Pedro de Água Branca, SPAB 114, 117 – 119; **Tocantins:** Araguaína, CFBH 11425, 11435; MAP 2666, 5347; ZUFMS-AMP 7949, 7950; Babaçulândia – Wanderlândia road, CFBH 8168, 28515; Barra do Ouro, BO 125 – 130, 146 – 148; Brejinho de Nazaré, CFBH 28918; Colinas do Tocantins, CFBH 19886, 19887, 23494; Gurupi, CFBH 23509, 28926; Palmeirante, CFBH 28441, 28439; Porto Nacional, CFBH 33368 – 33370. **Pará:** Juruti, J346 – J351; Santarém, SANTAREM09, SANTAREM11. **Piauí:** URCA 2225, 2325, 2326, 2328, RIB, 161, 162, 175 – 182.

Boana rubrolineata (stat. nov.) (N= 169): **PERU: Madre de Dios,** Puerto Maldonado, MUBI 8153; Puerto Maldonado – El Prado, MUBI 8059, 8060; Puerto Maldonado – La cachuela, MUBI 8073; Pampas del Health, MUBI 11085, 11086, 128752, 12876.

BRAZIL: Amazonas: Manicoré, MG 270 – 274, 306 – 308. **Mato Grosso:** Araputanga, UFMT-A 5891, 5898, 5941, 5994, 6001, 6660, 11734; Brasnorte, UFMT-A 10126 – 10131; Cáceres, UFMT-A 1083, 1084, 1092; Jauru, UFMT-A 1214, 5944, 10836 – 10840, 10842 – 10846, 10849; Lambari D'Oeste, CFBH 21786; Nossa Senhora do Livramento, UFMT-A 4350, 5777, 5778; Poconé, UFMT-A 53, 279, 436, 507; Pontes e Lacerda, UFMT-A 15304, 15452, 16258; Reserva do Cabaçal, UFMT-A 2221, 2245, 2249, 2255, 2258; Tangará da Serra, UFMT-A 2240, 2252, 2271, 2289, 2296; Vale de São Domingos, UFMT-A 1915, 14717. **Mato Grosso do Sul:** Aquidauana, ZUFMS-AMP 1419, 1843, 1843(1); Bonito, ZUFMS-AMP 3521, 3522; Camapuã, MAP 203, ZUFMS-AMP 11172, 11718, 11719; Campo Grande, MAP 2613, 2614, ZUFMS-AMP 1076, 1079, 1417, 1619, 3309, 3326, 3530, 3532, 3535, 7951, 7955, 9697, 9699, 9700, 10177, 10178, 10180 – 10186, 10872; Corumbá, CFBH 4360, 4361, 40991 – 40993, 41003, 41004, 41008, 41010 – 41013, ZUFMS-AMP 1332, 1739, 11667, 11669, 11671, 11673; Costa Rica, ZUFMS-AMP 10854; Dourados, ZUFMS-AMP 10991; Itaporã, ZUFMS-AMP 478, 2333; Ladario, ZUFMS-AMP 1882, Nova Alvorada do Sul, ZUFMS-AMP 045, 467, 2302; Paraíso das Águas, ZUFMS-AMP 10855; PENRT-Taquari, ZUFMS-AMP 11511 – 11515; Porto Murtinho, ZUFMS-AMP 11891 – 11898; Sidrolândia-Niaoque, CFBH 14276. **Rondônia:** Costa Marques, INPA-H 6479, 6480, 6531, 6546; ESEC-Cuniã, Porto Velho, INPA-H 6667, 6669, 6696, 6697, 6699; Pimenta Bueno, UFMT-A 19314; Pimenteiras do Oeste, INPA-H 6744, 6746, 6747; Vilhena, INPA-H, 7167, UFMT-A 13737, 13780. **São Paulo:** Teodoro Sampaio - Parque Morro do Diabo, CFBH 12014, 12016 – 12020.

Supplemental Material

Table S8. List of species included in our phylogenetic tree and *B. punctata* complex samples used in species discovery analysis, along with their identification of Voucher or Field Number, GenBank accession, locality information. All data analysed here were produced during this study.

Species	Voucher/Field Number	GenBank Accession number	Locality
<i>Boana cinerascens</i>	CTGA_N3165	To be submitted	Serra do Divisor-Acre
<i>Boana cinerascens</i>	ROM42238	To be submitted	Kurupukari, Essequibo- Gyana
<i>Boana cinerascens</i>	ROM43209	To be submitted	Kaieteur National Park - Gyana
<i>Boana cinerascens</i>	ROM42213	To be submitted	Kaieteur National Park - Gyana
<i>Boana punctata</i>	TAS1357	To be submitted	Meta/Maripan – Colômbia
<i>Boana punctata</i>	TAS850	To be submitted	Casanare/Aguazul - Colômbia
<i>Boana punctata</i>	PCC12	To be submitted	Pilcopata-Peru
<i>Boana punctata</i>	CAN20	To be submitted	Rio Purus, Canutama/AM-Brazil
<i>Boana punctata</i>	CTGA_6516	To be submitted	Comunidade Puruzinho/AM-Brazil
<i>Boana punctata</i>	SOL.83	To be submitted	Sto Antonio do Içá/AM- Brazil
<i>Boana punctata</i>	YUR04	To be submitted	Yurimaguas- Peru
<i>Boana punctata</i>	J351	To be submitted	Juruti/PA- Brazil
<i>Boana punctata</i>	MAPT123	To be submitted	Ribeirão/PE-Brazil
<i>Boana punctata</i>	CFBH2018	To be submitted	Estreito/MA-Brazil
<i>Boana punctata</i>	RIB181	To be submitted	Ribeiro Gonçalves / PI-Brazil
<i>Boana punctata</i>	MTR14588	To be submitted	EESGT (Estação Ecológica Serra Geral)/ TO-Brazil
<i>Boana punctata</i>	PARN030	To be submitted	Araioses/MA-Brazil
<i>Boana</i>	SPAB117	To be submitted	São Pedro de

