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Ronildo Alves Benício

**Influência de processos ecológicos e evolutivos na estruturação de
comunidades de anfíbios na Mata Atlântica Brasileira**

São Carlos – SP

2019

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comunidades de anfíbios na Mata Atlântica Brasileira**

Tese apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais do Centro de Ciências Biológicas e da Saúde como parte dos requisitos necessários para a obtenção do título de Doutor em Ciências, área de concentração em Ecologia e Recursos Naturais.

Orientador: Prof. Dr. Fernando Rodrigues da Silva

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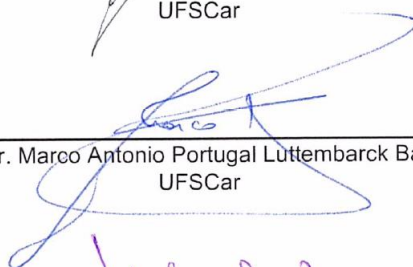
Centro de Ciências Biológicas e da Saúde
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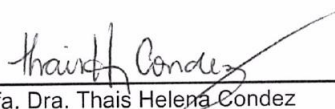
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“É necessário sempre acreditar que o sonho é possível, que o céu é o limite e você é imbatível, que o tempo ruim vai passar, é só uma fase, que o sofrimento alimenta mais a sua coragem, que a sua família precisa de você, lado a lado se ganhar, pra te apoiar se perder!”

FORMATAÇÃO:

Esta tese segue as exigências do Regimento Interno do Programa de Pós-Graduação em Ecologia e Recursos Naturais (PPGERN) da Universidade Federal de São Carlos – UFSCAR (<http://www.ppgern.ufscar.br/>). Nela consta: i) Introdução geral, com o embasamento conceitual necessário para o entendimento da tese como um todo; ii) dois capítulos referentes aos estudos realizados e redigidos no formato para apreciação em periódicos científicos (Capítulo 1 – *Ecology & Evolution* ISSN: 2045-7758; Capítulo 2 – *Journal of Tropical Ecology* ISSN: 1469-7831); e iii) Considerações finais. Apenas os capítulos estão em inglês, conforme normas dos periódicos científicos. Cada capítulo é um trabalho individual, mas alguns métodos podem se repetir ao longo da tese (por exemplo, os locais, o desenho amostral, a filogenia e as variáveis climáticas são as mesmas usadas nos capítulos 1 e 2). Realizei algumas mudanças para facilitar o entendimento do leitor. Por exemplo, as figuras e tabelas foram inseridas no corpo do texto. Além disso, algumas informações que serão disponibilizadas como materiais suplementares nas submissões dos artigos, foram trazidas ao texto, tornando-se figuras e tabelas. Nos apêndices é possível encontrar todo o material suplementar dos dois manuscritos. Nos anexos estão as normas dos periódicos referente a cada capítulo e o relatório das atividades acadêmicas realizadas durante meu período de doutoramento (2015 – 2019).

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Resumo

Minha tese é composta por dois capítulos independentes, mas que juntos, fornecem um arcabouço teórico dos processos ecológicos e evolutivos responsáveis pela estruturação de comunidades de anuros na Mata Atlântica Brasileira. No primeiro capítulo, avalio a importância relativa do clima (atual e Pleistoceno) e da topografia na distribuição espacial da estrutura filogenética das comunidades. No segundo capítulo, avalio como o clima (atual e Pleistoceno) e topografia afetam a distribuição espacial dos atributos funcionais (modos reprodutivos) das espécies de anuros. Os resultados mostram que os padrões de estrutura filogenética das comunidades e os atributos funcionais dos anuros não estão distribuídos aleatoriamente na Mata Atlântica Brasileira. As unidades de conservação localizadas em regiões com altas temperaturas e marcante sazonalidade na precipitação e baixa variação topográfica apresentaram comunidades agrupadas filogeneticamente e espécies com modos reprodutivos que permitem maior resistência à dessecação. Por outro lado, as unidades de conservação localizadas em áreas com temperaturas mais amenas e precipitação constante ao longo do ano, consideradas como refúgios durante o Pleistoceno, e com grande variação topográfica apresentaram comunidades dispersas filogeneticamente e espécies com modos reprodutivos mais especializados, tal como desenvolvimento direto. Assim, estes resultados sugerem que os gradientes climáticos e topográficos limitam a distribuição de espécies de anuros de determinadas linhagens com modos reprodutivos mais especializados.

Abstract

My thesis is composed of two independent chapters, but together they provide a theoretical framework of the ecological and evolutionary processes responsible for the anuran community structure in the Brazilian Atlantic Forest. In the first chapter, I evaluated the relative importance of climate (current and Pleistocene) and topography in the spatial distribution of the phylogenetic structure of communities. In the second chapter, I evaluate how the climatic gradient affects the spatial distribution of functional traits (reproductive modes) of anuran species. The results show that the patterns of phylogenetic structure of the communities and the functional traits of anurans are not distributed randomly in the Brazilian Atlantic Forest. Protected areas located in regions with high temperatures and marked seasonality in precipitation, and in areas with low topographic variation presented phylogenetic clustered communities and species with reproductive modes that allow greater resistance to desiccation. On the other hand, protected areas located in areas with milder temperatures and constant precipitation throughout the year, considered as refuges during the Pleistocene, and with great topographic variation presented phylogenetically dispersed communities and species with more specialized reproductive modes, such as direct development. Thus, these results suggest that climatic and topographic gradients limit the distribution of anuran species of certain lineages with more specialized reproductive modes.

Introdução geral

Ecologia de comunidades é o estudo de padrões na diversidade, abundância e composição de espécies nas comunidades e dos processos subjacentes a esses padrões (Vellend 2010). Uma das principais críticas à ecologia de comunidades é nossa incapacidade de fazer declarações gerais sobre conexões de padrões à processos (Lawton 1999, Simberloff 2004). Apesar do enorme número de mecanismos que supostamente sustentam padrões em comunidades ecológicas, todos esses mecanismos envolvem apenas quatro tipos distintos de processos (Vellend 2010): i) seleção - que representa diferenças de aptidão determinística entre as espécies, ii) deriva - que representa mudanças estocásticas na abundância de espécies, iii) especiação - que cria novas espécies; e iv) dispersão - que representa o movimento de organismos no espaço.

Revelar os mecanismos que impulsionam a montagem de comunidades ecológicas tem uma longa e contenciosa história em ecologia (e.g., Diamond & Case 1986, Chase & Leibold 2003, Chave 2004). O conceito tradicional de montagem de comunidades reflete a noção de que as espécies não coocorrem aleatoriamente, mas são moldadas por padrões históricos de especiação, extinção, dispersão, gradientes abióticos e interações bióticas (Götzenberger et al. 2012). A incorporação de análises filogenéticas (Pavoine & Bonsall 2011, Mouquet et al. 2012) e de atributos biológicos (McGill et al. 2006) em ecologia de comunidades têm fornecido novos *insights* para explicar os padrões de coocorrência de espécies (Pausas & Verdú 2010). Por exemplo, informações sobre atributos biológicos permitem gerar hipóteses sobre diferenças no desempenho (e.g., aptidão) e no nicho que levam ao agrupamento ou repulsão filogenética nas comunidades (Cavender-Bares et al. 2004, 2009, Emerson & Gillespie 2008, Losos 2008). Se tomadas conjuntamente, esta abordagem integrada permite testar se a conservação de nicho filogenético (e.g., espécies filogeneticamente próximas são ecologicamente mais similares) ou a convergência (e.g., atributo biológico semelhante presente em linhagens não aparentadas) é o principal mecanismo determinando a montagem de comunidades.

O arcabouço teórico da ecofilogenética propõe que caso as espécies que coocorrem sejam mais próximas filogeneticamente do que o esperado ao acaso, filtros ambientais (e.g., tolerância ao ambiente abiótico) seriam um importante processo determinando a montagem de comunidades locais (Wiens & Donoghue 2004). Por exemplo, Pellissier et al. (2013) registraram comunidades de borboletas filogeneticamente agrupadas em altitudes elevadas sugerindo que temperaturas frias agem como filtro permitindo que apenas espécies filogeneticamente próximas e adaptadas a essas condições ocupem esses ambientes. Por outro lado, as interações competitivas (e.g., similaridade limitante) limitariam a coocorrência de espécies filogeneticamente próximas, fazendo com que a comunidade local seja constituída de espécies distantes (Swenson et al. 2007, Yang et al. 2014). Por exemplo, Cavender-Bares et al. (2004) registraram comunidades de carvalhos dispersos filogeneticamente, com espécies distantemente relacionadas entre e dentro do mesmo clado. Contudo, estudos recentes têm chamado à atenção que a estrutura filogenética das comunidades não é determinada somente por processos locais como competição e filtro ambiental, e que o mesmo padrão pode ser originado por diferentes processos (Mayfield & Levine 2010). Este argumento leva a uma reinterpretação dos processos de montagem inferidos da estrutura filogenética da comunidade (Gerhold et al. 2015).

Muitos padrões e processos evolutivos e biogeográficos dependem da escala filogenética (Graham et al. 2018) ou espacial analisadas (Emerson & Gillespie 2008, Cavender-Bares et al. 2009, Kissling et al. 2012). Assim, a distribuição espacial de espécies, características funcionais e relações filogenéticas fornecem abordagens complementares para estudar padrões de biodiversidade e ajudar a desvendar os mecanismos que impulsionam a assembléia comunitária (Corbelli et al. 2015). Tomados em conjunto, o uso de uma abordagem combinada da distribuição espacial de espécies, características funcionais e relações filogenéticas provou ser útil para descrever como a biodiversidade varia no espaço, desvendando mecanismos de montagem (Cadotte et al. 2013) e entendendo os fatores que explicam a estrutura da comunidade (por exemplo, Pavoine et al. 2009 para peixes, Graham et al. 2012 para beija-flores, Huang et al. 2012 para mamíferos, Bernard-

Verdier et al. 2013 para plantas, Cavalheri et al. 2015 para serpentes, Jiménez-Robles et al. 2017 para anfíbios, Cavender-Bares et al. 2018 para carvalhos, Henckel et al. 2019 para aves).

Os anfíbios são o grupo de vertebrados mais ameaçado globalmente, com cerca de um terço das espécies sendo atualmente ameaçadas de extinção e metade delas em declínio (Catenazzi 2015). As principais ameaças incluem doenças fúngicas, destruição e alteração de habitat e mudanças climáticas (Catenazzi 2015). Além disso, muitas espécies evolutivamente distintas são propensas à extinção, fazendo dos anfíbios um grupo de alta preocupação de conservação (Wake & Vredenburg 2008). Como os planos de conservação bem-sucedidos devem visar preservar tanto a riqueza de espécies quanto a função no ecossistema e o potencial evolucionário das assembléias (por exemplo, Forest et al. 2007, Campos et al. 2017, Ouchi et al. 2018), compreender a distribuição espacial das comunidades e suas relações com a escala é fundamental para orientar o futuro planejamento sistemático da conservação para este grupo de vertebrados ameaçado (da Silva et al. 2016).

Considerando o arcabouço teórico apresentado acima, estruturei minha tese para, ao longo dos dois capítulos, avaliar os principais processos ecológicos e evolutivos estruturando as comunidades de anfíbios anuros na Mata Atlântica Brasileira.

No primeiro capítulo eu avaliei a importância relativa do clima (atual e Pleistoceno) e da topografia na distribuição espacial da estruturação filogenética das comunidades de anfíbios anuros na Mata Atlântica Brasileira. Eu encontrei que as comunidades filogeneticamente agrupadas estão localizadas em áreas com altas temperaturas e marcante sazonalidade na precipitação, e com baixa variação topográfica, enquanto que as comunidades filogeneticamente dispersas estão localizadas em áreas com temperaturas mais amenas e precipitação constante ao longo do ano, e grande variação topográfica. O clima passado (Pleistoceno) não influenciou a distribuição da estrutura filogenética das comunidades de anfíbios anuros.

No segundo capítulo eu avaliei como o gradiente climático (clima atual e Pleistoceno) e topografia afetam a distribuição espacial dos atributos funcionais (modos reprodutivos) das espécies de anuros. As espécies de anuros apresentaram sinal filogenético para os modos reprodutivos

indicando que o parentesco filogenético pode servir como um proxy razoável para a similaridade de atributos. As unidades de conservação que ocorrem em áreas com temperaturas mais amenas e precipitação constante ao longo do ano desde o Pleistoceno até o clima atual, e ampla variação topográfica abrigam espécies de anuros com modos reprodutivos especializados dependentes de alta umidade, como por exemplo desenvolvimento direto no solo, enquanto que as unidades de conservação localizadas em regiões com altas temperaturas, marcante sazonalidade na precipitação e baixa variação topográfica abrigam espécies de anuros com modos reprodutivos especializados e mais resistentes à dessecação.

Portanto, estes resultados demonstram que gradientes ambientais e topografia limitam a distribuição de espécies de anuros de determinadas linhagens com modos reprodutivos dependentes de umidade na Mata Atlântica Brasileira.

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Capítulo 1

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Topography and current climate drive the phylogenetic structure of amphibians communities in the Brazilian Atlantic Forest

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27 Abstract

28 One of the challenges of ecological research is to integrate different types of data to evaluate
29 ecological and evolutionary processes that influence the rules of community assembly. Here, we
30 explore the spatial distribution of the phylogenetic structure of anuran amphibian communities
31 along climatic gradient (historical and current) and topography. For that, we recorded species of
32 anurans in 14 Protected Areas (PAs) in the Brazilian Atlantic Forest between December 2014 and
33 February 2017. To determine phylogenetic structure, we calculated the mean pairwise distance
34 (MPD) that measure the mean pairwise phylogenetic distance (MPD) of taxa in a sample, and mean
35 nearest taxon distance (MNTD) that measure the phylogenetic distance to the nearest taxon in a
36 sample. We assessed the significance of MPD and MNTD using the Net Relatedness Index (NRI)
37 and Nearest Taxon Index (NTI). We found that the spatial distribution of the phylogenetic structure
38 of the anuran communities in the Brazilian Atlantic Forest was influenced by the environmental
39 gradients. Our analysis showed that NTI was more sensitive than NRI indicating that relatively
40 recent nodes (e.g., genus) rather than deep nodes (e.g., orders and families) are related to anuran
41 community assembly. The current climate explained 25% of variation on spatial distribution of NTI
42 values while topographic variables explained 2%. Phylogenetically clustered communities were
43 related to high temperatures and marked seasonality in precipitation, and areas with low
44 topographic variation, while phylogenetically dispersed communities were related to mild
45 temperatures and constant precipitation throughout the year, and areas with great topographic
46 variation. Our results suggest that topography and current climate plays an important role
47 structuring amphibian local communities along the Brazilian Atlantic Forest.

48

49 KEYWORDS

50 anurans, community assembly, climate, topographic, Biodiversity hotspot, Protected Areas.

51

52

53 1. INTRODUCTION

54 One of the greatest challenges in ecology is to define the relative importance of ecological and
55 evolutionary processes driving community assembly (Ricklefs, 1987). Evolutionary processes such
56 as speciation, extinction, and biogeographic dispersal, contribute to shape biodiversity patterns,
57 adding or removing species across time (Ricklefs, 2004). On the other hand, ecological processes
58 such as environmental filtering, biotic interactions and interspecific trade-offs are considered over
59 contemporary time scales and can largely determine patterns of species diversity and composition
60 (Chase & Myers, 2011). Ecological and evolutionary processes are expected to drive variation in
61 the phylogenetic structure of assemblages. For example, if rates of speciation or extinction are
62 associated with ecological traits, speciation or extinction may produce an unbalanced phylogeny,
63 with species increasingly concentrated within a few clades over time (Qian et al., 2017). On the
64 other hand, distributions of species along environmental gradients reflect their ability to persist in a
65 particular set of environmental conditions such as cold or drought, which are often phylogenetically
66 conserved (Kellermann et al., 2012). Cold and drought tolerances mostly evolved after the initiation
67 of global cooling in the early but only in the relatively few lineages that extended or shifted their
68 distributions into cold and/or dry environments (Latham & Ricklefs, 1993). Thus, in incorporating
69 phylogenetic information into community ecology, one can quantify the evolutionary relatedness of
70 species co-occurring within and across regions, and then associate the community phylogenetic
71 structure to niche-related, neutral and historical processes that have mediated species coexistence
72 (Cavender-Bares et al., 2009).

73 Darwin (1859) suggested that closely related species are ecologically more similar than
74 distantly related species, and therefore exert greater negative influence on each other. Such
75 competitive interactions limit the similarity of coexisting species and lead to phylogenetic
76 overdispersion in assemblages at local scales (Cavender-Bares et al., 2006). Phylogenetic
77 overdispersion in local assemblages is consistent with the limiting similarity hypothesis (MacArthur
78 & Levins, 1967) and is frequently explained as a result of negative biotic interactions among closely

79 related species (Swenson et al., 2007; Yang et al., 2014). However, new analyzes have shown that
80 the same pattern can be originated by different processes or the same process can generate different
81 patterns (HilleRisLambers et al., 2012). For example, Mayfield & Levine (2010) argue that two
82 types of species differences determine competitive exclusion with opposing effects on relatedness
83 patterns. This means that competition can sometimes eliminate more different and less related taxa,
84 even when the traits underlying the relevant species differences are phylogenetically conserved.
85 This argument leads to a reinterpretation of the assembly processes inferred from community
86 phylogenetic structure. Furthermore, is still a challenge the interpretation of the assembly processes
87 of the communities inferred from communities in tropical regions.

88 Here, we explored the spatial distribution of the phylogenetic structure of anuran communities
89 along environmental gradients in the Brazilian Atlantic Forest. Brazilian Atlantic Forest is one of
90 the most diverse biomes and considered a highly threatened global biodiversity hotspot in the world
91 (Mittermeier et al., 2005) with more than 500 known amphibian species, of which 88% are endemic
92 (Haddad et al., 2013). We tested the following hypotheses that could explain the spatial distribution
93 of anuran phylogenetic structure:

94 i) Climate-diversity hypotheses – climatic variables that reflect present-day conditions are the
95 key drivers of current patterns of species distribution (Ricklefs, 1987; Wiens & Donoghue, 2004;
96 Hua & Wiens, 2013; da Silva et al., 2014). Harsh environments, as boreal and polar regions, and
97 deserts, with extreme drought conditions and high temperatures, should produce stronger
98 environmental filters (e.g., Herrera et al., 2010; Qian et al., 2017). This hypothesis have been shown
99 to be key environmental determinants of the spatial distribution of anuran communities
100 (Vasconcelos et al., 2010, 2014; Hawkins et al., 2003; da Silva et al., 2012, 2014). Thus, due to the
101 physiological constraints of amphibians and the selection of a few more related lineages adapted to
102 the harsh climate, we expected that communities located in sites with high temperatures and marked
103 seasonality in the precipitation will present strong phylogenetic clustering.

104 ii) Pleistocene refuge hypothesis (Haffer, 1969) – during the cold dry conditions of the Last
105 Glacial Maximum (LGM), approximately 21.000 yr BP, some areas in the Atlantic Forest
106 experienced less variability in temperature and precipitation (Martins, 2011; Carnaval & Moritz,
107 2008; Porto et al., 2013). The historical hypothesis postulates that the duration and extent of stable
108 climatic conditions in Earth’s history have allowed more opportunity for diversification due to high
109 speciation and/or low extinction rates (Svenning & Skov, 2005; Araújo et al., 2008). In regions with
110 a more harshness climate had a higher extinction rate over time or did not allow the dispersion of
111 species susceptible to these types of environments, and in regions with the more stable climate over
112 time, regions considered as forest refuge areas in the Pleistocene allowed the occurrence (lower
113 extinction rate) of a greater number of species. Thus, we expected that regions considered as a
114 refuge will present a greater number of species, and therefore a greater phylogenetic diversity,
115 which may or may not be related, soon, sites in these regions will present phylogenetic
116 overdispersion or random;

117 iii) Topographic hypothesis – historical events such as mountain uplift promote speciation
118 through habitat specialization and altitudinal isolation, which increases endemism and,
119 consequently, the discrepancy in species richness between sites within a region (Lomolino, 2001;
120 Rahbek & Graves, 2001; Ruggiero & Hawkins, 2008). Thus, due the elevation of mountains in the
121 Serra do Mar and, specifically, the wide variation in topography in the eastern Atlantic Forest (AF),
122 we expect that, due to historically limited anuran dispersal capabilities, speciation through habitat
123 specialization and altitudinal isolation, regions with extensive variation in topography harbor
124 different species of different non-related lineages, and, therefore, sites in these regions will present
125 phylogenetic overdispersion.

126

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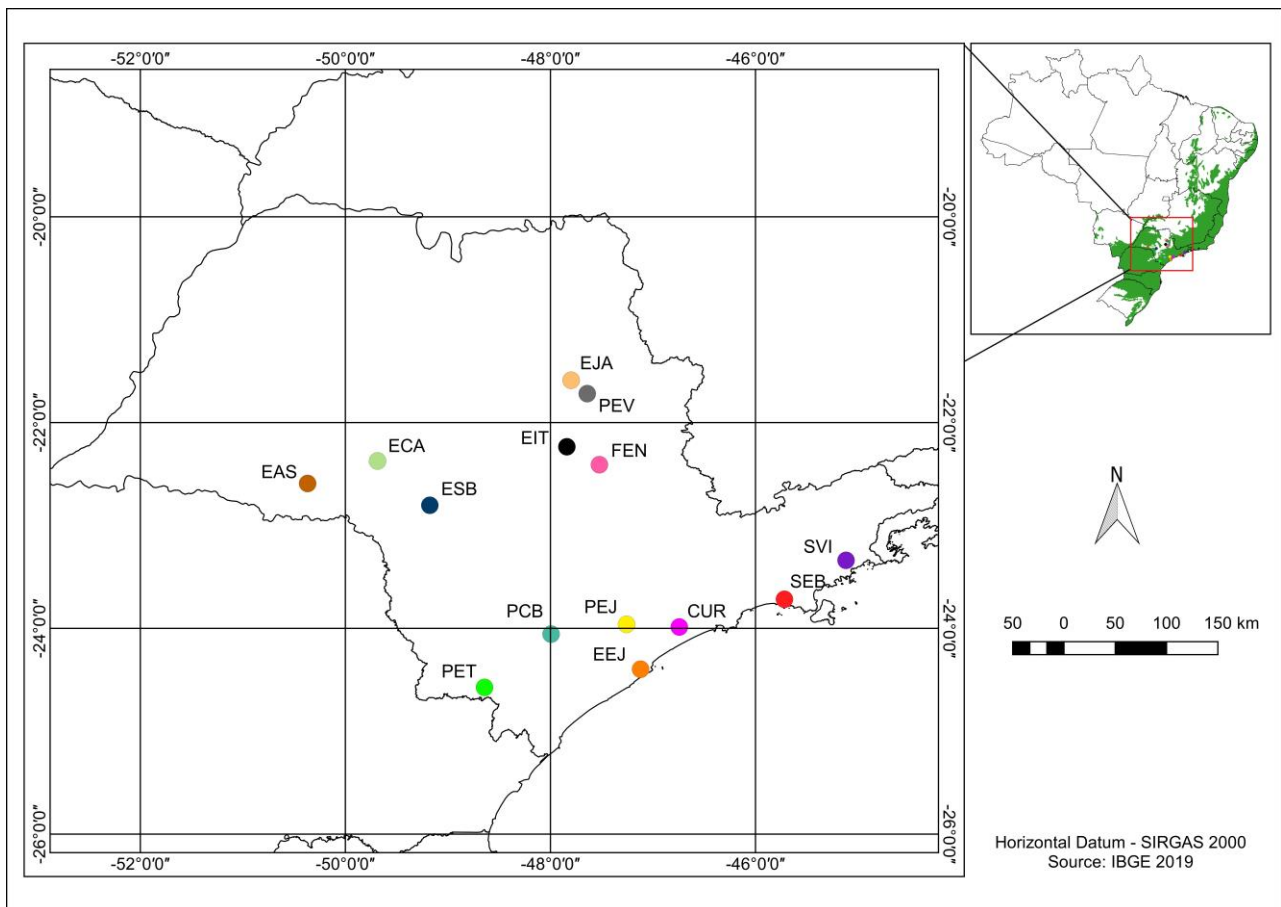
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130 **2. METHODS**

131 **2.1 Study area**

132 We used the species composition of 14 anuran communities along the longitudinal gradient in the
 133 state of São Paulo (Table S1). These communities were sampled at 14 Protected Areas (PAs) in the
 134 Brazilian Atlantic Forest (AF; Figure 1). The communities sampled are located along a current
 135 climatic gradient with high rainfall and constant temperatures throughout the year, mainly in the
 136 eastern region of Atlantic Forest, and high temperatures and pronounced precipitation seasonality
 137 throughout the year, located in inland areas, mainly in the northeast of the AF. In addition, the study
 138 area also features regions considered as a refuge due to the stable climate over time (during the
 139 Pleistocene), located mainly in the eastern AF, and a complex topography (elevation varies from sea
 140 level to 2.000 m a.s.l.).



141

142 **FIGURE 1** Distribution of 14 protected areas (PAs) sampled in Brazilian Atlantic Forest. ECA =

143 Estação Ecológica de Caetetus, EAS = Estação Ecológica de Assis, EIT = Estação Ecológica de

144 Itirapina, EJA = Estação Ecológica de Jataí, PEV = Parque Estadual Vassununga, FEN = Floresta
145 Edmundo Navarro, ESB = Estação Ecológica de Santa Bárbara, PCB = Parque Estadual Carlos
146 Botelho, EEJ = Estação Ecológica Jureia-Itatins, PEJ = Parque Estadual Jurupará, PET = Parque
147 Estadual Alto do Ribeira, CUR = Parque Estadual da Serra do Mar – Núcleo Curucutu, SEB =
148 Parque Estadual Serra do Mar – Núcleo São Sebastião, SVI = Parque Estadual da Serra do Mar –
149 Núcleo Santa Virgínia.

150

151 Although some PAs are within the Cerrado biome (inland areas), the specifically sampled
152 sites are remnants of Atlantic Forest. In these areas, the Atlantic Forest is represented by the
153 Seasonal Semideciduous Forest sensu stricto (Veloso et al., 1991; Pennington et al., 2006), which is
154 characterized by having 20-50% of tree species that lose part or all their leaves in the winter or
155 during the dry season. We used the definition of the limits of the Atlantic Forest proposed in
156 Ribeiro et al. (2009) that includes areas in the interior of the state of São Paulo, which are
157 considered as areas belonging to the Cerrado domain. The authors incorporated these regions
158 because, in addition to presenting savanna areas of the Cerrado domain (such as, for example, the
159 Itirapina Ecological Station, here called PA EIT), some of its plant formations correspond to
160 seasonal semideciduous forests of the Atlantic Forest, or transition areas between the two biomes
161 (Oliveira-Filho & Fontes, 2000; Ribeiro et al., 2009). For more details see Muylaert et al. (2018).
162 Furthermore, we choose sampling units with very similar physiognomic characteristics in the all
163 sites (Please, see above and Figure 2).

164

165 **2.2 Specimen sampling**

166 We visited six breeding sites (two ponds, two streams and two transects; Figure 2) in each protected
167 area between December, January, and February of 2014-2015, 2015-2016 and 2016-2017. To
168 determine the species composition at each breeding site, we used two sampling methods: i) survey
169 of adults (Scott Jr. & Woodward, 1994) during the turn of vocalization, between 19h and midnight.

170 At each visit, we searched for specimens with a combination of visual and auditory search; and ii)
171 larval surveys (Shaffer, 1994) between 10 and 18 h. In breeding habitats such as ponds and streams,
172 we used a hand dipnet with 3 mm mesh, passed intensively on the margins of reproductive habitats.
173 For species that reproduce by direct development (with spawning on the soil) or the deposit eggs
174 and / or tadpoles in bromeliads, we performed active searches in the transect areas used to survey of
175 adults. All adult specimens collected were anesthetized and killed with 10% lidocaine, fixed in 10%
176 formaldehyde and stored in 70% ethanol in the Coleção de Anfíbios do Departamento de Zoologia
177 da Universidade Estadual Paulista, campus de Rio Claro, state of São Paulo, Brazil (CFBH). The
178 tadpoles were anesthetized in lidocaine solution soon after collection, and preserved in 10%
179 formalin. All tadpole specimens were deposited in the Coleção de Anfíbios do Departamento de
180 Zoologia e Botânica da Universidade Estadual Paulista, campus São José do Rio Preto, state of São
181 Paulo, Brazil (DZSJRP).



182

183 **FIGURE 2** Example of sampled sites (da Silva et al., 2017).

184 **2.3 Phylogenetic inference**

185 We built a regional phylogeny using three mitochondrial genes - 12S (1100 bp), 16S (1573 bp) and
186 cytochrome oxidase subunit I (COI; 645 bp) - and one nuclear gene - recombination-activating gene
187 1 (RAG-1; 429 bp) (Table S3). Some species were sequenced here while others were obtained from
188 Genbank (Table S4). We calculate a dated phylogeny using Bayesian analyses in BEAST 2 version
189 2.4.7 (Figure S1). The dataset was analyzed using the GTRGAMMA model for all genes. Trees
190 were estimated using relaxed log-normal clock, Yule speciation process and five fossil calibration
191 points with a lognormal distribution. The fossil age constraints were obtained from Wiens (2011).
192 The calibrations included the most recent common ancestral (MRCA): i) Pipidae and all other frogs
193 145 Mya, ii) Bufonidae 55.8 Mya, and iii) “Terrana” 35 Mya. We run one Markov chain for 100
194 million generations, sampling every 10000 generations, and discarding the first 30 million
195 generations as burn-in to prevent sampling before reaching stationarity. The convergence of
196 parameter estimation was checked using Trace ver. 1.5. We also used TreeAnnotator ver. 1.8.0 to
197 produce maximum clade credibility trees from the post-burn-in trees.

198

199 **2.4 Climatic variables (current and historical)**

200 We obtained climatic variables related to historical and current climate from WorldClim (Hijmans
201 et al., 2005; <http://www.worldclim.org>). The climatic variables selected were: Annual Mean
202 Temperature (AMT), Temperature Seasonality (TS), Annual Precipitation (AP), Precipitation
203 Seasonality (PS) (Table S6). Climatic variables related to historical climate represents “bioclimatic
204 variables” of the Last Glacial Maximum (LGM, about 22,000 years ago). The four climate variables
205 correspond to BIO1, BIO12, BIO4 and BIO15, respectively, in the WorldClim database.
206 Furthermore, we used Google Earth (<https://www.google.com/earth/>) to obtain the following
207 topographical data: Maximum Elevation (MAEL), Minimum Elevation (MIEL), and Elevational
208 Range (difference between MAEL and MIEL: ElevR). These variables were used because they
209 describe the average trends as well as variation in temperature, precipitation and elevational range

210 which might represent physiological limits for amphibians (Wiens et al., 2006; Buckley & Jetz,
211 2007, 2008; da Silva et al., 2012).

212

213 **2.5 Data analysis**

214 **2.5.1 Phylogenetic community structure**

215 To determine phylogenetic structure, we calculated the mean pairwise distance (MPD) that measure
216 the mean pairwise phylogenetic distance of taxa in a sample; and mean nearest taxon distance
217 (MNTD) that measure the phylogenetic distance to the nearest taxon in a sample. MPD measures
218 phylogenetic relatedness among taxa at both the root and tips of a phylogenetic tree and emphasizes
219 phylogenetic relatedness among major clades (e.g., orders and families), whereas MNTD measures
220 phylogenetic relatedness near the tips (Webb, 2000; Webb et al., 2002; Webb et al., 2006). When
221 taking into account both deep and shallow nodes (representing older and more recent evolutionary
222 histories, respectively) in measuring phylogenetic relatedness (e.g., using MPD), phylogenetic
223 relatedness may increase or decrease as the climate gradient changes. In this sense, it is important to
224 take into account the different evolutionary scales within the phylogeny (Graham et al., 2018).

225 We assessed the significance of MPD and MNTD using the Net Relatedness Index (NRI) and
226 Nearest Taxon Index (NTI; Table 1). NRI and NTI are standardized effect sizes of MPD and
227 MNTD, respectively, estimated from 999 randomly generated null communities from the regional
228 phylogeny (Graham et al., 2009; Swenson, 2014). To generate randomized assemblages, we used
229 the null model 4 that randomizes co-occurrence while maintaining species richness and occurrence
230 frequency of each community (Kembel, 2009). Species tend to differ in their frequency of
231 occurrence and differences in species frequencies affect measures of species co-occurrence (Gotelli,
232 2000) as well as measures of phylogenetic community structure (Kembel & Hubbell, 2006; Hardy,
233 2008). Null model 4 was implemented using the independent swap algorithm (Gotelli, 2000).

234 A positive NRI or NTI indicates that species are more closely related than expected by chance
235 or phylogenetic clustering. Conversely, a negative NRI or NTI value indicates that species are more

236 distantly related than expected by chance or phylogenetic evenness or overdispersion. NRI and NTI
237 measure phylogenetic relatedness among species at different depths in the phylogeny: NRI
238 measures phylogenetic relatedness among taxa at both the root and tips of a phylogenetic tree and
239 emphasizes phylogenetic relatedness among major clades, whereas NTI measures phylogenetic
240 relatedness near the tips (Webb, 2000; Webb et al., 2002; Webb et al., 2006).

241

242 **2.5.2 Influence of historical and current climate and topography on phylogenetic structure**

243 First, in order to reduce the data dimensionality and multicollinearity, we performed a principal
244 component analysis (PCA) based on a correlation matrix of the data considering both for historical
245 and current climate and elevation-related variables. First axis explained 73.8% and second axis
246 15.6% of all data variation.

247 To test if NRI and NTI are correlated with climatic variables and topography, we used
248 Generalized Linear Models (GLM) to build alternative models with different combinations of
249 predictor variables. All models represented an a priori hypothesis about physiological limits for
250 amphibians (e.g., Wiens et al., 2006; Buckley & Jetz, 2007, 2008; da Silva et al., 2012). Then, we
251 used theoretical information approach to conduct a model selection procedure using Akaike
252 information criterion, corrected for small samples (AICc, Burnham & Anderson, 2002) to select the
253 best model. All models with $\Delta AICc < 2$ were considered as the best models (Burnham & Anderson,
254 2002). All analyzes were performed in R version 3.4 (R Core Team, 2019).

255

256 **2.5.3 Relative importance of historical and current climate and topography on phylogenetic** 257 **structure**

258 We examined the relative importance of historical and current climate and topography in explaining
259 phylogenetic structure (both for NRI and NTI) using a variance partitioning technique where the
260 total percentage of the variation of ordinary least-squares regressions is partitioned into unique and
261 common contributions of the sets of predictors (Borcard et al., 1992). The total variation of the

262 phylogenetic structure was divided into eight fractions: 1) variation explained purely by current
263 climate; 2) variation explained purely by historical climate; 3) variation explained purely by
264 topography; 4) variation explained by current climate and historical climate together; 5) variation
265 explained by current climate and topography together; 6) variation explained by historical climate
266 and topography together; 7) variation explained by current climate, historical climate and
267 topography together; and 8) unexplained (residual). All analyses were conducted in R 3.4 (R Core
268 Team, 2019).