<i>punctata</i>			Água Branca / MA- Brazil
<i>Boana punctata</i>	CFBH11275	To be submitted	Gurupi/TO- Brazil
<i>Boana punctata</i>	BO128	To be submitted	Barra do Ouro / TO- Brazil
<i>Boana punctata</i>	BO130	To be submitted	Barra do Ouro / TO- Brazil
<i>Boana punctata</i>	MNHC12876	To be submitted	Pampas del Heath/Peru
<i>Boana punctata</i>	H3060	To be submitted	UHE Jirau, Mutum/ RO- Brazil
<i>Boana punctata</i>	MG273	To be submitted	Manicoré/AM- Brazil
<i>Boana punctata</i>	MAP3004	To be submitted	Alcinópolis/MS- Brazil
<i>Boana punctata</i>	CFBH1088	To be submitted	Paraiso/MS- Brazil
<i>Boana punctata</i>	CFBH15116	To be submitted	Porto Murtinho/MS- Brazil
<i>Boana punctata</i>	RHO666	To be submitted	Jauru/MT- Brazil
<i>Boana punctata</i>	CFBH4742	To be submitted	Acorizal/MT - Brazil
<i>Boana punctata</i>	CFBH9537	To be submitted	Teodoro Sampaio/SP- Brazil
<i>Boana atlantica</i>	CFBH12827	To be submitted	Camumu/BA- Brazil
<i>Boana atlantica</i>	CFBH9240	To be submitted	Aurelino Leal/BA- Brazil
<i>Boana atlantica</i>	MTR35125	To be submitted	Parque Estadual serra do Condurú/BA- Brazil
<i>Boana atlantica</i>	CFBH15788	To be submitted	Ilheus/BA - Brazil

Tabela S2. Geographic association scheme used to define the association between morphological and genetic data to perform the iBPP species delimitation analysis. Columns indicates the samples identification, examined specimens and the geographic range including both genetic and morphological data.

Sample ID	Voucher/Field Number of specimens from morphometric data	Locality
BO128	CFBH11435; CFBH23494; CFBH28441; CFBH28439; ZUFMS-AMP7949; ZUFMS-AMP7950; BO_148; BO_147; BO129; BO_128; BO_125	Tocantins-Brazil
BO130	CFBH19886; CFBH19887; BO127; BO126; BO_130; BO_146	Tocantins-Brazil
CAN20	MTR19338; UFAC-452; UFAC-3039; UFAC-27; UFAC-207; UFAC-292; UFAC-4778; UFAC-293; UFAC-3344; UFAC-5429; UFAC-6196; UFAC-6197; UFAC-5296; UFAC-5297; UFAC-4066; UFAC-286; UFAC-287; UFAC-3345; UFAC-3343; UFAC-5530; UFAC-142; UFAC-32; UFAC-138; UFAC-42; UFAC-228; UFAC-43; UFAC-144; UFAC-221; UFAC-234; UFAC-30; UFAC-537; INPAH-30197	Southern Amazon – Purus River and Acre-Brazil
CFBH1088	ZUFMS-AMP11172; ZUFMS-AMP11718; ZUFMS-AMP11719; ZUFMS-AMP10855; MAP203	Mato Grosso do Sul-Taquari Basin-Brazil
CFBH11275	CFBH33370; CFBH28918; CFBH33368; CFBH23509; CFBH28926	Tocantins-Brazil
CFBH27814	CFBH27690; CFBH27814	Bahia- Brazil
CFBH15116	CFBH4361; CFBH4360; CFBH41003; CFBH41013; CFBH41004; CFBH41012; CFBH41008; CFBH41010; CFBH41011; CFBH40991; CFBH40992; CFBH40993; CFBH14276; ZUFMS-AMP11669; ZUFMS-AMP11667; ZUFMS-AMP11671; ZUFMS-AMP11673; ZUFMS-AMP1739; ZUFMS-AMP1332; ZUFMS-AMP11897; ZUFMS-AMP11898; ZUFMS-AMP11896; ZUFMS-AMP11891; ZUFMS-AMP11892; ZUFMS-AMP11895; ZUFMS-AMP11893; ZUFMS-AMP11894; ZUFMS-AMP1076; ZUFMS-AMP1417; ZUFMS-AMP1619; ZUFMS-AMP10872; ZUFMS-AMP3535; ZUFMS-AMP3326; ZUFMS-AMP3532; ZUFMS-AMP3530; ZUFMS-AMP3309; ZUFMS-AMP9697; ZUFMS-AMP7951; ZUFMS-AMP7955; ZUFMS-AMP9700; ZUFMS-AMP9699; ZUFMS-AMP10182; ZUFMS-AMP10178; ZUFMS-AMP10184; ZUFMS-AMP10181; ZUFMS-AMP10183; ZUFMS-AMP10186; ZUFMS-AMP1079; ZUFMS-AMP10177; ZUFMS-AMP10185; ZUFMS-AMP10180; ZUFMS-AMP1843(1); ZUFMS-	Mato Grosso do Sul-Cerrado, Pantanal Domain - Brazil