269

270 **3 RESULTS**

271 We recorded 112 amphibian species (Tables S2). The number of species (ntaxa) varied between 16
272 and 46 on 14 Protected Areas (PAs) in the Brazilian Atlantic Forest (Table 1). We found that the
273 spatial distribution of the phylogenetic structure of the anuran communities in the Brazilian Atlantic
274 Forest was influenced by the environmental gradients (Table 1, Figure 3). Protected areas located in
275 regions with high temperatures and marked seasonality in the precipitation presented a phylogenetic
276 distribution clustered, while the protected areas located in areas with topographic complexity, mild
277 temperatures and constant precipitation throughout the year presented dispersed phylogenetic
278 distribution (Table 1, Figure 3). Our analysis has also shown that NTI was more sensitive compared
279 to NRI (Table 1, Figure 3). This suggest that dissimilarity on species composition are due to
280 lineages of recent nodes (e.g., genus) rather than relatively deep nodes (e.g. for orders or families).

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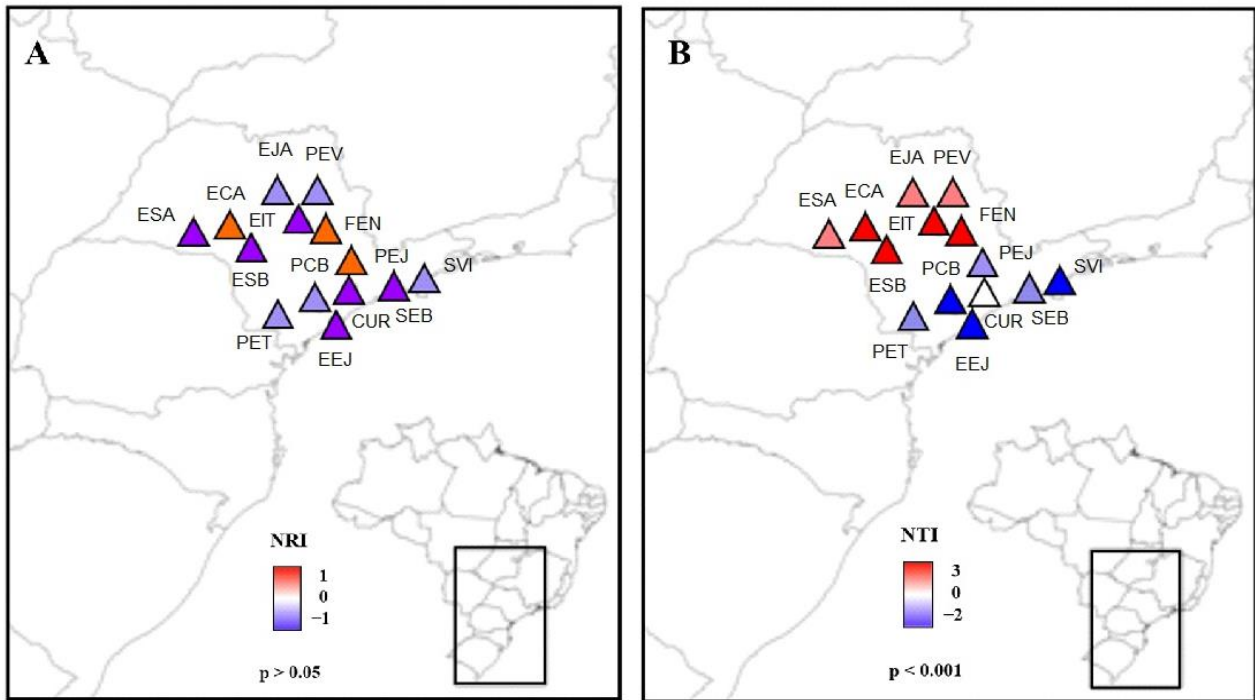
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286

287 **TABLE 1** Results of Net Relatedness Index (NRI) and Nearest Taxon Index (NTI) for 14 analyzed
 288 Protected Areas (PAs), in the Brazilian Atlantic Forest.

| PAs | ntaxa | NRI | | NTI | |
|-----|-------|------------|-------|------------|--------------|
| | | Z | P | Z | P |
| ECA | 25 | 1.2598055 | 0.095 | 2.1172129 | 0.015 |
| EAS | 24 | -0.5127119 | 0.702 | 1.1884610 | 0.126 |
| EIT | 26 | -0.2024404 | 0.614 | 2.7160171 | 0.003 |
| EJA | 18 | 0.1503854 | 0.486 | 1.2904991 | 0.101 |
| PEV | 24 | 0.7895137 | 0.239 | 2.8314198 | 0.003 |
| FEN | 16 | 1.7672863 | 0.943 | 1.5717186 | 0.067 |
| ESB | 25 | -0.3314914 | 0.634 | 3.0720916 | 0.001 |
| PCB | 46 | 0.1793798 | 0.428 | -1.2146431 | 0.876 |
| EEJ | 36 | -0.4794581 | 0.682 | -1.6829509 | 0.961 |
| PEJ | 44 | 1.2606218 | 0.105 | -0.5119124 | 0.682 |
| PET | 41 | 0.3518768 | 0.385 | 0.3060072 | 0.382 |
| CUR | 34 | -0.8925821 | 0.200 | 1.5285526 | 0.071 |
| SEB | 44 | -0.8455643 | 0.784 | 0.1125574 | 0.455 |
| SVI | 42 | 0.3791504 | 0.355 | -0.4933643 | 0.681 |

289 The P-value that indicates if the observed value of phylogenetic diversity (MPD or MNTD) is lower
 290 or higher than expected by chance. The values NRI or NTI are the Z values. The higher Z values
 291 (positive values), more phylogenetically clustered are the analyzed communities. The lower the Z
 292 values (negative values), more phylogenetically overdispersed are the analyzed communities. ECA
 293 = Estação Ecológica de Caetetus; EAS = Estação Ecológica de Assis; EIT = Estação Ecológica de
 294 Itirapina; EJA = Estação Ecológica de Jataí; PEV = Parque Estadual Vassununga; FEN = Floresta
 295 Edmundo Navarro; ESB = Estação Ecológica de Santa Bárbara; PCB = Parque Estadual Carlos
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 297 Estadual Alto do Ribeira; CUR = Parque Estadual da Serra do Mar – Núcleo Curucutu; SEB =
 298 Parque Estadual Serra do Mar – Núcleo São Sebastião; SVI = Parque Estadual da Serra do Mar –
 299 Núcleo Santa Virgínia. In bold values of $P < 0.05$.



300

301 **FIGURE 3** Map of Atlantic Forest with communities. Colors of the triangles represent the result of
 302 the phylogenetic structure for NRI (A) and for NTI (B). ECA = Estação Ecológica de Caetetus;
 303 EAS = Estação Ecológica de Assis; EIT = Estação Ecológica de Itirapina; EJA = Estação Ecológica
 304 de Jataí; PEV = Parque Estadual Vassununga; FEN = Floresta Edmundo Navarro; ESB = Estação
 305 Ecológica de Santa Bárbara; PCB = Parque Estadual Carlos Botelho; EEJ = Estação Ecológica
 306 Jureia-Itatins; PEJ = Parque Estadual Jurupará; PET = Parque Estadual Alto do Ribeira; CUR =
 307 Parque Estadual da Serra do Mar – Núcleo Curucutu; SEB = Parque Estadual Serra do Mar –
 308 Núcleo São Sebastião; SVI = Parque Estadual da Serra do Mar – Núcleo Santa Virgínia.

309

310 **Influence of historical and current climate and topography on phylogenetic structure**

311 None of the models evaluated were related to the distribution of NRI values (Table 2). In contrast,
 312 we found that the model including current climate and topography explained 74% of the variation
 313 of NTI values (Table 3).

314

315 **TABLE 2** Generalized Linear Models (GLM) used to investigate the influence of environmental
 316 conditions in explaining the phylogenetic structure (NRI) of amphibian communities in the
 317 Brazilian Atlantic Forest.

| GLM Models | ΔAICc | wAICc | R^2 |
|----------------------|---------------------|-------|-------|
| Null | 0.0 | 0.84 | - |
| CCLim + HClim | 6.8 | 0.02 | 0.04 |
| CCLim + Topo | 6.8 | 0.02 | 0.04 |
| HClim + Topo | 7.0 | 0.02 | 0.02 |
| CCLim + HClim + Topo | 11.8 | 0.00 | 0.04 |

318 ΔAICc = variation AICc; wAICc = model weight; R^2 = Pseudo R squared. CCLim = current climate,
 319 HClim = historical climate, Topo = topographic complexity.

320

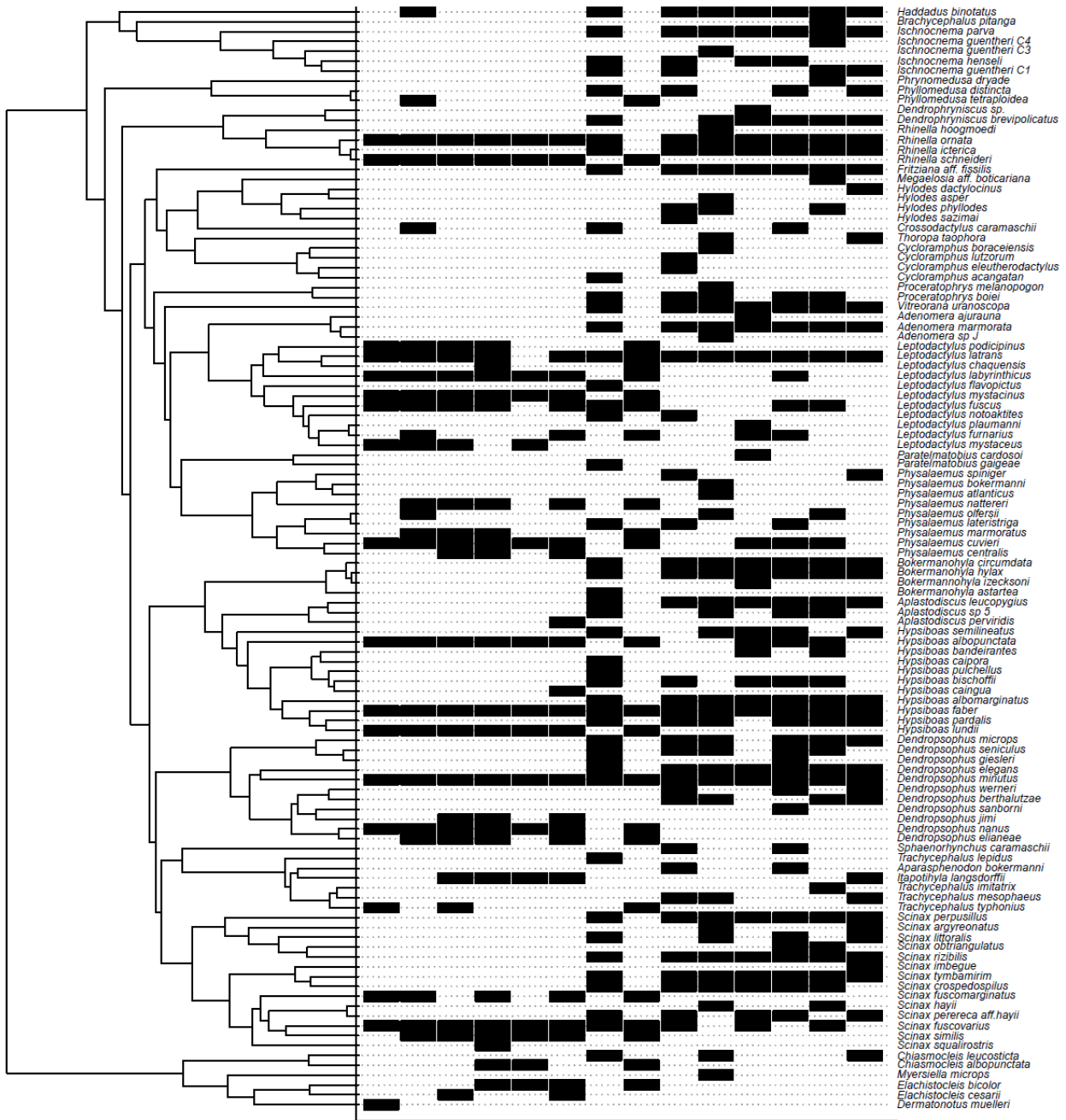
321 **TABLE 3** Generalized Linear Models (GLM) used to investigate the influence of environmental
 322 conditions in explaining the phylogenetic structure (NTI) of amphibian communities in the
 323 Brazilian Atlantic Forest.

| GLM Models | ΔAICc | wAICc | R^2 |
|----------------------|---------------------|-------|-------------|
| CCLim + Topo | 0.0 | 0.55 | 0.74 |
| CCLim + HClim | 1.9 | 0.21 | 0.71 |
| CCLim + HClim + Topo | 4.6 | 0.05 | 0.75 |
| HClim + Topo | 9.3 | 0.00 | 0.51 |
| Null | 12.0 | 0.00 | - |

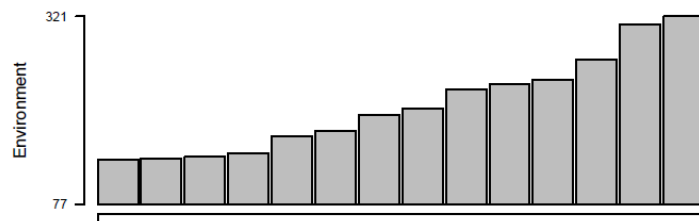
324 ΔAICc = variation AICc; wAICc = model weight; R^2 = Pseudo R squared. CCLim = current climate,
 325 HClim = historical climate, Topo = topographic complexity. In bold the best models ($\Delta\text{AICc} < 2$).

326

327 The effect of climate and topography variables on the NTI seem to affect some lineages that
 328 include the species of the genera *Adenomera*, *Bokermanohyla*, *Brachycephalus*, *Cycloramphus*,
 329 *Dendrophryniscus*, *Fritziana*, *Gastrotheca*, *Hylodes*, *Ischnocnema*, *Megaelosia*, *Myersiella*,
 330 *Paratelmatobius*, *Phrynomedusa*, *Proceratophrys*, *Sphaenorhynchus*, *Thoropa*, and *Vitreorana*, that
 331 do not occurs in the sites located in areas with harshness climate conditions (Figure 4). Species of
 332 these genera occurred exclusively in sites located in areas with milder climate conditions, while
 333 only two genera (*Dermatonotus* and *Elachistocleis*) were recorded exclusively in sites located in
 334 areas with harshness climate conditions (Figure 4).



ECA EAS EIT EJA PEV FEN ESB PCB EEJ PEJ PET CUR SEB SVI



335

336 **FIGURE 4** Distribution of amphibian lineages in the 14 Protected Areas (PAs), along a climatic
 337 gradient, in the Brazilian Atlantic Forest. ECA = Estação Ecológica de Caetetus; EAS = Estação
 338 Ecológica de Assis; EIT = Estação Ecológica de Itirapina; EJA = Estação Ecológica de Jataí; PEV =
 339 Parque Estadual Vassununga; FEN = Floresta Edmundo Navarro; ESB = Estação Ecológica de

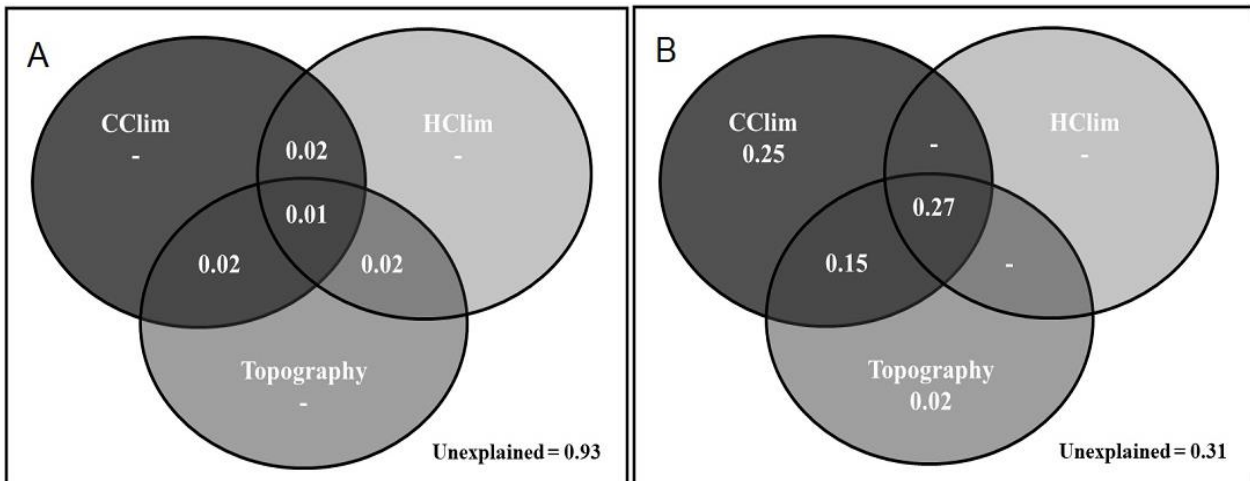
340 Santa Bárbara; PCB = Parque Estadual Carlos Botelho; EEJ = Estação Ecológica Jureia-Itatins;
 341 PEJ = Parque Estadual Jurupará; PET = Parque Estadual Alto do Ribeira; CUR = Parque Estadual
 342 da Serra do Mar – Núcleo Curucutu; SEB = Parque Estadual Serra do Mar – Núcleo São Sebastião;
 343 SVI = Parque Estadual da Serra do Mar – Núcleo Santa Virgínia.

344

345 **Relative importance of historical and current climate and topography on phylogenetic**
 346 **structure**

347 The deviance partitioning indicates that there was no significant influence of environmental
 348 gradients on the spatial distribution of NRI values (Figure 5A). Although, we found that a combined
 349 effect of the current climate and topographic variables accounted for the largest fraction (27%) of
 350 the variation of NTI values (Figure 5B), the current climate gradient alone accounted for 25% of the
 351 variation of NTI values while topographic gradient alone accounted for 2% (Figure 5B).

352



353

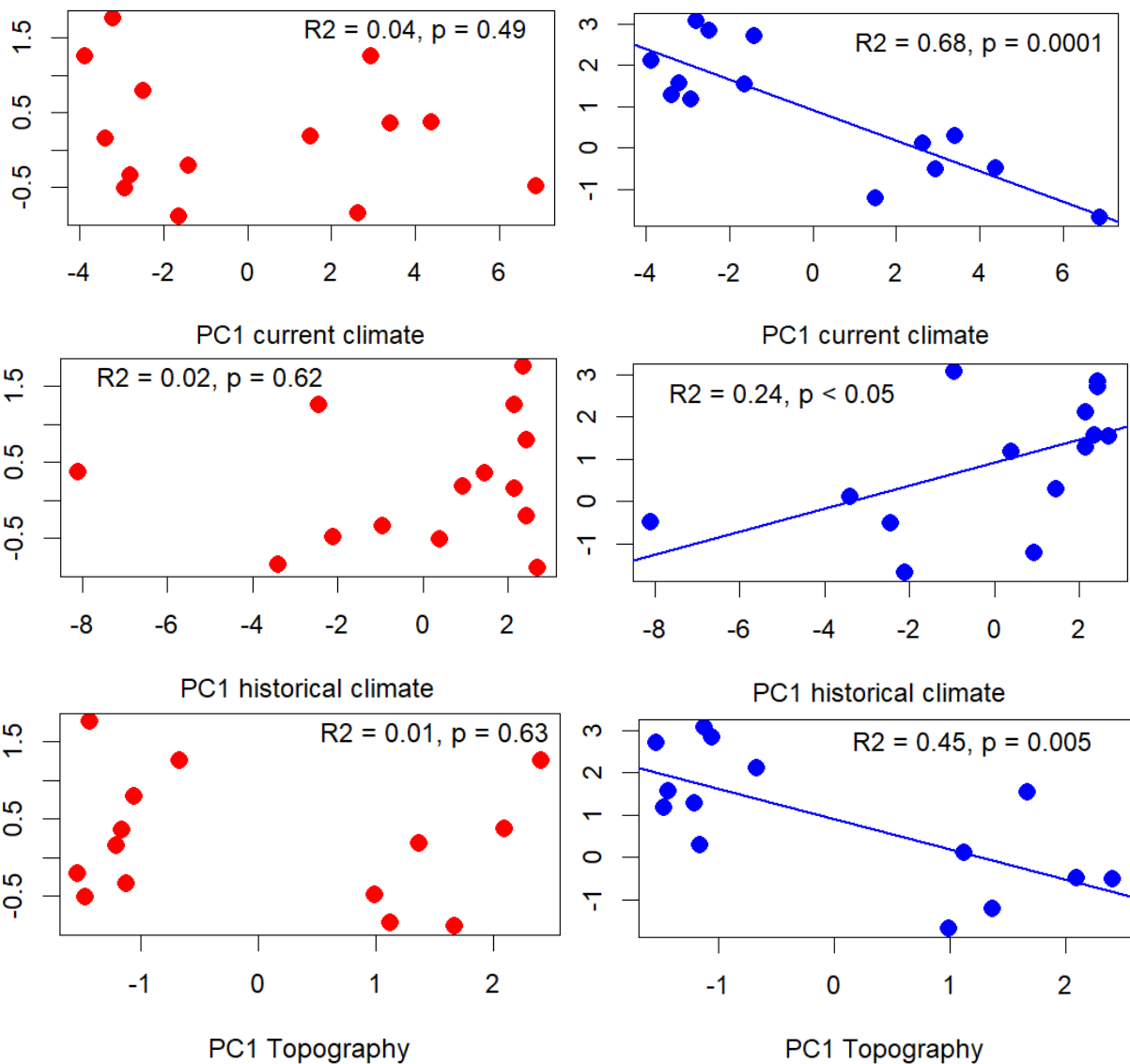
354 **FIGURE 5** Partition of the variance of phylogenetic metrics (A – NRI, B – NTI) explained by
 355 current climate (CClim), historical climate (Hclim) and topography for 14 sites in Brazilian Atlantic
 356 Forest. “-” = variation explained < 0.

357

358

359 When analyzed separately, the same pattern was maintained, with no significant influence of
 360 environmental gradients on the spatial distribution of NRI values (Figure 6) and with the current
 361 climate (68%, $p=0.0001$) and topography (45%, $p=0.005$) explaining alone an largest fraction of the
 362 variation of NRI values (Figure 6). Same singly the historical climate explained little (24%,
 363 $p=0.05$), compared to the current climate and topography.

364



367

368 **FIGURE 6** Principal component analysis (PCA) based on current climate, historical climate and
 369 topography and phylogenetic metrics (NRI – red circles, and NTI – blue circles).

370

371

372 4 DISCUSSION

373 Our results show that the climate and topography affected the spatial distribution of the
374 phylogenetic structure of the anuran communities in the Brazilian Atlantic Forest. Sites with high
375 temperatures and marked seasonality in the precipitation presented strong phylogenetic clustering.
376 Sites with higher topographic heterogeneity (e.g., altitudinal variation) presented phylogenetic
377 overdispersion. Our results suggest that current climate and topography are important drivers in the
378 assembly of anuran amphibian communities in the Atlantic Forest.

379 Our analysis has also shown that NTI, which measures phylogenetic relatedness based on
380 relatively recent nodes, was more sensitive compared to NRI. NRI and NTI measure different
381 evolutionary characteristics of communities. The positive relationship between NRI and NTI that
382 was found in the present study suggests that effect of the environmental variables that determine the
383 assembly of species in the clustered of species in different communities is consistent between the
384 deep and shallow nodes throughout the phylogenetic tree. These results indicate that anuran
385 communities in the Atlantic Forest tend to be more closely related in warmer and / or drier climates.
386 When more recent evolutionary histories are considered in phylogeny (NTI), phylogenetic
387 clustering is more strongly related to climate. This finding corroborates with other studies in the
388 literature (e.g., Qian et al., 2017) and is consistent with the prediction of the hypothesis of
389 phylogenetic niche conservatism (TNC). For example, the variation of the values of NTI was higher
390 along environmental gradients, suggest that ecological traits responsible for heat and drought
391 tolerance (in this case) are generally conserved at relatively recent nodes (e.g., for species) rather
392 than relatively deep nodes (e.g. for orders or families) (Qian et al., 2017). In addition, the pattern of
393 increased phylogenetic relatedness in relation to environment variables is more sensitive in
394 communities located in species-poor regions.

395 The current climate is well known to strongly affect the spatial distribution of phylogenetic
396 relationship between species on a large scale (e.g., Qian et al., 2017). Here, we demonstrate that
397 there was also a strong effect of the current climate on the phylogenetic structure of local anuran

398 communities in the Atlantic Forest and that this may be a result of extreme drought conditions
399 producing stronger environmental filters (Hypothesis 1). Most species in the region with a
400 harshness climate (high temperatures and marked seasonality in precipitation) are a subset of the set
401 of species that can tolerate very hot climate, with reproductive modes involving protection of eggs
402 with foam nests that allow greater resistance to desiccation, and are generally widely distributed
403 longitudinally in the Atlantic Forest (e.g., species of the family Leptodactylidae). In contrast, in
404 regions with milder climates (mild temperatures and constant precipitation throughout the year), in
405 the coastal region of the Atlantic Forest, harbor species of anurans with specialized reproductive
406 modes, such as basin constructions close to reproductive habitats and direct development (e.g.,
407 species belonging to the families Brachycephalidae, Craugastoridae, and Hylidae). In this sense,
408 current climatic seems to act as filter limiting the spatial distribution of anurans along the
409 environmental gradients of the Brazilian Atlantic Forest. This finding is consistent with the TNC
410 hypothesis. This result is similar to that found by da Silva et al. (2012), who showed that moister
411 sites in the Atlantic Forest harbored a greater phylogenetic diversity of amphibians than drier sites.
412 Thus, spatial distribution patterns of the phylogenetic structure of these communities result from a
413 combination of variation in speciation rates and extinction and environmental filtering.

414 Historical effects are also recognized processes that influence the spatial distribution of
415 communities (e.g., da Silva et al., 2014) and, consequently, can determine which lineages reside in a
416 given region (Kennedy et al., 2017). Although the phylogenetic dispersion of communities in the
417 eastern Atlantic Forest is in agreement with the historical forest refuges planned for the Pleistocene,
418 we found no effect of past climate on the phylogenetic structuring of communities (Hypothesis 2).
419 One explanation for this is that the diversification of amphibians in the Atlantic Forest (e.g., 70 myr
420 for Brachycephalidae, 75 myr for Hylidae, and 78 myr for Leptodactylidae, Hutter et al., 2017) is
421 much older than Pleistocene (21.000 years before the present), and due to the maintenance of a
422 more stable climate over time, the regions considered areas of forest refuge in the Pleistocene
423 allowed the occurrence (lower extinction rate) of a greater number of species of different unrelated

424 lineages. So, these historically stable areas must retain high levels of endemism for several taxa,
425 including amphibians. In addition, it is expected that the higher the long-term climatic stability in an
426 area, the more new clades will persist without being extinguished or reuniting with other clades
427 (Jansson, 2003). On the other hand, in areas with more harshness climates over time may have
428 limited the dispersion / occurrence of species more sensitive to these types of environments. For
429 example, 17 genera (*Adenomera*, *Bokermanohyla*, *Brachycephalus*, *Cycloramphus*,
430 *Dendrophryniscus*, *Fritziana*, *Gastrotheca*, *Hylodes*, *Ischnocnema*, *Megaelosia*, *Myersiella*,
431 *Paratelmatobius*, *Phrynomedusa*, *Proceratophrys*, *Sphaenorhynchus*, *Thoropa*, and *Vitreorana*)
432 were recorded exclusively in sites located in areas with milder climate conditions, while only two
433 genera (*Dermatonotus* and *Elachistocleis*) were recorded exclusively in sites located in areas with
434 harshness climate conditions. Although the analyzes were unable to confirm this, these results
435 suggest that the past climate may have maintained (low extinction rate, rather than promoting high
436 diversification) the phylogenetic diversity in communities located in areas with milder climate and
437 selecting the few lineages of species of anurans able to survive in areas with a more harshness
438 climate.

439 Patterns in species diversity along elevational gradients are also well recognized and may vary
440 between climatic areas and differ within and between taxonomic groups, reflecting both physical
441 and physiological constraints (Guo et al., 2013). Due the elevation of mountains in the Serra do Mar
442 and, specifically, the wide variation in topography in the eastern Atlantic Forest (AF), and the
443 historically limited anuran dispersal capabilities, speciation through habitat specialization and
444 altitudinal isolation, regions with extensive variation in topography harbor different species of
445 different non-related lineages, and, therefore, sites in these regions will present phylogenetic
446 overdispersion (Hypothesis 3). Our results show that the highest rates of amphibian endemism
447 within AF were in the eastern region, compared to the northwestern region which is composed
448 mainly of specialists in open habitat. Regions with higher amplitudes in elevation promote
449 speciation through habitat specialization and altitudinal isolation, which increases endemism and,

450 consequently, the discrepancy in species richness between sites within a region (Haddad & Prado,
451 2005; Lomolino, 2001; Rahbek & Graves, 2001; Ruggiero & Hawkins, 2008). Here, we found that
452 the wide variation in topography limited the dispersion of some lineages of amphibians more
453 sensitive to more harshness environments in the northwest region of the Atlantic Forest, and
454 allowed the occurrence of a greater number of different lineages of species living along gradients in
455 the southeast region of the Atlantic Forest. In this sense, altitudinal variation also explain the spatial
456 distribution of the phylogenetic structure of anuran communities in the Atlantic Forest.

457 In conclusion, we found that the patterns of phylogenetic structure of the anuran communities
458 are not randomly distributed in the Brazilian Atlantic Forest. Our results suggest that current
459 climatic and topography drives phylogenetic structure of the anuran communities limiting the
460 distribution of species of anurans of certain lineages in more harshness climates. Thus,
461 diversification and limitation in dispersion are important processes that organize the spatial
462 distribution of anurans along the environmental gradients of the Brazilian Atlantic Forest.

463

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471

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Capítulo 2

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Niche conservatism and environmental filters explain the diversity of reproductive traits of amphibians along the Brazilian Atlantic Forest

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27 **Abstract**

28 One of the challenges in ecological research is to integrate phylogeny, traits, geography,
29 environmental and species composition to assess ecological and evolutionary processes that
30 influence the community assembly rules. Here, we evaluated the relationship among reproductive
31 modes of anuran communities along climatic and topographic gradients in a geographic and
32 phylogenetic context in the Brazilian Atlantic Forest. We found that current and past climate and
33 topography influences the distribution of the reproductive modes of anurans in the Brazilian
34 Atlantic Forest. Communities located in the eastern region of the Atlantic Forest, which present
35 constant precipitation throughout the year, absence of seasonality in temperature and wide
36 altitudinal variation, harbor anuran species with specialized reproductive modes, such as basin
37 constructions close to reproductive habitats and direct development. On the other hand,
38 communities located in the western region present high temperatures and marked seasonality in the
39 precipitation, harbor species of anurans with reproductive modes involving protection of eggs with
40 foam nests that allow greater resistance to desiccation. Our results demonstrate that temperature,
41 precipitation, and elevation are important factors that limit the distribution of species of anurans
42 through their effects on reproductive modes. Thus, niche conservatism (i.e., tendency of lineages to
43 retain their niche-related traits through speciation events) and environmental filters seem to be
44 important processes organizing local amphibian communities in the Brazilian Atlantic Forest.

45

46 **Keywords:** community assembly; abiotic factors; phylogeny; ecological traits; reproductive modes;
47 anurans

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53 **Introduction**

54 There are two main ideas related to community assembly processes (Chase & Myers 2011): i)
55 deterministic theories suggest that niche-based processes, such as environmental filtering and
56 species interactions, determine patterns of species diversity and composition; and ii) stochastic
57 theories emphasize the importance of chance colonization, random extinction and ecological drift.
58 Niche theory proposes that species differences underlie both coexistence within communities and
59 the differentiation in species composition among communities via limiting similarity and
60 environmental filtering (Baraloto *et al.* 2012). However, it has been difficult to extend niche theory
61 to species-rich communities because of the empirical challenge of quantifying niches for many
62 species (Baraloto *et al.* 2012). This has motivated the development of functional and phylogeny-
63 based approaches in community ecology (Kraft & Ackerly 2010), which represent two different
64 means of approximating niche attributes. Alternatively, species may drift neutrally in abundance,
65 yet co-occur over long periods of time (Hubbell 2001), or biotic interactions and filtering may
66 balance each other to produce seemingly random, or neutral, patterns (Purves & Pacala 2005). In
67 this sense, the incorporation of phylogenetic perspectives (Pavoine & Bonsall 2011; Mouquet *et al.*
68 2012) and ecological traits (McGill *et al.* 2006) into community ecology are approaches particularly
69 suited to address the role that species differences in ecological strategy play (e.g., Swenson &
70 Enquist 2009; Kraft & Ackerly 2010).

71 Functional traits capture essential aspects of species' morphology, ecophysiology, and life-
72 history strategy (McGill *et al.* 2006; Violle *et al.* 2007). Approaches based on functional traits have
73 been used to demonstrate the importance of environmental filtering in structuring diverse
74 ecological communities, including fish (Bellwood *et al.* 2006, Mouillot *et al.* 2007, Ingram &
75 Shurin 2009, Villéger *et al.* 2010), tropical trees (Kraft *et al.* 2008, Paine *et al.* 2011) and temperate
76 woody plants (Diaz *et al.* 2004, Cornwell *et al.* 2006, Cornwell & Ackerly 2009). Although the
77 inferences made in many studies (e.g., Freckleton *et al.* 2002, Webb *et al.* 2002, Moles *et al.* 2005,
78 Donoghue 2008) rest upon the same central assumption, often termed phylogenetic conservatism,

79 that is, closely related species are more ecologically and functionally similar; in many other studies
80 closely-related species can differ greatly in some functional traits as a result of rapid evolution or
81 ecological convergence (Cavender-Bares *et al.* 2004, Losos 2008, 2011). Losos (2008) clearly
82 shows the importance of testing the assumption of phylogenetic conservatism before identifying
83 which trait states and which lineages are filtered by the environment.