	AMP1843; ZUFMS-AMP1419; ZUFMS-AMP1882; ZUFMS-AMP3521; ZUFMS-AMP3522; MAP2613; MAP2614	
CFBH15788	CFBH34086; CFBH39425; CFBH42514; CFBH42513; CFBH42517; CFBH42516; CFBH42515; CFBH32060; CFBH35716	Ilheus-Bahia-Brazil
CFBH2018	CFBH8168; CFBH28515; CFBH11425; CFBH25935; CFBH9160; CFBH8121; MAP5347; MAP2652; MAP2666	Tocantins-Brazil Maranhão-Brazil
CFBH4742	UFMT-A1084; UFMT-A1083; UFMT-A436; UFMT-A507; UFMT-A279; UFMT-A53; UFMT-A2296; UFMT-A5777; UFMT-A1092; UFMT-A5778; UFMT-A2252; UFMT-A2289; UFMT-A2271; UFMT-A2240; UFMT-A4350	Mato Grosso-Brazil
CFBH18725	CFBH18725; CFBH18724; CFBH18726	Aurelino Leal-Bahia- Brazil
CFBH9537	CFBH12020; CFBH12018; CFBH12017; CFBH12014; CFBH12016; CFBH12019; ZUFMS-AMP478; ZUFMS-AMP2333; ZUFMS-AMP10991; ZUFMS-AMP2302; ZUFMS-AMP467; ZUFMS-AMP0045	Paraná River Basin- Brazil
CTGA_N6516	MANAC0381; CZPBAA970; CZPBAA620; CZPBAA971; CZPBAA654; CZPBAA505; CZPBAA969; CTGAN_6516; MANAC0411; MANAC0413; INPAH-1827; INPAH-1826; INPAH-1825; INPAH-1823; INPAH-1824	Lower Solimões Amazonas River
H3060	UFMT-A19314; INPAH-6669; INPAH-6479; INPAH-6699; INPAH-6667; INPAH-6696; INPAH-6480; INPAH-6531; INPAH-7167; INPAH-6546; INPAH-6697	Rondônia-Brazil
J351	SANTAREM11; SANTAREM09; J347; J346; J349; J348; J351; J350	Para- Brazil
MAP3004	ZUFMS-AMP10854; ZUFMS-AMP11513; ZUFMS-AMP11515; ZUFMS-AMP11514; ZUFMS-AMP11511; ZUFMS-AMP11512	Taquari- Mato Grosso do Sul-Brazil
MAPT123	CFBH2483; CFBH2514	Igarassu-Pernambuco-Brazil
MG273	MG306; MG273; MG307; MG308; MG274; MG270; MG272; MG271	Manicore-Amazonas-Brazil
MNHC12876	MUBI_8059; MUBI_8060; MUBI_8073; MUBI_8153; MUBI_11085; MUBI_11086; MUBI_12875; MUBI_12876	Madre de Dios- Peru
MTR14588	CFBH33369	Porto Nacional

		Tocantins- Brazil
MTR35125	MTR35125; CFBH23379; CFBH32366; CFBH13262; CFBH32462; CFBH34050; CFBH13256; CFBH13260; CFBH32365; CFBH13259; CFBH37964; CFBH13258	Uruçuca and Serra do Conduru- Bahia- Brazil
CFBH26616	PARN030	Barreirinha- Maranhão- Brazil
PCC12	MUBI_12715; MUBI_15179; MUBI_15181; MUBI_15182; MUBI_15183	Loreto and Ucayali- Peru
RHO666	CFBH21786; UFMT-A10846; UFMT-A10131; UFMT- A10128; UFMT-A10844; UFMT-A5891 UFMT-A10837; UFMT-A11734; UFMT-A10130; UFMT-A5944; UFMT-A10838; UFMT-A10129; UFMT-A12127; UFMT-A10126; UFMT-A10849; UFMT-A10839; UFMT-A10843; UFMT-A10846; UFMT-A10836; UFMT-A1214; UFMT-A10840; UFMT-A10845; UFMT-A5984; UFMT-A6001; UFMT- A6660; UFMT-A15452; UFMT-A5898; UFMT-A1915; UFMT-A15304; UFMT-A5941; UFMT-A2221; UFMT- A2245; UFMT-A5994; UFMT-A16258; UFMT- A13737; UFMT-A13780; UFMT-A14717; UFMT- A2249; UFMT-A2255; UFMT-A10842; UFMT-A2258; INPAH-6746; INPAH-6744; INPAH-6747	Pantanal- Guaporé Basin- Brazil
RIB181	CFBH14014; MAP2651; MAP2650; UFMT-A11220; URCA_2325; URCA_2328; URCA_2326; URCA_2225; BAL_123; RIB_161; RIB_162; RIB_175; RIB_176; RIB_177; RIB_178; RIB_179; RIB_180; RIB_181; RIB182	Maranhão and Tocantins- Brazil
SOL83	MTR36266; MTR35799; MTR35750; MTR36706; MUBI_8821; MUBI_8842; MUBI_8843; MUBI_8844; MUBI_8845; MUBI_8846; MUBI_8847; MUBI_8848; MUBI_8849; MUBI_10066; MUBI_10067; INPAH- 28539; INPAH-28533; INPAH-28536; INPAH-28538; INPAH-28540; INPAH-28537; INPAH-28534; RSOL99; RSOL101; RSOL105; RSOL103; RSOL100; RSOL106; RSOL104; RSOL10; RSOL110; RSOL111, RSOL53; RSOL45; RSOL44; RSOL97; RSOL83; RSOL92; RSOL82; RSOL79; ACRE27; RSOL57; RSOL67; RSOL56; RSOL55; RSOL58; RSOL71; RSOL76; RSOL72; RSOL70	Upper Amazon Brazil and Peru
SPAB117	SPAB_114; SPAB_117; SPAB_118; SPAB_119	São Pedro de Água Branca- Maranhão
TAS_1357	QCAZ30939; QCAZ18178; QCAZ406; QCAZ2874; QCAZ177; QCAZ57222; QCAZ25487; QCAZ9021;	Ecuador – Colômbia