84 The Brazilian Atlantic Forest (AF) is one of the most diverse biomes and a global biodiversity
85 hotspot (Mittermeier *et al.* 2005). Its broad geographical variation (latitudinal distribution of 25° C)
86 result in a climatic gradient related to the annual rainfall (from approximately 800–4.000 mm),
87 mean annual temperatures (averages from 15 to 25 °C), which influence floristic distributions
88 (Oliveira-Filho & Fontes 2000), and topographic variation (elevation varies from sea level to 2.000
89 m a.s.l.). Amphibians are usually less tolerant of desiccation and require higher humidity levels than
90 reptiles or other terrestrial vertebrates (Ludwig 1945). The reason for these differences is that
91 amphibians have highly permeable skin, complex life cycles that are typically dependent on both
92 aquatic and terrestrial environments (Wilbur 1980, Becker *et al.* 2007, da Silva *et al.* 2012). These
93 characteristics explain why humidity-related variables, such as mean annual rainfall, tend to be
94 more important for amphibian species (Rodríguez *et al.* 2005, Vasconcelos *et al.* 2010) than for
95 other terrestrial vertebrates (Rahbek & Graves 2001, Zhao *et al.* 2006). The diversity of
96 reproductive modes among amphibians constitutes a striking example of how differences in the
97 biology of species provide important explanations for species distribution patterns on a broad scale.
98 A great diversity in reproductive modes (hereafter termed RMs) is a distinctive feature of the class
99 Amphibia, particularly of the order Anura (da Silva *et al.* 2012). Only in the AF there are
100 approximately 550 anuran species that have 39 reproductive modes, most of which are specific at
101 the species, genus, or even family level (Haddad & Prado 2005, Haddad *et al.* 2013). This high
102 diversity of reproductive modes is attributed to the successful utilization of the diversified and
103 humid microhabitats present in this biome (Haddad & Prado 2005).

104 Anuran reproductive modes is a combination of traits that includes oviposition site, ovum and
105 clutch characteristics, rate and duration of development, stage and size of hatchling, and type of
106 parental care, if any (Salthe & Duellman 1973), and represent an important functional trait to
107 understand the distribution of species along marked environmental gradients, since there is a close
108 relationship between reproductive characteristics and habitat use (Haddad & Prado 2005). In this
109 biome it has already been demonstrated that the gradient of species richness and number of
110 reproductive modes is not randomly distributed, being that anuran species richness and the number
111 of reproductive modes were associated with total annual rainfall and temperature (Vasconcelos *et*
112 *al.* 2010, da Silva *et al.* 2012). Therefore, to understand which species occur in local communities
113 and how environmental filters select these species, it is necessary to consider how key climatic
114 factors restrict or facilitate the occurrence of certain characteristics, such as types of reproductive
115 modes.

116 As the great richness and endemism of amphibians in this region are usually attributed to the
117 unusual extent of the ranges of topography (elevation varies from sea level to 2000 m a.s.l.), the
118 climatic conditions, as well as regions which served as a large climatic refugium in the late
119 Pleistocene (Carnaval *et al.* 2009, da Silva *et al.* 2012), and assuming the premise that, in general,
120 taxonomic diversity (species richness) is related to functional diversity (i.e., herein, the number of
121 reproductive modes) - some communities show remarkably similar relationships between species
122 richness and functional diversity (e.g., Petchey & Gaston 2002, Cilleros *et al.* 2016, Arnan *et al.*
123 2017), here, we evaluate the same hypotheses proposed in chapter 1, but now test the influence of
124 the climate (current and Pleistocene) and topography on the spatial distribution of the functional
125 attributes (reproductive modes) of the anuran communities in the Brazilian Atlantic Forest:

126 i) Climate-diversity hypotheses - climatic variables that reflect present-day conditions are the
127 key drivers of speciation, extinction and dispersal rates, and also influencing current patterns of
128 diversity of the reproductive modes. Thus, we expect that throughout the climate gradient,
129 communities located in regions with more harshness climate (high temperatures and marked

130 seasonality in precipitation) harbor species of anurans with more specialized reproductive modes
131 desiccation, while communities located in regions with a more stable climate allow a greater
132 richness of species of different reproductive modes.

133 ii) Pleistocene refuge hypothesis (Haffer 1969) - during the cold dry conditions of the Last
134 Glacial Maximum (LGM), approximately 21.000 yr BP, some areas in the Atlantic Forest
135 experienced less variability in temperature and precipitation (Martins 2011, Carnaval & Moritz
136 2008, Porto *et al.* 2013). The historical hypothesis postulates that the duration and extent of stable
137 climatic conditions in Earth's history have allowed more opportunity for diversification due to high
138 speciation and/or low extinction rates (Svenning & Skov 2005, Araújo *et al.* 2008). In regions with
139 a more harshness climate had a higher extinction rate over time or did not allow the dispersion of
140 species susceptible to these types of environments, and in regions with the more stable climate over
141 time, regions considered as forest refuge areas in the Pleistocene allowed the occurrence (lower
142 extinction rate) of a greater number of species of different reproductive modes.

143 iii) Topographic hypothesis - historical events such as mountain uplift promote speciation
144 through habitat specialization and altitudinal isolation, which increases endemism and,
145 consequently, the discrepancy in species richness between sites within a region (Lomolino 2001,
146 Rahbek & Graves 2001, Ruggiero & Hawkins 2008). Thus, due the elevation of mountains (in the
147 Serra do Mar) and, specifically, the wide variation in topography in the eastern Atlantic Forest
148 (AF), we expected that regions with extensive variation in topography would harbor different
149 species of different reproductive modes.

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156 **Methods**

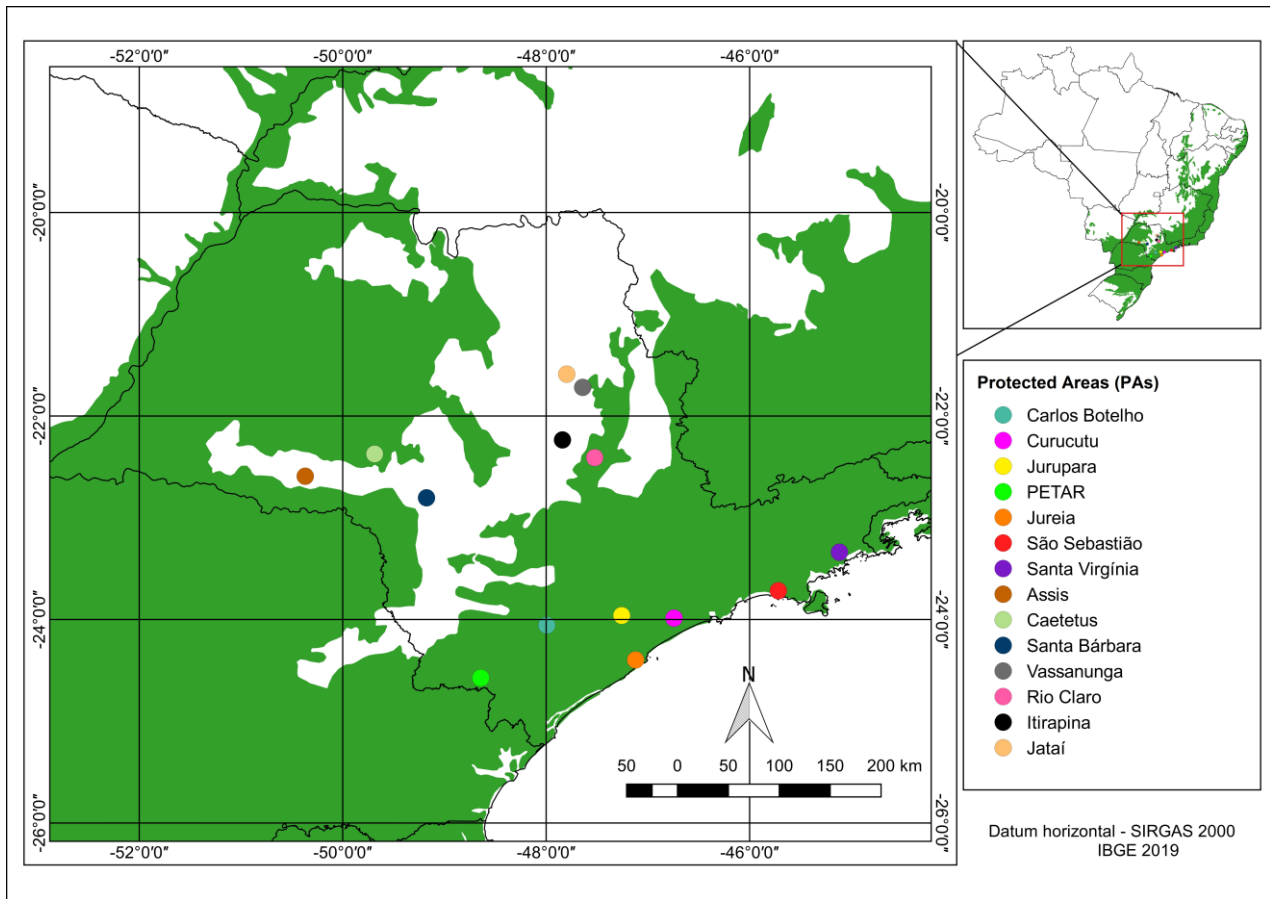
157 The sites, sampling design, phylogeny, and climatic variables are the same as those used in chapter
158 1. For further details, see above and supplementary material.

159

160 **Study area and anuran surveys**

161 We surveyed anuran species in 14 Protected Areas distributed along a longitudinal gradient in the
162 state of São Paulo, southeastern Brazil (Table S1) between December and February 2014-2017. In
163 each PA, we surveyed six sites (two ponds, two streams, and two transects) using three sampling
164 methods: i) survey of adults (Scott Jr. & Woodward 1994) at breeding sites, between 19h and
165 midnight; ii) larval surveys (Shaffer 1994) between 10 and 18 h using a hand dipnet with 3 mm²
166 mesh, passed intensively on the margins of ponds and streams; iii) visual encounter surveys (Crump
167 & Scott Jr. 1994) in the transects looking for species that reproduce by direct development or
168 deposit eggs and/or tadpoles in bromeliads. All adult specimens collected were anesthetized and
169 killed with 10% lidocaine, fixed in 10% formaldehyde, and stored in 70% ethanol in the Coleção de
170 Anfíbios do Departamento de Zoologia da Universidade Estadual Paulista, campus de Rio Claro,
171 São Paulo, Brazil (CFBH). The tadpoles were anesthetized in lidocaine solution soon after
172 collection, and preserved in 10% formalin, and deposited in the Coleção de Anfíbios do
173 Departamento de Zoologia e Botânica da Universidade Estadual Paulista, campus São José do Rio
174 Preto, São Paulo, Brazil (DZSJRP).

175



176

177 **Figure 1.** Map showing the 14 Protected Areas (PAs) in the Brazilian Atlantic Forest.

178

179 **Phylogenetic inference**

180 We built a phylogeny for 112 anuran species using three mitochondrial genes - 12S (1100 bp), 16S
 181 (1573 bp), and cytochrome oxidase subunit I (COI; 645 bp) and one nuclear gene: recombination-
 182 activating gene 1 (RAG-1; 429 bp) (Table S3). We generated some sequences while others were
 183 obtained from Genbank (Table S4). We inferred a dated phylogeny using Bayesian Inference in
 184 BEAST 2 version 2.4.7 (Figure 1S). The aligned sequence block was analyzed using the GTR+ Γ
 185 model. Trees were estimated using relaxed log-normal clock, Yule speciation process, and three
 186 fossil calibration points with a lognormal distribution. The fossil age constraints were obtained from
 187 Wiens (2011). The calibrations included the most recent common ancestral (MRCA) of: i) pipidae
 188 and all other frogs 145 Mya, ii) Bufonidae 55.8 Mya, and iii) “Terrana” 35 Mya. We run one
 189 Markov chain Monte Carlo for 100 million generations, sampling every 10,000 generations, and

190 discarding the first 30 million generations as burn-in. The convergence of parameter estimation was
191 checked using Tracer ver. 1.5. We also used TreeAnnotator ver. 1.8.0 to produce maximum clade
192 credibility trees from the post-burn-in trees.

193

194 **Climatic variables**

195 We obtained climatic variables related to historical and current climate from WorldClim (Hijmans
196 *et al.* 2005, <http://www.worldclim.org>). The climatic variables selected were: Annual Mean
197 Temperature (AMT), Temperature Seasonality (TS), Annual Precipitation (AP), Precipitation
198 Seasonality (PS) (Table S6). Climatic variables related to historical climate represents “bioclimatic
199 variables” of the Last Glacial Maximum (LGM, about 22,000 years ago). The four climate variables
200 correspond to BIO1, BIO12, BIO4 and BIO15, respectively, in the WorldClim database.
201 Furthermore, we used Google Earth (<https://www.google.com/earth/>) to obtain the following
202 topographical data: Maximum Elevation (MAEL), Minimum Elevation (MIEL), and Elevational
203 Range (difference between MAEL and MIEL: ElevR). These variables were used because they
204 describe the average trends as well as variation in temperature, precipitation and elevational range
205 which might represent physiological limits for amphibians (Wiens *et al.* 2006, Buckley & Jetz
206 2007, 2008, da Silva *et al.* 2012).

207

208 **Reproductive modes (RMs)**

209 We followed the classification of reproductive modes (Table 1) according to Haddad & Prado
210 (2005) and Haddad *et al.* (2013) to create a matrix with presence / absence of each RM by species
211 (Table S5). We consider each RM characteristic (i.e., oviposition site, ovum and clutch
212 characteristics, rate of development, stage and size of hatchlings, and type of parental care, if any)
213 as an trait and create a matrix with the presence / absence of the characteristics of each reproductive
214 mode for each species in order to unite the closest species in its most general attributes (e.g., nest
215 eggs), but at the same time differentiating them from a more specific filter within the common

216 characteristics of RM (e.g., nest eggs of foam + exotrophic tadpoles in ponds or nest eggs of foam +
 217 exotrophic tadpoles in streams; Table S5).

218

219 **Table 1.** Reproductive modes (RMs) in anurans (adapted from Haddad & Prado 2005; Haddad *et*
 220 *al.* 2013) observed in the Atlantic Forest.

| RM | Description of the RM |
|-----------|---|
| 1 | Eggs and tadpoles in lentic water |
| 2 | Eggs and tadpoles in lotic water |
| 3 | Eggs and early larval stages in chambers; tadpoles in streams |
| 4 | Eggs and early larval stages in basins; tadpoles in ponds or streams |
| 5 | Eggs and early larval stages in subterranean nests; tadpoles in ponds or streams |
| 6 | Eggs and exotrophic tadpoles in water in tree holes or aerial plants |
| 8 | Eggs and endotrophic tadpoles in water in tree holes or aerial plants |
| 10 | Bubble nest floating on pond; tadpoles in ponds |
| 11 | Foam nest on pond; tadpoles in ponds |
| 12 | Foam nest on pond; tadpoles in streams |
| 13 | Foam nest on water accumulated in constructed basins; tadpoles in ponds |
| 14 | Foam nest on water on the axils of terrestrial bromeliads; tadpoles in ponds |
| 18 | Eggs on ground or rock above water; upon hatching, tadpoles move to water |
| 19 | Eggs on rock or tree roots above water; semi-terrestrial tadpoles |
| 20 | Eggs hatch into tadpoles that are carried to water by adult |
| 21 | Eggs hatch into tadpoles that complete their development in the nest |
| 23 | Direct development of terrestrial eggs |
| 24 | Eggs hatch into tadpoles that drop in lentic water |
| 25 | Eggs hatch into tadpoles that drop in lotic water |
| 27 | Eggs hatch into froglets |
| 28 | Foam nest on the humid forest floor; tadpoles in ponds |
| 30 | Foam nest with eggs and early larval stages in nests; tadpoles in ponds |
| 32 | Foam nest in subterranean nests; tadpoles complete development in nest |
| 36 | Eggs carried on dorsum or in dorsal pouch of female; tadpoles in bromeliads or bamboo |
| 37 | Eggs carried on dorsum or in dorsal pouch of female; direct development |

221

222 **Statistical analyses**

223 **Phylogenetic signal of RMs**

224 We decomposed the Rao's quadratic entropy (Rao 1982) along the nodes of the phylogeny (Pavoine
225 *et al.* 2010) to test for phylogenetic signal in reproductive modes. This analysis measures trait
226 diversity of descendants from a given node (Pavoine *et al.* 2010). Trait diversity is measured by the
227 quadratic entropy index with distances among species calculated from differences among trait
228 states. The contribution to trait diversity of a particular node is equal to the trait diversity among the
229 n groups of species descending from it multiplied by an abundance weight (either proportional to
230 the number of descendant species). Afterwards, used the root skewness test to test if trait diversity
231 is significantly skewed towards the root of the phylogeny, meaning that all descendants will have
232 similar trait values (Pavoine *et al.* 2010).

233 If the representation of trait diversity on the phylogenetic tree highlights a nonrandom pattern,
234 then it is important to test and reject the null hypothesis that the structure associated with trait
235 diversity and phylogenetic relationships among species is not simply a random distribution of trait
236 values across species. First, we test if the trait values of the species in the phylogeny are organized
237 so that only one node expresses the whole diversity (test for concentration of trait diversity on a
238 single node, referred to as SN = "single-node skewness test"). Second, we test if the trait diversity
239 values are evenly distributed across nodes. More precisely, this FN = "few-nodes skewness test"
240 complements the single-node skewness test to determine whether only a few nodes have high
241 contributions to trait diversity while many have low or no contributions. Third, we test if the values
242 of the species are organized within the phylogeny so that the diversity is either clustered near the
243 root or near the tips = Ro (the test is referred to as the "tips skewness test" when phylogenetic
244 skewness is biased toward the tips, the "root skewness test" when skewness is concentrated toward
245 the root node, or the "tips/root skewness test" for a two-sided test if the node contributions are
246 either skewed significantly toward the tips or toward the root). This third test provides a description
247 of phylogenetic signal where species have more different trait values if they are distantly related on

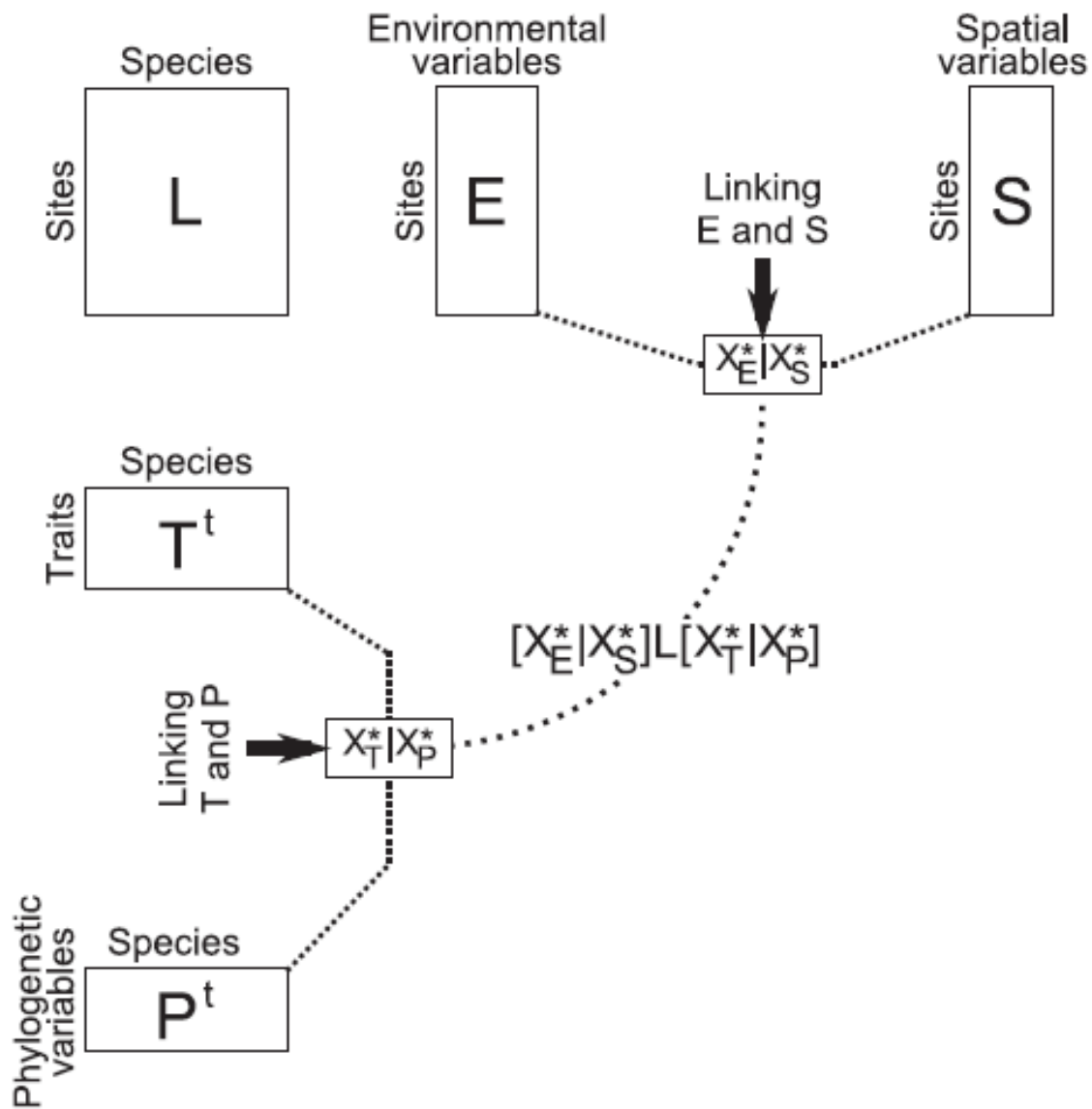
248 the phylogeny (trait diversity is skewed to root) and a description of the absence of phylogenetic
249 signal where closely related species have highly different trait values (e.g., due to convergent
250 evolution and trait diversity is then skewed to tips). All tests are performed by permuting the
251 species across the tips of the phylogenetic tree. Overall, studying trait diversity in a phylogenetic
252 context allows the link between current ecological processes and lineage-dependent historical
253 evolutionary factors to be thoroughly investigated (Pavoine *et al.* 2010).

254

255 **Influence of climatic variables on RMs**

256 We used an extended version of the RLQ (Pavoine *et al.* 2011) to evaluate the relationship between
257 environmental gradients and distribution of reproductive modes. The traditional RLQ analysis (R-
258 mode Linked to Q-mode; Dolédec *et al.* 1996) is an extends of the co-inertia approach (Dolédec &
259 Chessel 1994) to deal with three table ordination technique instead of two (Dolédec *et al.* 1996). As
260 such, it maximizes the covariation between linear combinations of the columns of the
261 environmental (sites by variables, Q matrix) and trait (species by trait, R matrix) matrices using a
262 species composition matrix (species by site) as a link (L matrix). The first step in the RLQ is to
263 analyze each table (R, L, and Q) separately using an appropriate ordination technique to deal with
264 each type of data (Dray *et al.* 2014). The extended RLQ (Pavoine *et al.* 2011) analyzes the
265 correlation between traits and environment controlling for phylogenetic autocorrelation in traits and
266 spatial autocorrelation in environmental variables. The extended RLQ assesses the association
267 between environmental variables and traits using five matrices: i) matrix E with environmental
268 variables of sites; ii) matrix S with geographical coordinates of sites; iii) matrix T with traits of
269 species; iv) matrix P with phylogenetic distance of species; and v) matrix L with presence and
270 absence of species in sites (Pavoine *et al.* 2011, Figure 2). Then, it creates a matrix combining the
271 environmental and space variables, and a matrix combining attributes and phylogeny (Pavoine *et al.*
272 2011). We analyzed with a Principal Component Analysis (PCA, Legendre & Legendre 2012) the
273 reduced matrix of environmental variables; we extracted a distance matrix from phylogeny and

274 calculated a Principal Coordinates Analysis (PCoA, Legendre & Legendre 2012) to extract
275 phylogenetic eigenvectors; and we analyzed with a Correspondence Analysis (CA) the species
276 composition matrix, where the presence of each individual analyzed was placed in rows and
277 localities as columns. This new method combines the original environmental (E), trait (T),
278 phylogenetic distance (P), and geographical coordinates (S) matrices using factorial analysis. Then,
279 each matrix is standardized by dividing it by the square root of its first eigenvector. Afterward, the
280 standardized spatial and environmental matrices are juxtaposed to become matrix R, while matrix Q
281 is defined by the juxtaposition of the standardized trait and phylogenetic matrices (Pavoine *et al.*
282 2011). Finally, these new R and Q matrices are analyzed using the regular RLQ method (Dolédec *et*
283 *al.* 1996). The RLQ is a good method to analyze how traits relate to environmental gradients at the
284 species level (Kleyer *et al.* 2012). The results of the RLQ are interpreted in terms of the first
285 ordination axis, using Spearman rank correlation of scores of ordinal traits (reproductive modes)
286 and Pearson correlation for the scores of continuous climatic variables along the first. We
287 performed all the analyzes in the R v. 3.2.2 (R Core Team 2019) package ade4 (Dray & Dufour
288 2007) using the self-written functions available in Pavoine *et al.* (2011).



289

290 **Figure 2.** Schematic summary of combined analysis of the geographic space (S), environmental
 291 variables (E), species compositions in sampling units (L), biological traits (T) and phylogeny (P). T^t
 292 and P^t are the transposed matrices of T and P, respectively. The notations $[X_E^* | X_S^*]$ and $[X_T^* | X_P^*]$
 293 mean that matrices E and S and matrices T and P, respectively, are transformed in a way that allows
 294 their linking (Taken from Pavoine *et al.* 2011).

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298 **Results**

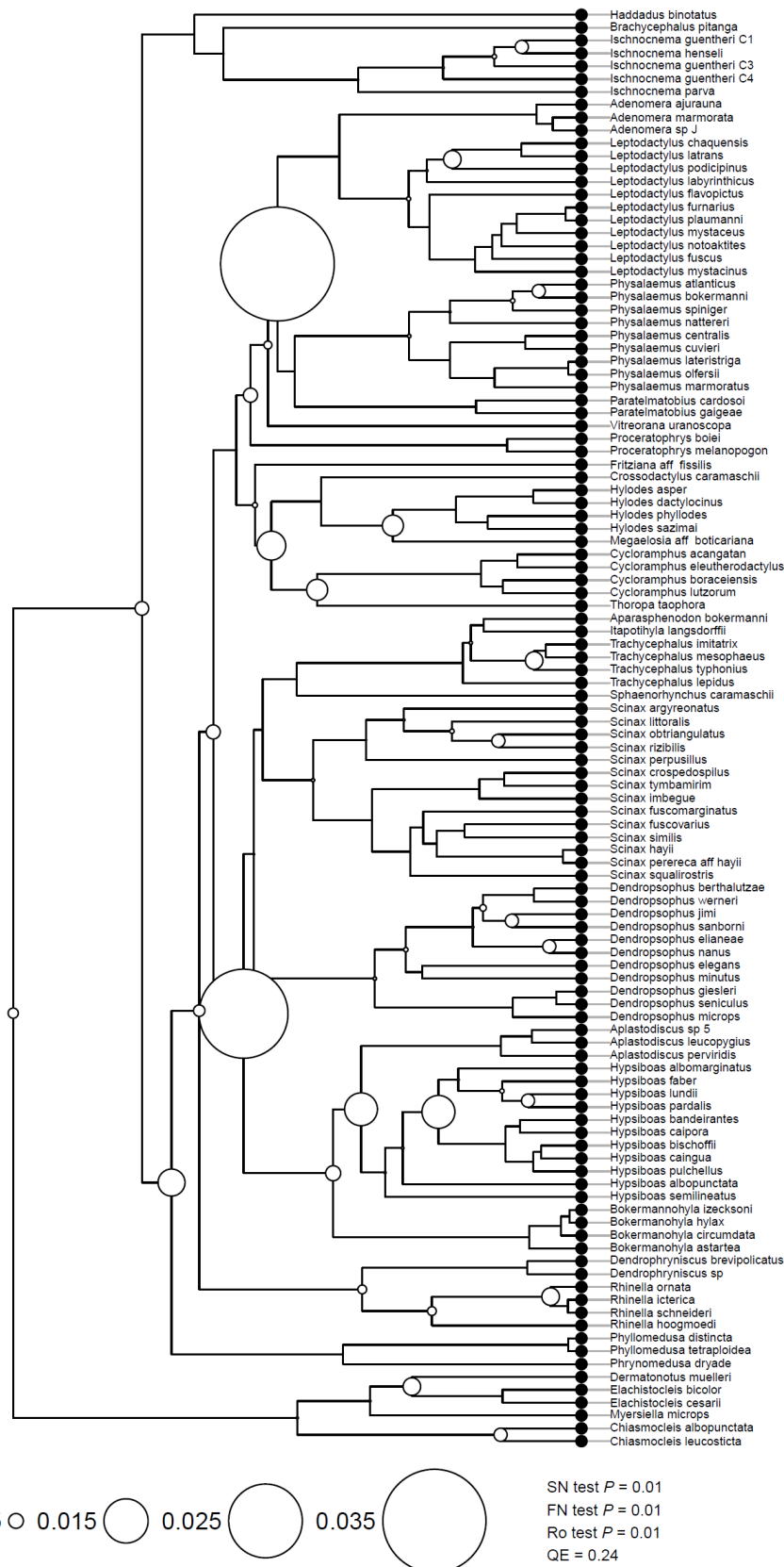
299 **Phylogenetic signal of RMs**

300 We recorded 112 amphibian species, which had 21 reproductive modes (Tables S2 and S5). Trait
301 diversity was significantly shewed towards the root, meaning that reproductive modes had
302 phylogenetic signal (Figure 3). This result indicates that higher taxonomic ranks (e.g., families and
303 subfamilies) have different reproductive modes while lower taxonomic ranks (e.g., genus) have
304 similar trait values.

305

306 **Influence of climatic variables on RMs**

307 The protected areas were distributed along the first RLQ axis in an east-west gradient (Figure 3 and
308 4). The first axis of the RLQ explained 87% of the variation in the data. We found that current and
309 past climate and topography influences the distribution of the reproductive modes of anurans in the
310 Brazilian Atlantic Forest. Communities located in the eastern region of the Atlantic Forest, which
311 present constant precipitation throughout the year, absence of seasonality in temperature and wide
312 altitudinal variation (Figure 4 and 5), harbor anuran species with specialized reproductive modes,
313 such as basin constructions close to reproductive habitats and direct development (e.g., Modes 4
314 and 23, Table S5). These species belong to the families Brachycephalidae, Craugastoridae, and
315 Hylidae. On the other hand, communities located in the western region of the Atlantic Forest, which
316 present high temperatures and marked seasonality in the precipitation (Figure 4 and 5), harbor
317 species of anurans with reproductive modes involving protection of eggs with foam nests that allow
318 greater resistance to desiccation (e.g., Modes 11 and 30, Table S5). These species belong to the
319 family Leptodactylidae.



320

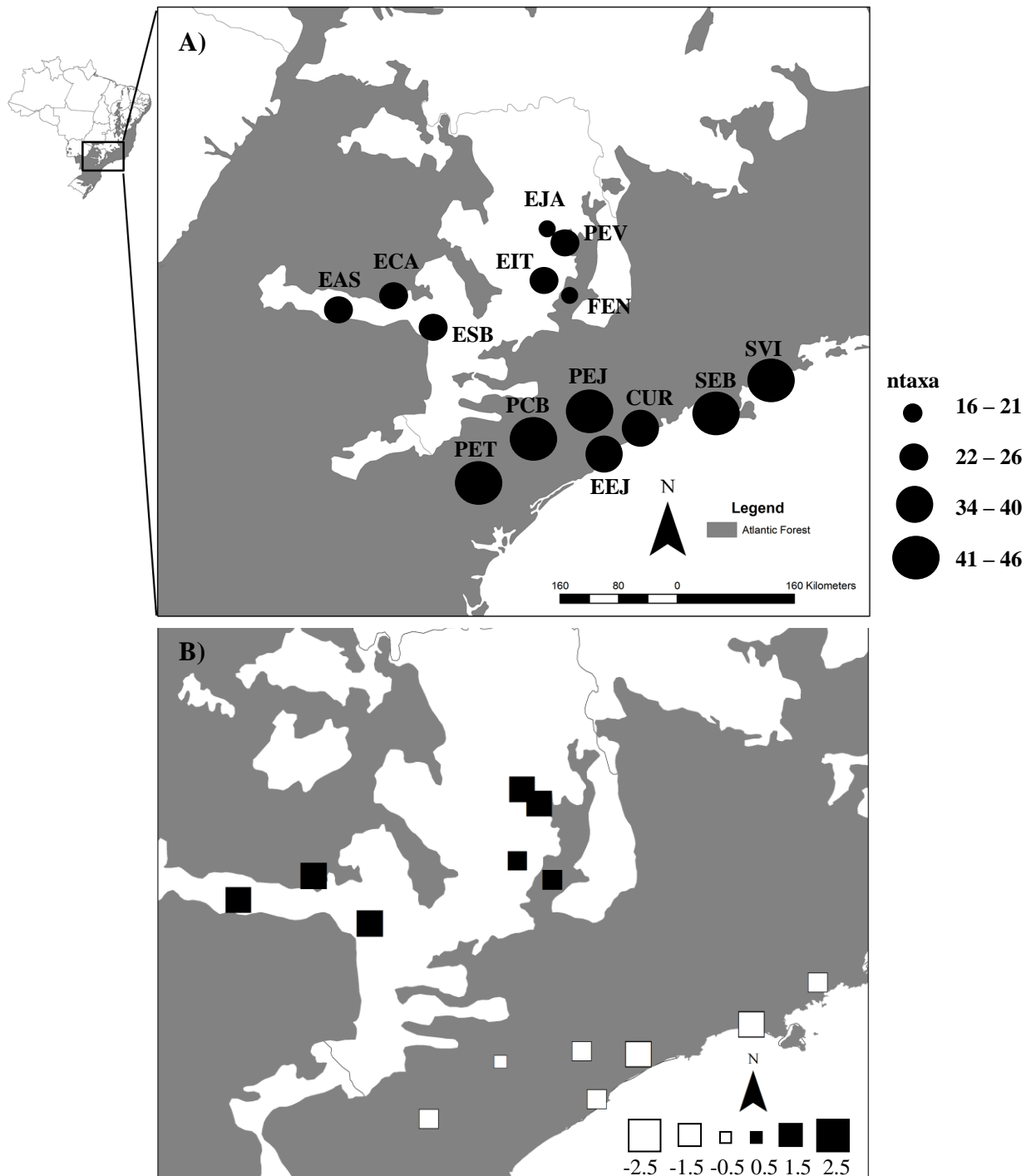
321 **Figure 3.** Decomposition of the diversity of reproductive modes among the nodes of the
 322 phylogenetic tree for the species of anurans sampled in the Brazilian Atlantic Forest. Circles
 323 represent the node's contribution to total diversity. The scale referring to the circles is in the

324 inferential part of the figure. The sum of all contributions is equal to the total trait diversity as
325 measured by the quadratic entropy (QE values). Results of the permutation tests are given at the
326 bottom of figure: SN = single-node skewness test; FN = few-nodes skewness test; Ro = root/tips
327 skewness test.