	QCAZ39441; QCAZ53762; QCAZ54450; QCAZ54452; QCAZ54454; QCAZ54453; QCAZ52608; QCAZ52607; QCAZ52603; QCAZ52604; QCAZ46401; QCAZ46424; QCAZ46425; QCAZ46402; QCAZ52374; QCAZ55358; QCAZ62207; QCAZ54676; QCAZ63544; QCAZ61770; QCAZ43790; QCAZ489; QCAZ22753; QCAZ43792; QCAZ51622; QCAZ6470; QCAZ43105; QCAZ43117; QCAZ43119; QCAZ8029; QCAZ43118; QCAZ43122; UF_108635; UF_8517	Llanos
TAS_850	UF_8518_1; UF_8518_2; UF_8518_3; UF_8518_4; UF_8518_5 UF_8518_6; UF_8519	Colômbia- Oriquia Llanos
YUR04	MUBI_9029; MUBI_9030; MUBI_9098; MUBI_9099; MUBI_9101; MUBI_9103; MUBI_9104; MUBI_9105; MUBI_9107; MUBI_9108; MUBI_9109; MUBI_9110; MUBI_9113; MUBI_9114; MUBI_9116; YURI12; YURI11; YURI05; YURI01; YURI02	Loreto- Peru

CONSIDERAÇÕES FINAIS

Os resultados obtidos nos três capítulos desta tese trazem importantes contribuições acerca da sistemática, diversidade e taxonomia do grupo *B. punctata*, redelimitando este agrupamento taxonômico e revelando um complexo de espécies dentro do que hoje é conhecido como *B. punctata*, com base em evidências genéticas, morfológicas e biogeográficas.

Acessada pela primeira vez por dados genéticos, a posição filogenética de *Boana hobbsi* não só confirma o status de espécie plena, como também a reposiciona no grupo *Boana benitezi*, sugerindo que esta não tenha parentesco próximo às espécies do grupo *B. punctata*. Além disso, identificamos caracteres morfológicos que corroboram essa relação.

Ainda, na topologia obtida, o posicionamento de *Boana jimenezi* (grupo *B. punctata*) também foi recuperado no grupo *B. benitezi* resultado que confirma suposições prévias de que esta espécie também não tem parentesco com ao conjunto de espécies ao qual ela era assinalada até o presente estudo. Das espécies que compõem o grupo *B. punctata*, apenas a posição de *Boana alemani* não foi testada formalmente, embora o status taxonômico dessa espécie seja incerto. Dessa forma, propusemos uma reformulação do grupo *B. punctata* contendo o complexo *B. cinerascens* e o complexo *B. punctata*, e de forma provisória *B. alemani* e *B. sibleszi* que continuam com posicionamentos filogenéticos incertos. Consequentemente, esse resultado também implica na reformulação do grupo *B. benitezi* que a partir deste trabalho compreende as espécies: *B. hobbsi*, *B. benitezi*, *B. lemai*, *B. rhythmica*, *B. jimenezi*, *B. ornatissima*, *B. tepuiana*, *B. nympa*, *B. microderma* e *B. roraima*.

No capítulo 2 desta tese, identificamos através dos métodos de delimitação de espécies a existência de pelo menos seis linhagens, das quais cinco possuem ao menos duas fontes de evidência (genética e fenotípica) que as confirmam como espécies candidatas. Tais linhagens possuem divergência genética significativa e apresentaram clara estrutura

geográfica, sem sobreposição dos limites de distribuição destas entidades evolutivas. Esses resultados, confirmam a existência de um complexo de espécies dentro do binômio *B. punctata*, e em conjunto com as análises dos dados morfológicos e acústicos permitem a reavaliação do status taxonômico de *Hyla punctata rubro-lineata* elevando-a ao status de espécie plena sob o nome *Boana rubrolineata*. Dessa forma, o complexo *B. punctata* passa a conter três espécies reconhecidas: *Boana punctata*, que compreende as linhagens da Amazônia, do Escudo Guianas, do norte do estado do Maranhão, e da zona litorânea da Mata Atlântica ao norte do rio São Francisco no nordeste brasileiro; *Boana atlantica*, aqui redefinida apenas às linhagens que compreendem as populações do litoral baiano da Mata Atlântica; e *Boana rubrolineata* que corresponde às linhagens relacionadas às áreas alagadas das bacias dos rios Madre de Dios - Madeira - Guaporé no Peru, Brasil e Bolívia, ao Pantanal, e nas bacias dos rios Paraguai e Paraná na região dos Chacos da Argentina e Paraguai.

Por fim no capítulo 3, utilizando dados moleculares nucleares e análises de delimitação de espécies, obtivemos uma reconstrução filogenética bem suportada que nos permitiu identificar haver uma diversidade ainda maior que a esperada no complexo *B. punctata*. Esse resultado confirma o status das espécies nominais do complexo, e a ocorrência de pelo menos duas espécies candidatas, uma, na região dos llanos da Colômbia e outra envolvendo as linhagens do norte do Cerrado, na bacia dos Rios Tocantins e Parnaíba e norte da Mata Atlântica no estado de Pernambuco. Além disso, para *B. rubrolineata* os resultados apontam uma divergência entre as populações que compõem a linhagem da bacia dos Rios Madre de Dios - Madeira em relação às populações mais ao sul que ocorrem no Pantanal e bacias dos rios Paraguai e Paraná. Os padrões filogeográficos observados e as análises de datação proporcionam evidências do papel importante dos eventos que moldaram a paisagem da América do Sul do final do Mioceno até o Pleistoceno, na diversificação deste complexo de espécies. As dinâmicas geológicas da região do sistema Pebas, responsáveis pela

estruturação atual das bacias de drenagem da Amazônia coincidem com as fases iniciais de divergência das linhagens do complexo, e posteriormente os eventos de flutuações climáticas que levaram a expansão de áreas de savanas ou de vegetação aberta coincidem com a dispersão das linhagens nas regiões dos atuais, Pantanal, Cerrado e Mata Atlântica no sul e na dispersão para a região norte do nordeste brasileiro.

Os resultados desta tese, fornecem importante base teórica para uma possível revisão taxonômica do complexo *B. punctata*, que ainda abriga sinonímias a serem avaliadas com a inclusão de amostras de dados genômicos para regiões das Guianas, e de outras lacunas de amostragem que podem fornecer melhor definição dos limites de distribuição das linhagens encontradas. Além disso, a delimitação de espécies dentro desse complexo, evidenciam a ocorrência dessas de linhagens e espécies putativas em regiões sob forte ameaça como a porção norte do Cerrado, norte da Mata Atlântica e Pantanal. Tais regiões sofrem atualmente com avanço do desmatamento ilegal, expansão agrícola e queimadas, e possivelmente abrigam diversas espécies ainda “escondidas” sob o binômio de táxons amplamente distribuídos. Dessa forma, fica clara a importâncias desses ambientes como áreas críticas para a conservação, uma vez que o avanço das ameaças é intenso em comparação com a capacidade de pesquisadores descobrirem essas espécies.

Referências Bibliográficas

- Aleixo, A. (2009) Conceitos de espécie e suas implicações para a conservação. *Megadiversidade*, 5, 87-95.
- Angulo, A., & Icochea, J. (2010). Cryptic species complexes, widespread species and conservation: Lessons from Amazonian frogs of the *Leptodactylus marmoratus* group (Anura: Leptodactylidae). *Systematics and Biodiversity*, 8(3), 357–370. <https://doi.org/10.1080/14772000.2010.507264>
- Antonelli, A., Ariza, M., Albert, J., Andermann, T., Azevedo, J., Bacon, C., Faurby, S., Guedes, T., Hoorn, C., Lohmann, L.G., Matos-Maraví, P., Ritter, C.D., Sanmartín, I., Silvestro, D., Tejedor, M., ter Steege, H., Tuomisto, H., Werneck, F.P., Zizka, A. & Edwards, S.V. (2018a) Conceptual and empirical advances in Neotropical diversity research. *PeerJ*, 6, e5644. <https://doi.org/10.7717/peerj.5644>
- Antonelli, A., Zizka, A., Carvalho, F.A., Schran, R., Bacon, C.D., Silvestro, D. & Condamine, F.L. (2018b) Amazonia is the primary source of Neotropical biodiversity. *PNAS*, 115, 23, 6034–6039. <https://doi.org/10.1073/pnas.1713819115>
- Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., ... Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, 22(3), 148–155. <https://doi.org/10.1016/j.tree.2006.11.004>
- Bicudo, T. C., Sacek, V., Almeida, R. P. De, Bates, J. M., & Ribas, C. C. (2019). Andean Tectonics and Mantle Dynamics as a Pervasive Influence on Amazonian Ecosystem. *Scientific Reports*, (November), 1–11. <https://doi.org/10.1038/s41598-019-53465-y>
- Burbrink F. T. & Ruane, S. (2021) Contemporary phylosophy and methods for studying speciation and delimitating species. *Ichthyology & Herpetology*, 109 (3), 874 – 894.
- Caminer, M.A. & Ron, S.R. (2014) Systematic of the *Hypsiboas calcaratus* and *Hypsiboas fasciatus* species complex (Anura, Hylidae) with description of four new species. *Zookeys*, 370, 1, 1–68. <https://doi.org/10.3897/zookeys.370.6291>
- Caminer, M.A. & Ron, S.R. (2020) Systematics of the *Boana semilineata* species group (Anura: Hylidae), with a description of two new species from Amazonian Ecuador. *Zoological Journal of the Linnean Society*, zlaa002. <https://doi.org/10.1093/zoolinnea/zlaa002>
- Carstens, B.; Pelletier, T. A.; Reid, N. M. & Salter, J. D. (2013) How to fail at species delimitation. *Molecular Ecology*, 22, 4269 – 4383.