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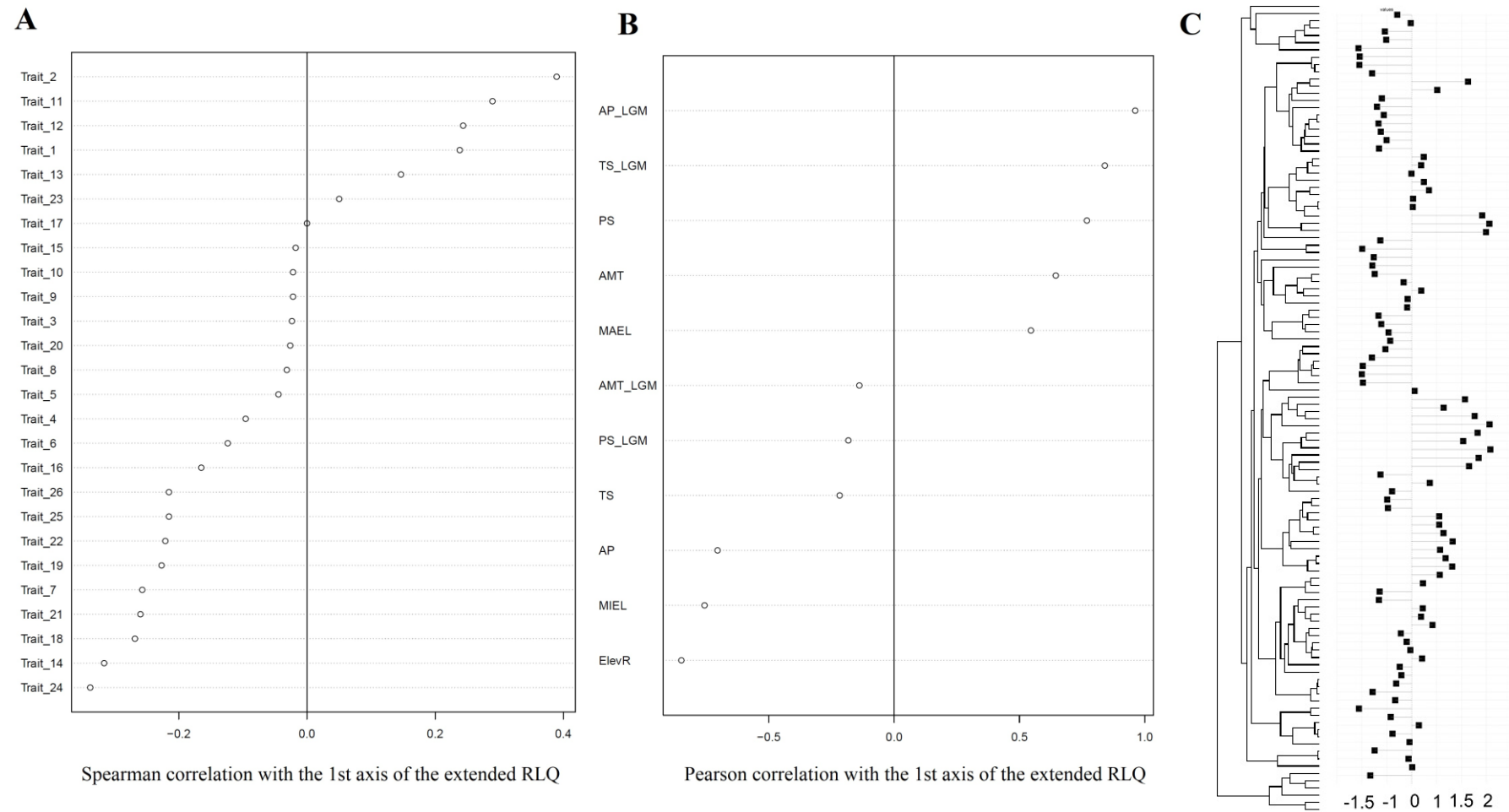
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331

332 **Figure 4.** A) Distribution of the 14 Protected Areas sampled along the longitudinal distribution of
 333 the Brazilian Atlantic Forest. ECA = Estação Ecológica de Caetetus, EAS = Estação Ecológica de
 334 Assis, EIT = Estação Ecológica de Itirapina, EJA = Estação Ecológica de Jataí, PEV = Parque
 335 Estadual Vassununga, FEN = Floresta Edmundo Navarro, ESB = Estação Ecológica de Santa
 336 Bárbara, PCB = Parque Estadual Carlos Botelho, EEJ = Estação Ecológica Jureia-Itatins, PEJ =
 337 Parque Estadual Jurupará, PET = Parque Estadual Alto do Ribeira, CUR = Parque Estadual da Serra

338 do Mar – Núcleo Curucutu, SEB = Parque Estadual Serra do Mar – Núcleo São Sebastião, SVI =
339 Parque Estadual da Serra do Mar – Núcleo Santa Virgínia. Size of the circles is proportional to the
340 number of species in each community (ntaxa). B) Global coordinates of sites (i.e., combination of
341 environmental and spatial variables) relative to the first axis of the extended RLQ. Black and white
342 squares represent negative and positive coordinates, respectively. The size of the squares is
343 proportional to the absolute values of the coordinates.



344

345 **Figure 5.** Details of the distribution of environmental variables, reproductive modes and phylogenetic tree of anuran species along the first axis of the
 346 extended RLQ analysis. A) Spearman correlation between the reproductive modes and the coordinates of the first axis of the extended RLQ. B)
 347 Pearson correlation between the environmental variables and the coordinates of the first axis of the extended RLQ. C) Phylogenetic tree and

348 coordinates of the first axis of the extended RLQ. From this figure we deduce that some species that occur in sites with high levels of precipitation and
349 mild temperatures (negative correlations in Fig. B) present specialized reproductive modes for direct development and basin constructions close to
350 reproductive habitats (negative correlations Figs. A and C). On the other hand, species that occurred in places with high temperatures and marked
351 seasonality in precipitation (positive correlations in Fig. B) present more resistant reproductive methods to desiccation with protection by foam nests
352 (positive correlations Figs. A and C).

353 **Discussion**

354 Our results show a strong effect of environmental gradients on reproductive modes along the east-
355 west Atlantic Forest (AF) climate gradient. The specialized reproductive modes for streams,
356 bromeliads and soil occurred in areas with high moisture levels and mild temperatures located in
357 the eastern region of the AF, while specialized reproductive modes to be more resistant to
358 desiccation occurred in regions with high temperatures and marked seasonality in the precipitation
359 located in the western region of the AF. The reproductive modes associated with high temperatures
360 and marked seasonality in precipitation were exclusive of leptodactylids, while traits associated
361 with milder temperatures and constant precipitation throughout the year were shared by species of
362 the families Brachycephalidae, Craugastoridae, and Hylidae. The association of amphibians with
363 humid areas is expected based on their physiological requirements (moist environments prevent
364 desiccation of the animal, and allow cutaneous gas exchange) and also based on their reproductive
365 requirements (an amniotic eggs that depend on water bodies or moist environments for an adequate
366 development), which are unique characteristics of this animal group (Duellman & Trueb 1994).
367 Phylogenetically related species often have similar functional trait values, and it is expected that
368 them to co-occur more often in the same communities, reflecting their shared environmental
369 tolerances (Andersen *et al.* 2012). Recent studies have supported these results. For example, Li &
370 Wiens (2017) found that hotter and seasonally changing environments influenced traits of lizard
371 species by reducing the temperature range. Furthermore, for several other groups it was shown that
372 the environmental gradient drives a strong influence on the assembly of the communities (e.g.,
373 Graham *et al.* 2009, Hoiss *et al.* 2012, Dehling *et al.* 2014, Qian & Ricklefs 2016, Qian *et al.* 2017).
374 Thus, environmental hardness in the western sites may prevent the colonization of new species
375 (with non-climate-adapted reproductive modes that are more harshness) from the eastern region,
376 which is recognized for its high rates of speciation and endemism.

377 The reproductive modes had phylogenetic signal. This suggests that phylogenetic relatedness,
378 at the scale of the entire tree, can serve as a reasonable proxy for trait similarity. Furthermore, both

379 trait and phylogenetic community signal were consistent with an interpretation of environmental
380 filtering. These results are indicative that phylogenetic proximity may be a surrogate for niche
381 overlap (Cavender-Bares *et al.* 2004, Vamosi *et al.* 2009, Pausas & Verdú 2010). For example, in
382 tropical rain forest tree communities, a consistent phylogenetic signal for climate niche has been
383 shown to occur along regional rainfall gradients and to be well correlated among continents,
384 indicating that initial adaptations for particular climatic conditions tend to be well conserved within
385 at least some major clades (Hardy *et al.* 2012). Although establishing of the phylogenetic signal
386 does not demonstrate the existence of phylogenetic niche conservatism (Losos 2008), once
387 ecological traits have been found to display various phylogenetic signals from convergence to
388 conservatism (Losos 2008), herein, this convergence lead to a phylogenetic signal so that both
389 phylogenetic clustering and trait clustering was found locally. Trait variation can be resulted both
390 from the effect of niche conservatism and unique and independent adaptive responses of each
391 species to environmental conditions. Thus, these results confirm an important contribution of
392 climate niche conservatism to explain the spatial distribution of the diversity of functional attributes
393 (reproductive modes) in the Atlantic Forest, mainly due to the inability of most amphibian lineages
394 with more sensitive reproductive modes (southeastern Atlantic Forest), which is probably related to
395 intolerance to the harsh environment.

396 Our results suggest that niche conservatism and environmental filters are important processes
397 organizing anuran communities in the Brazilian Atlantic Forest. They demonstrate that
398 environmental gradients limit the distribution of anuran species with reproductive modes dependent
399 on humidity. Several studies have demonstrated the expected drastic effect of climate change on the
400 distribution of anurans' richness in the Atlantic Forest (e.g., Lemes *et al.* 2014, Loyola *et al.* 2014,
401 Vasconcelos *et al.* 2018, 2019). Thus, due to the physiological, morphological or behavioral
402 restrictions of amphibians, and given that reproductive modes are associated with susceptibility to
403 desiccation, our results help to understand the spatial distribution of anuran communities in the

404 Brazilian Atlantic Forest and indicate that the effects of climate change may also affect the
405 distribution of the reproductive modes of species of anurans more sensitive to the warmer climate.

406

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Considerações finais

Nossos resultados demonstram que os gradientes climáticos e topográficos limitam a distribuição de espécies de anuros de determinadas linhagens com modos reprodutivos dependentes da umidade. Assim, estes resultados sugerem que o conservadorismo de nicho (isto é, a tendência das linhagens de reter suas características relacionadas ao nicho através de eventos de especiação) e os filtros ambientais são importantes processos que organizam as comunidades de anfíbios.

Portanto, nossos resultados ajudam a distinguir a importância relativa dos diferentes processos ecológicos e evolutivos que auxiliam na compreensão de como a biodiversidade varia no espaço, desvendando mecanismos de montagem e entendendo os fatores que explicam a estrutura das comunidades de anfíbios na Mata Atlântica Brasileira.

Apêndices – Supplementary material

Table S1. Description of the localities sampled in this study.

| Protected Areas (PAs) | Abbreviations |
|---|----------------------|
| Parque Estadual Carlos Botelho | PCB |
| Parque Estadual Alto do Ribeira | PET |
| Parque Estadual da Serra do Mar – Núcleo Curucutu | CUR |
| Parque Estadual da Serra do Mar – Núcleo Santa Virgínia | SVI |
| Parque Estadual da Serra do Mar – Núcleo São Sebastião | SEB |
| Estação Ecológica Jureia-Itatins | EEJ |
| Parque Estadual Jurupará | PEJ |
| Parque Estadual Vassununga | PEV |
| Estação Ecológica de Santa Bárbara | ESB |
| Estação Ecológica de Assis | EEA |
| Estação Ecológica de Jataí | EJA |
| Estação Ecológica de Itirapina | E EI |
| Estação Ecológica de Caetetus | EEC |
| Floresta Edmundo Navarro | FEN |

Table S2. List of species with abundance for the 112 species of anurans recorded in the 14 Protected Areas, in the Brazilian Atlantic Forest. ECA = Estação Ecológica de Caetetus; EAS = Estação Ecológica de Assis; EIT = Estação Ecológica de Itirapina; EJA = Estação Ecológica de Jataí; PEV = Parque Estadual Vassununga; FEN = Floresta Edmundo Navarro; ESB = Estação Ecológica de Santa Bárbara; PCB = Parque Estadual Carlos Botelho; EEJ = Estação Ecológica Jureia-Itatins; PEJ = Parque Estadual Jurupará; PET = Parque Estadual Alto do Ribeira; CUR = Parque Estadual da Serra do Mar – Núcleo Curucutu; SEB = Parque Estadual da Serra do Mar – Núcleo São Sebastião; SVI = Parque Estadual da Serra do Mar – Núcleo Santa Virgínia.

| Species | ECA | EAS | EIT | EJA | PEV | FEN | ESB | PCB | EEJ | PEJ | PET | CUR | SEB | SVI |
|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <i>Adenomera marmorata</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 12 | 8 | 2 | 20 | 70 | 32 |
| <i>Adenomera</i> sp. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 |
| <i>Adenomera ajurauna</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 |
| <i>Aparasphenodon bokermanni</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 5 | 0 | 0 | 0 |
| <i>Aplastodiscus</i> sp. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 3 | 0 | 0 | 1 | 4 |
| <i>Aplastodiscus leucopygius</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 5 | 2 | 1 | 2 | 10 |
| <i>Aplastodiscus perviridis</i> | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Bokermanohyla astartea</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Bokermanohyla circumdata</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 1 | 2 | 1 | 4 | 1 | 6 |
| <i>Bokermanohyla hylax</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 2 | 9 | 1 | 4 | 9 | 4 |
| <i>Bokermannohyla izecksoni</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| <i>Brachycephalus pitanga</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 |
| <i>Chiasmocleis albopunctata</i> | 0 | 5 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Chiasmocleis leucosticta</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 2 | 0 |
| <i>Crossodactylus caramaschii</i> | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 0 | 3 | 0 | 0 | 0 | 0 |
| <i>Cycloramphus acangatan</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

| | | | | | | | | | | | | | | |
|--|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| <i>Cycloramphus boraceiensis</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 |
| <i>Cycloramphus eleutherodactylus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| <i>Cycloramphus lutzorum</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 |
| <i>Dendrophryniscus brevipolicatus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 1 | 1 | 0 | 8 | 1 | 1 |
| <i>Dendrophryniscus sp.</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| <i>Dendropsophus berthalutzae</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 23 | 1 |
| <i>Dendropsophus elegans</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 21 | 30 | 20 | 2 | 3 | 1 |
| <i>Dendropsophus elianeae</i> | 2 | 2 | 1 | 0 | 5 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Dendropsophus giesleri</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 2 | 0 | 0 | 0 | 0 |
| <i>Dendropsophus jimi</i> | 0 | 0 | 5 | 0 | 1 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Dendropsophus microps</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 15 | 20 | 24 | 0 | 2 | 37 |
| <i>Dendropsophus minutus</i> | 11 | 40 | 43 | 2 | 5 | 25 | 25 | 16 | 9 | 23 | 18 | 7 | 6 | 26 |
| <i>Dendropsophus nanus</i> | 41 | 65 | 8 | 20 | 25 | 10 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Dendropsophus sanborni</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| <i>Dendropsophus seniculus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 1 | 5 | 0 | 1 | 15 |
| <i>Dendropsophus weneri</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 33 | 35 | 0 | 0 | 0 |
| <i>Dermatonotus muelleri</i> | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Elachistocleis bicolor</i> | 0 | 15 | 15 | 0 | 0 | 5 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Elachistocleis cesarii</i> | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Fritziana aff. fissilis</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 6 | 19 | 8 | 22 | 10 | 9 |
| <i>Haddadus binotatus</i> | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 6 | 3 | 3 | 7 | 4 | 3 |
| <i>Hylodes asper</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 |
| <i>Hylodes dactylocinus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| <i>Hylodes phyllodes</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 15 | 10 |
| <i>Hylodes sazimai</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| <i>Hypsiboas albomarginatus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 32 | 22 | 23 | 8 | 15 | 5 |
| <i>Hypsiboas albopunctata</i> | 1 | 5 | 9 | 7 | 5 | 9 | 30 | 0 | 0 | 11 | 0 | 40 | 0 | 3 |

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|-----------------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| <i>Myersiella microps</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| <i>Paratelmatoobius cardosoi</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 |
| <i>Paratelmatoobius gaigeae</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Phrynomedusa dryade</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| <i>Phyllomedusa distincta</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 1 | 3 | 7 | 0 | 0 | 0 |
| <i>Phyllomedusa tetraploidea</i> | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Physalaemus atlanticus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 |
| <i>Physalaemus bokermanni</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 |
| <i>Physalaemus centralis</i> | 0 | 0 | 5 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Physalaemus cuvieri</i> | 17 | 25 | 25 | 10 | 2 | 13 | 6 | 0 | 0 | 14 | 0 | 25 | 0 | 7 |
| <i>Physalaemus lateristriga</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 27 | 26 | 0 | 0 | 0 |
| <i>Physalaemus marmoratus</i> | 1 | 11 | 2 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Physalaemus nattereri</i> | 2 | 19 | 7 | 0 | 5 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Physalaemus olfersii</i> | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 14 |
| <i>Physalaemus spiniger</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 8 | 0 | 0 | 0 |
| <i>Proceratophrys boiei</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 0 | 14 | 2 | 0 | 2 | 1 |
| <i>Proceratophrys melanopogon</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| <i>Rhinella hoogmoedi</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| <i>Rhinella icterica</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 10 | 2 | 15 | 16 | 2 | 27 |
| <i>Rhinella ornata</i> | 11 | 0 | 2 | 4 | 4 | 19 | 2 | 2 | 13 | 7 | 17 | 11 | 14 | 5 |
| <i>Rhinella schneideri</i> | 4 | 6 | 2 | 4 | 3 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax argyreonatus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 |
| <i>Scinax crospedospilus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 2 | 2 | 1 | 5 | 5 |
| <i>Scinax fuscomarginatus</i> | 8 | 8 | 3 | 2 | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax fuscovarius</i> | 11 | 45 | 24 | 1 | 23 | 9 | 3 | 2 | 0 | 0 | 4 | 1 | 0 | 1 |
| <i>Scinax hayii</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 26 |
| <i>Scinax imbegue</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 | 0 |