- Cracraft, J. (1983) Species concept and speciation analysis. *Current Ornithology*, 1, 159 – 187.
- Darwin, C. (1859). On the origin of species by means of natural selection, or, the preservation of favoured races in the struggle for life. London: J. Murray.
- Dayrat, B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society*. Soc. 85: 407–415.
- De Queioz, K. (2007) Species concepts and species delimitation. *Systematic Biology*. 56 (6), 879 – 886.
- Diniz-Filho, J.A.F., Loyola, R.D., Raia, P., Mooers, A.O. & Bini, L.M. (2013) Darwinian shortfall in biodiversity conservation. *Trends in Ecology and Evolution*, 28, 12, 689–695. <https://linkinghub.elsevier.com/retrieve/pii/S0169534713002127>
- Dobzhansky, T. (1970) Genetics of the evolutionary process. Columbia University Press, New York.
- Duellman, W.E.; Marion, A.B. & Hedges, S.B. (2016) Phylogenetics, classification, and biogeography of the treefrogs (Amphibia: Anura: Arboranae). *Zootaxa*, 4140, 1, 1–109. <https://doi.org/10.11646/zootaxa.4104.1.1>
- Faivovich, J., Köhler, G., Araujo-Vieira, K., Blotto, B. L., Baêta, D., Haddad, C. F. B., ... Campbell, J. A. (2018). On the Monophyly and Relationships of Several Genera of *Hylini* (Anura: Hylidae: Hylinae), with Comments on Recent Taxonomic Changes in Hylids. *South American Journal of Herpetology*, 13(1), 1–32. <https://doi.org/10.2994/sajh-d-17-00115.1>
- Faivovich, J., D.P. Pinheiro, P., Lyra, M. L., Pereyra, M. O., Baldo, D., Muñoz, A., ... Haddad, C. F. B. (2021). Phylogenetic relationships of the *Boana pulchella* Group (Anura: Hylidae). *Molecular Phylogenetics and Evolution*, 155. <https://doi.org/10.1016/j.ympev.2020.106981>
- 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, 1–240. [https://doi.org/10.1206/0003-0090\(2005\)294\[0001:srotff\]2.0.co;2](https://doi.org/10.1206/0003-0090(2005)294[0001:srotff]2.0.co;2)
- Faivovich, J., McDiarmid, R.W. & Myers, C.W. (2013) Two new species of *Myersiohyla* (Anura: Hylidae) from Cerro de la Neblina, Venezuela, with comments on other species of the genus. *American Museum Novitates*, 3792, 1–63. <https://doi.org/10.1206/3792.1>
- Ferrão, M., Colatreli, O., de Fraga, R., Kaefer, I. L., Moravec, J., & Lima, A. P. (2016). High

- Species Richness of *Scinax* Treefrogs (Hylidae) in a Threatened Amazonian Landscape Revealed by an Integrative Approach. *PLOS ONE*, 11(11), e0165679. <https://doi.org/10.1371/journal.pone.0165679>
- Fouquet, A., Gilles, A., Vences, M., Marty, C., Blanc, M. & Gemmell, N.J. (2007) Underestimation of species richness in Neotropical frogs revealed by mtDNA analyses. *PLoS ONE*, 2, e1109. <https://doi.org/10.1371/journal.pone.0001109>
- Fouquet, A., Martinez, Q., Zeidler, L., Courtois, E.A., Gaucher, P., Blanc, M., Lima, J.D., Souza, S.M., Rodrigues, M.T. & Kok, P.J.R. (2016) Cryptic diversity in the *Hypsiboas semilineatus* species group (Amphibia, Anura) with description of a new species from eastern Guiana Shield. *Zootaxa*, 4084, 1, 79–104. <http://biotaxa.org/Zootaxa/article/view/zootaxa.4084.1.3>
- Fouquet, A., Noonan, B.P., Blanc, M. & Orrico, V.G.D. (2011) Phylogenetic position of *Dendropsophus gaucheri* (Lescure and Marty 2000) highlights the need for an in-depth investigation of the phylogenetic relationships of *Dendropsophus* (Anura: Hylidae). *Zootaxa*, 3035, 59–67. <http://dx.doi.org/10.11646/zootaxa.3035.1.3>
- Frost, D. R., Grant, T., Faivovich, J., Bain, R. H., Haas, A., Haddad, C. F., ... & Wheeler, W. C. (2006). The amphibian tree of life. *Bulletin of the American Museum of natural History*, 2006(297), 1-291.
- Frost, D.R. (2022) Amphibians Species of the World: an Online Reference. Version 6.1. American Museum of Natural History, New York, USA. Eletronic Database accessible at <https://amphhibiansoftheworld.amnh.org/index.php> (accessed 7 April 2020) <https://doi.org/10.5531/db.vz.0001>
- Fujita, M. K., Leaché, A. D., Burbrink, F. T., McGuire, J. A., & Moritz, C. (2012). Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology & Evolution*, 27(9), 480-488. <https://doi.org/10.1016/j.tree.2012.04.012>
- Funk, C. W., Caminer, M., & Ron, S. R. (2012). High levels of cryptic species diversity uncovered in Amazonian frogs. *Proceedings of the Royal Society B: Biological Sciences*, 279(1734), 1806–1814. <https://doi.org/10.1098/rspb.2011.1653>
- Guarnizo, C.E, Paz, A., Muñoz-Ortiz, A., Flechas, S.V., Méndez-Narváez, J. & Crawford, A.J. (2015) DNA Barcoding Survey of Anurans across the Eastern Cordillera of Colombia and the Impact of the Andes on Cryptic Diversity. *PLoS ONE*, 10, 5, e0127312. <https://doi.org/10.1371/journal.pone.0127312>
- Hao, X., Jiang, R., & Chen, T. (2011). Clustering 16S rRNA for OTU prediction: a method of unsupervised Bayesian clustering. *Bioinformatics*, 27(5), 611 – 618.

- Henning, R. G. (1966) Phylogenetic systematics. University of Illinois Press, Urbana.
- Hillis, D. M. (2019) Species delimitation in Herpetology. *Journal of Herpetology*, 53 (1), 3 – 12.
- Hillis, D. M., Chambers, E. A., & Devitt, T. J. (2021). Contemporary methods and evidence for species delimitation. *Ichthyology & Herpetology*, 109(3), 895-903.
- Hoorn, C., Wesselingh, F. P., Ter Steege, H., Bermudez, M. A., Mora, A., Sevink, J., ... Antonelli, A. (2010). Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science*, 330(6006), 927–931. <https://doi.org/10.1126/science.1194585>
- Hortal, J., De Bello, F., Diniz-Filho, J. A. F., Lewinsohn, T. M., Lobo, J. M., & Ladle, R. J. (2015). Seven Shortfalls that Beset Large-Scale Knowledge of Biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, 46, 523–549. <https://doi.org/10.1146/annurev-ecolsys-112414-054400>
- IUCN. 2021. The IUCN Red List of Threatened Species. Version 2021-3. <https://www.iucnredlist.org>. Accessed on [13th May, 2022].
- Jansen, M., Bloch, R., Schulze, A. & Pfenninger, M. (2011) Integrative inventory of Bolivia's lowland anurans reveals hidden diversity. *Zoologica Scripta*, 40, 6, 567–583. <https://doi.org/10.1111/j.1463-6409.2011.00498.x>
- Jenkins, C. N., Pimm, S. L., & Joppa, L. N. (2013). Global patterns of terrestrial vertebrate diversity and conservation. *Proceedings of the National Academy of Sciences of the United States of America*, 110(28), E2603–E2610. <https://doi.org/10.1073/pnas.1302251110>
- Kapli, P., Lutteropp, S., Zhang, J., Kobert, K., Pavlidis, P., Stamatakis, A., & Flouri, T. (2017). Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics* (Oxford, England), 33(11), 1630–1638. <https://doi.org/10.1093/bioinformatics/btx025>
- Lima, L.R., Dubeux, M.J.M., Nascimento, F.A.C.D., Bruschi, D.P. & Mott, T. (2019) Uncovering Neotropical treefrog diversity: Integrative taxonomy reveal paraphyly in *Boana atlantica* (Amphibia, Anura, Hylidae). *Amphibia Reptilia*, 40, 4, 511–521. <https://doi.org/10.1163/15685381-20191109>
- Lomolino, M.V. (2004) Conservation Biogeography. In: Lomolino MV, Heaney LR (eds) *Frontiers of Biogeography: New Directions in the Geography of Nature*. Sinauer, Sunderland, pp 293–296.

- Luo, A., Ling, C., Ho, S. Y., & Zhu, C. D. (2018). Comparison of methods for molecular species delimitation across a range of speciation scenarios. *Systematic Biology*, 67(5), 830-846.
- Magalhães, R. F. de, Rocha, P. C., Santos, F. R., Strüssmann, C., & Giaretta, A. A. (2018). Integrative taxonomy helps to assess the extinction risk of anuran species. *Journal for Nature Conservation*, 45(July), 1–10. <https://doi.org/10.1016/j.jnc.2018.07.001>
- Mayr, E. (1942) Systematic and origin of species. Columbia University Press, New York.
- Mayer, M., da Fonte, L.F.M. & Lötters, S. (2019) Mind the gap! A review of Amazonian anurans in GenBank. *Salamandra*, 55, 2, 89–96. <http://doi.org/10.5281/zenodo.2840666>
- Meseguer, A. S., Antoine, P., Fouquet, A., Delsuc, F., & Condamine, F. L. (2020). The role of the Neotropics as a source of world tetrapod biodiversity. *Global Ecology and Biogeography*, (April), 1–14. <https://doi.org/10.1111/geb.13141>
- Morrone, J.J. (2014) Biogeographic regionalization of the Neotropical region. *Zootaxa*, 3782, 1, 1–110. <http://biotaxa.org/Zootaxa/article/view/zootaxa.3782.1.1>
- Padial, J.M., Miralles, A., De la Riva, I. & Vences, M. (2010) The integrative future of taxonomy. *Frontiers in Zoology*, 7, 16. <https://doi.org/10.1186/1742-9994-7-16>
- Peloso, P.L.V., Oliveira, R.M., Sturaro, M.J., Rodrigues, M.T., Lima-Filho, G.R., Bitar, Y.O.C., Wheeler, W.C. & Aleixo, A. (2018) Phylogeny of map tree frogs, *Boana semilineata* species group, with new Amazonian species (Anura: Hylidae). *South American Journal of Herpetology*, 13, 2, 150–169. <http://doi.org/10.2994/SAJH-D-17-00037.1>
- Pinheiro, P.D.P., Kok, P.J.R., Noonan, B.P., Means, D.B., Haddad, C.F.B. & Faivovich, J. (2019) A new genus of Cophomantini, with comments on the taxonomic status of *Boana liliae* (Anura: Hylidae). *Zoological Journal of the Linnean Society*, 185, 226–245. <https://doi.org/10.1093/zoolinnean/zly030>
- Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. J. M. E. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, 21(8), 1864-1877.
- Pyron, R.A. & Wiens, J.J. (2011) A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Biology and Evolution*, 61, 543–583. <https://doi.org/10.1016/j.ympev.2011.06.012>
- Ramos, E.K.S., Magalhães, R.F., Marques, N.C.S., Baêta, D., Garcia, P.C.A. & Santos, F.R. (2019) Cryptic diversity in Brazilian endemic monkey frogs (Hylidae, Phyllomedusinae,

- Pithecopus*) revealed by multispecies coalescent and integrative approaches. *Molecular Phylogenetics and Evolution*, 132, 105–116.
<https://doi.org/10.1016/j.ympev.2018.11.022>
- Rannala B, Yang Z. (2003). Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics* 164: 1645–1656.
- Rannala B.H. 2015. The art and science of species delimitation. *Current Zoology*, 61:846–853.
- Sandwith, N.Y. (1925) Humboldt and Bonpland's itinerary in Venezuela. *Bulletin of Miscellaneous Information*, 7: 295–310.
- Spix, J.B. (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, 1–53 pp.
<https://doi.org/10.5962/bhl.title.63182>
- Sturaro, M.J., Costa, J.C.L., Maciel, A.O., Lima-Filho, G.R., Rojas-Runjaic, F.J.M., Mejia, D.P. & Peloso, P.L.V. (2020) Resolving the taxonomic puzzle of *Boana cinerascens* (Spix, 1824), with resurrection of *Hyla granosa gracilis* Melin, 1941 (Anura:Hylidae). *Zootaxa*, 4750, 1, 1–30. <https://doi.org/10.11646/zootaxa.4750.1.1>
- Sukumaran, J., & Knowles, L. L. (2017). Multispecies coalescent delimits structure , not species. *PNAS*, 114(7), 1607–1612.
<https://doi.org/https://doi.org/10.1073/pnas.1607921114>
- Trontelj, P., & Fier, C. (2009). Cryptic species diversity should not be trivialised. *Systematics and Biodiversity*, 7(1), 1–3. <https://doi.org/10.1017/S1477200008002909>
- Vacher, J., Chave, J., Ficetola, F. G., Sommeria-Klein, G., Tao, S., Thébaud, C., ... Fouquet, A. (2020). Large-scale DNA-based survey of frogs in Amazonia suggests a vast underestimation of species richness and endemism. *Journal of Biogeography*, 47(8), 1781–1791. <https://doi.org/10.1111/jbi.13847>
- Vences, M., Meike, T., Van der Meijden, A., Chiari, Y. & Vieites, D. (2005) Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology*, 2, 7–12. <https://doi.org/10.1186/1742-9994-2-5>
- Wakeley, J. (2008) Coalescent theory: An Introduction. Greenwood Village, Roberts & Company Publishers, CO, 326 pp.
- Wallace, A. R. (1876). The geographical distribution of animals; with a study of the relations of living and extinct faunas as elucidating the past changes of the Earth's surface. New York: Harper & Brothers. Volume 1.

- Werneck, F. P. (2011). The diversification of eastern South American open vegetation biomes: Historical biogeography and perspectives. *Quaternary Science Reviews*, 30(13–14), 1630–1648. <https://doi.org/10.1016/j.quascirev.2011.03.009>
- Whittaker, R.J., Araújo, M.B., Paul, J., Ladle, R.J., Watson, J.E.M. & Willis, K.J. (2005) Conservation biogeography: Assessment and prospect. *Diversity and Distribution*, 11, 3–23. <https://doi.org/10.1111/j.1366-9516.2005.00143.x>
- Wiens, J. J., Graham, C. H., Moen, D. S., Smith, S. A., & Reeder, T. W. (2006). Evolutionary and ecological causes of the latitudinal diversity gradient in hylid frogs: treefrog trees unearth the roots of high tropical diversity. *The American Naturalist*, 168(5), 579–596.
- Wiens, J.J., Fetzner Jr.J.W., Parkinson, C.L. & Reeder, T. (2005) Hylid frogs phylogeny and sampling strategies for speciose clades. *Systematic Biology*, 54, 5, 719–748.
- 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
- Yang, Z. & Rannala, B. (2014) Unguided species delimitation using DNA sequence data from multiple loci. *Molecular Biology and Evolution*, 31, 3125 – 3135.
- Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, 29(22), 2869–2876.