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|--|-----|-----|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <i>Scinax littoralis</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 16 | 2 | 0 | 0 | 28 | 0 |
| <i>Scinax obtriangulatus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 |
| <i>Scinax perereca</i> aff. <i>hayii</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 5 | 20 | 11 | 8 | 0 | 0 |
| <i>Scinax perpusillus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 3 | 7 | 6 | 52 | 5 | 20 |
| <i>Scinax rizibilis</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 3 | 24 | 46 | 1 | 4 | 4 |
| <i>Scinax similis</i> | 1 | 2 | 1 | 0 | 13 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax squalirostris</i> | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax tymbamirim</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 28 | 28 | 12 | 36 | 22 | 5 |
| <i>Sphaenorhynchus caramaschii</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 25 | 0 | 0 | 0 |
| <i>Thoropa taophora</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 1 | 0 |
| <i>Trachycephalus imitatrix</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| <i>Trachycephalus mesophaeus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 1 | 0 |
| <i>Trachycephalus lepidus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Trachycephalus typhoni</i> | 0 | 7 | 0 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Vitreorana uranoscopa</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 10 | 9 | 6 | 20 | 12 | 24 |
| Total abundance | 195 | 382 | 201 | 74 | 134 | 130 | 166 | 452 | 330 | 484 | 452 | 442 | 403 | 441 |

Table S3. Details of the primers including: name of the primer, sequence and authors.

| Gene | Primers | Direction | Sequence (5' for 3') | Authors |
|------|------------|-----------|-------------------------------------|---------------------------|
| Cytb | MVZ 15-L | Forward | GAACTAATGGCCCACACWWTACGNAA | Moritz et al. 1992 |
| Cytb | H15149 | Reverse | AAACTGCAGCCCCTCAGAAATGATATTTGTCCTCA | Kocher et al. 1989 |
| 12S | t-Phe-frog | Forward | ATAGCRCTGAARAYGCTRAGATG | Wiens et al. 2005 |
| 12S | t-Val-frog | Reverse | TGTAAGCGARAGGCTTTKGTTAAGCT | Wiens et al. 2005 |
| 16S | SC | Forward | GTRGGCCTAAAAGCAGCCAC | Darst and Cannatella 2004 |
| 16S | BR | Reverse | CCGGTCTGAACTCAGATCACGT | Palumbi et al. 1991 |
| RAG1 | TG1F | Forward | GAGAAGTCTACAAAAVGGCAAAG | Faivovich et al. 2005 |
| RAG1 | TG1R | Reverse | GAAGCGCCTGAACAGTTTATTAC | Faivovich et al. 2005 |

Table S4. Details of the primers including: species, name of the primer and sequence.

| Species | 12S | 16S | COI | RAG1 |
|-----------------------------------|------------|------------|------------|------------|
| <i>Adelophryne baturitensis</i> | JX298249.1 | JX298281.1 | KU494301 | JX298149.1 |
| <i>Adelophryne maranguapensis</i> | JX298252.1 | JX298285.1 | KU494312 | JX298152.1 |
| <i>Adelophryne pachydactyla</i> | JX298259.1 | JX298294.1 | CFBHT16634 | JX298161.1 |
| <i>Adenomera marmorata</i> | KC470092.1 | KC477242.1 | KF674784.1 | KF674474.1 |
| <i>Adenomera sp.</i> | CFBHT19356 | CFBHT19356 | CFBHT19356 | CFBHT19356 |
| <i>Adenomera ajurauna</i> | KF675179 | KF675179.1 | KF674771 | KF674458 |
| <i>Adenomera andreae</i> | KC520683.1 | KC520683.1 | KC520689.1 | KF674220.1 |
| <i>Agalychnis callidryas</i> | AY843563 | AY843563 | FJ766570.1 | * |
| <i>Agalychnis granulosa</i> | AY843687.1 | AY843687.1 | KU494462 | AY844469.1 |
| <i>Allobates femoralis</i> | DQ283045.1 | DQ283045.1 | DQ502916.1 | DQ503326.1 |
| <i>Allobates zaparo</i> | DQ502026.1 | DQ502026.1 | DQ502752.1 | DQ503305.1 |
| <i>Allophryne ruthveni</i> | AY843564.1 | AY843564.1 | KU494330 | AY844361.1 |
| <i>Ameerega flavopicta</i> | DQ502124.1 | DQ502124.1 | DQ502831 | DQ503340.1 |
| <i>Ameerega picta</i> | KJ940455 | KJ940455.1 | TG255-3 | TG255-3 |
| <i>Aparasphenodon bokermanni</i> | CFBHT19320 | CFBHT19320 | CFBHT19320 | CFBHT19320 |
| <i>Aparasphenodon brunoi</i> | AY843567.1 | AY843567.1 | KU494340 | AY844364.1 |
| <i>Aplastodiscus albosignatus</i> | KU184037 | KU184037 | KU184064 | KU184086 |
| <i>Aplastodiscus arildae</i> | KU184012 | KU184012 | KU184062 | KU184084 |
| <i>Aplastodiscus cavicola</i> | KU184023 | KU184023 | KU184044 | KU184089 |
| <i>Aplastodiscus cochranae</i> | AY843568 | AY843568 | KU184043 | AY844365 |
| <i>Aplastodiscus eugenioi</i> | KU184030 | KU184030 | KU184066 | KU184088 |
| <i>Aplastodiscus flumineus</i> | KU184013 | KU184013 | KU184072 | KU184092 |
| <i>Aplastodiscus leucopygius</i> | KU184033 | KU184033 | KU184055 | KU184080 |
| <i>Aplastodiscus perviridis</i> | KU184020 | KU184020 | KU184057 | KU184082 |
| <i>Aplastodiscus sp 5</i> | CFBHT19296 | CFBHT19296 | CFBHT19296 | CFBHT19296 |
| <i>Aplastodiscus weygoldti</i> | KU184026 | KU184026 | KU184069 | KU184090 |
| <i>Arcovomer passarellii</i> | TG402 | TG402 | TG402 | * |
| <i>Barycholos ternetzi</i> | A561 | JX267466.1 | KU494355 | A561 |

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|--|------------|------------|------------------|------------------|
| <i>Bokermannohyla alvarengai</i> | AY843677.1 | AY843677.1 | CFBHT08264 | AY844461.1 |
| <i>Bokermannohyla izecksoni</i> | CFBH17677 | MLL-A69 | CFBHT04412 | MLLA69 |
| <i>Bokermannohyla astartea</i> | A1321 | MLL-A1302 | CFBHT17905/A1321 | A1302/1325 |
| <i>Bokermannohyla circumdata</i> | AY549328.1 | AY549328.1 | CFBHT04376 | AY844409.1 |
| <i>Bokermannohyla hylax</i> | CFBHT19426 | CFBHT19268 | CFBHT03848 | CFBHT19268 |
| <i>Brachycephalus ephippium</i> | AY326008.1 | AY326008.1 | CFBHT02424/A192 | A192 |
| <i>Brachycephalus pitanga</i> | HQ435686.1 | HQ435699.1 | CFBHT14865 | CFBHT19898 |
| <i>Ceratophrys aurita</i> | KP295606.1 | KP295606.1 | KP295687.1 | KP295587.1 |
| <i>Ceratophrys cranweli</i> | KP295609.1 | KP295609.1 | KP295690.1 | KP295590.1 |
| <i>Ceratophrys joazeirensis</i> | KP295617.1 | KP295617.1 | KP295692.1 | KP295594.1 |
| <i>Ceratophrys ornata</i> | AY326013.1 | AY326013.1 | KP295693.1 | KP295595.1 |
| <i>Chiasmocleis albopunctata</i> | TGA579 | TGA579 | TGA579 | * |
| <i>Chiasmocleis crucis</i> | CFBHT16631 | CFBHT16631 | CFBHT15820 | * |
| <i>Chiasmocleis leucosticta</i> | CFBHT19367 | CFBHT19367 | CFBHT11667 | * |
| <i>Chiasmocleis mantiqueira</i> | MLL1252 | MLLA1252 | CFBHT14478/A1252 | * |
| <i>Corythomantis greeningi</i> | AY843578.1 | AY843578.1 | A451 | MLLA451 |
| <i>Crossodactylus caramaschii</i> | CFBHT19072 | CFBHT19072 | KU494385 | KC604005.1 |
| <i>Crossodactylus schmdti</i> | AY843579.1 | AY843579.1 | CFBH | AY844375.1 |
| <i>Cycloramphus acangatan</i> | A1051 | MLLA1051 | A1051 | A1051 |
| <i>Cycloramphus boraceiensis</i> | DQ283097.1 | DQ283097.1 | DQ502856.1 | DQ503357.1 |
| <i>Cycloramphus eleutherodactylus</i> | CFBHT19228 | CFBHT19228 | CFBHT10534 | CFBHT19228 |
| <i>Cycloramphus lutzorum</i> | CFBHT19221 | CFBHT19221 | CFBHT19204/A1022 | CFBHT19221/A1022 |
| <i>Dasypops schirchi</i> | DQ283095.1 | DQ283095.1 | KU494399 | * |
| <i>Dendrobates auratus</i> | AY843581.1 | AY843581.1 | FJ766698.1 | * |
| <i>Dendrobates leucomelas</i> | EU342648.1 | EU342648.1 | DQ502850.1 | * |
| <i>Dendrobates tinctorius</i> | DQ502248.1 | DQ502248.1 | DQ502918.1 | DQ503387.1 |
| <i>Dendrophryniscus brevipolicatus</i> | MLLA28 | AF375515.1 | KU494405 | A028 |
| <i>Dendrophryniscus leucomystax</i> | TG416 | TG416 | CFBHT14571/TG416 | TG416 |
| <i>Dendrophryniscus sp.</i> | CFBHT19412 | CFBHT19412 | CFBHT19412 | CFBHT19412 |
| <i>Dendropsophus anceps</i> | AY843597.1 | AY843597.1 | CFBHT10908 | AY844386.1 |
| <i>Dendropsophus berthaltutzae</i> | AY843607.1 | AY843607.1 | KU494409 | AY844397.1 |

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|------------------------------------|------------|------------|-----------------|------------|
| <i>Dendropsophus ebraccatus</i> | AY843624.1 | AY843624.1 | KP149221.1 | AY844415.1 |
| <i>Dendropsophus elegans</i> | DQ380355.1 | AF308102.1 | CFBHT14315/A622 | A622 |
| <i>Dendropsophus elianeae</i> | CFBHT19014 | CFBHT19014 | CFBHT00949 | CFBHT19014 |
| <i>Dendropsophus giesleri</i> | AY843629.1 | AY843629.1 | CFBHT15819 | AY844417.1 |
| <i>Dendropsophus jimi</i> | CFBHT18968 | CFBHT18968 | CFBHT10273 | CFBHT18968 |
| <i>Dendropsophus microcephalus</i> | AY843643.1 | AY843643.1 | KP149218.1 | AY844430.1 |
| <i>Dendropsophus microps</i> | CFBHT19199 | CFBHT19199 | KU494411 | CFBHT19199 |
| <i>Dendropsophus minutus</i> | FRSG022 | AY549345.1 | KJ940047.1 | FRS022 |
| <i>Dendropsophus nanus</i> | AY549346.1 | AY549346.1 | CFBHT07711 | AY844437.1 |
| <i>Dendropsophus parviceps</i> | AY843652.1 | AY843652.1 | MTR_ALCX209P71 | AY844440.1 |
| <i>Dendropsophus rubicundulus</i> | AY843661.1 | AY843661.1 | CFBHT | AY844449.1 |
| <i>Dendropsophus sanborni</i> | AY843663.1 | AY843663.1 | CFBHT05313 | AY844450.1 |
| <i>Dendropsophus seniculus</i> | CFBHT19134 | CFBHT19134 | CFBHT09034 | CFBHT19231 |
| <i>Dendropsophus triangulum</i> | AY843680.1 | AY843680.1 | MTR_ALCX182P23 | AY844464.1 |
| <i>Dendropsophus werneri</i> | CFBHT19216 | CFBHT19216 | CFBHT04969 | CFBHT19216 |
| <i>Dermatonotus muelleri</i> | MLLA381 | MLL-A381 | KU494424 | AY571647.1 |
| <i>Elachistocleis bicolor</i> | CFBHT18971 | CFBHT18971 | KU494428 | * |
| <i>Elachistocleis cesarii</i> | CFBHT18972 | CFBHT18972 | KU494431 | * |
| <i>Engystomops petersi</i> | DQ337234.1 | DQ337234.1 | * | GQ375543.1 |
| <i>Euparkerella brasiliensis</i> | JX298276.1 | JX267468.1 | KF625079.1 | JX298185.1 |
| <i>Fritziana aff. fissilis</i> | CFBHT 3863 | CFBHT3863 | CFBHT 3863 | CFBHT 3863 |
| <i>Fritziana goeldii</i> | MW31 | MW31 | MW31 | MW31 |
| <i>Fritziana ohausi</i> | CFBHT 6528 | CFBHT6528 | CFBHT 6528 | CFBHT 6528 |
| <i>Gastrotheca cornuta</i> | AY843591.1 | AY843591.1 | KC014657.1 | DQ679280.1 |
| <i>Gastrotheca fissipes</i> | AY843592.1 | AY843592.1 | CFBHT00377 | * |
| <i>Gastrotheca pulchra</i> | AY326051.1 | AY326051.1 | KU494441 | * |
| <i>Haddadus binotatus</i> | DQ283092.1 | DQ283092.1 | KU494446 | A575 |
| <i>Holoaden bradei</i> | EF493378.1 | EF493366.1 | * | JX298186.1 |
| <i>Holoaden luederwaldti</i> | EU186728.1 | EU186710.1 | KU494456 | A104 |
| <i>Hylodes asper</i> | A171 | KM390792.1 | KU494458/A171 | A171 |
| <i>Hylodes dactylocinus</i> | FRS1068 | FRS1068 | CFBH | FRS1068 |
| <i>Hylodes hayeri</i> | CC067 | CC067 | CFBH | CC067 |

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|---------------------------------|------------|------------|------------|------------|
| <i>Hylodes phyllodes</i> | FRS904 | FRS904 | CFBH | FRS904 |
| <i>Hylodes sazimai</i> | AY143344.1 | MLL-A801 | KJ961565.1 | KJ961603.1 |
| <i>Hypsiboas albomarginatus</i> | CFBHT19422 | CFBHT19422 | CFBH | CFBHT19422 |
| <i>Hypsiboas albopunctata</i> | AY549317.1 | AY549317.1 | CFBH | * |
| <i>Hypsiboas alfaroi</i> | KF955303.1 | KF955305.1 | KF955306.1 | * |
| <i>Hypsiboas bandeirantes</i> | CFBHT19382 | CFBHT19382 | CFBHT19382 | CFBHT19382 |
| <i>Hypsiboas bischoffii</i> | AY549324.1 | AY549324.1 | KU494467 | AY844398.1 |
| <i>Hypsiboas caingua</i> | CBHT19051 | CFBHT19051 | CBHT19051 | CBHT19051 |
| <i>Hypsiboas caipora</i> | CFBHT19239 | CFBHT19239 | CFBHT19239 | CFBHT19239 |
| <i>Hypsiboas calcaratus</i> | AY326056.1 | AY326056.1 | CFBH | * |
| <i>Hypsiboas crepitans</i> | AY843621.1 | AY843621.1 | KU494470 | AY844412.1 |
| <i>Hypsiboas ericae</i> | AY549332.1 | AY549332.1 | CFBH | AY844416.1 |
| <i>Hypsiboas faber</i> | AY549334.1 | AY549334.1 | JQ627303.1 | TG368 |
| <i>Hypsiboas fasciatus</i> | AY819427.1 | AY549335.1 | CFBH | * |
| <i>Hypsiboas joaquina</i> | AY549340.1 | AY549340.1 | CFBH | AY844421.1 |
| <i>Hypsiboas lanciformis</i> | AY843636.1 | AY843636.1 | KP149169.1 | * |
| <i>Hypsiboas leptolineatus</i> | AY549341.1 | AY549341.1 | CFBH | AY844424.1 |
| <i>Hypsiboas lundii</i> | AY843639.1 | AY843639.1 | CFBH | A666 |
| <i>Hypsiboas marginatus</i> | AY549342.1 | AY549342.1 | CFBH | AY844426.1 |
| <i>Hypsiboas multifasciatus</i> | AY843648.1 | AY843648.1 | CFBH | AY844436.1 |
| <i>Hypsiboas pardalis</i> | AY843651.1 | AY843651.1 | KU494481 | A648 |
| <i>Hypsiboas polytaenius</i> | AY843655.1 | AY843655.1 | CFBH | AY844443.1 |
| <i>Hypsiboas pulchellus</i> | AY549352.1 | AY549352.1 | CFBHT14792 | AY844445.1 |
| <i>Hypsiboas semilineatus</i> | FRS912 | AY843779.1 | FRS912 | FRS912 |
| <i>Hypsiboas semiguttata</i> | AY549357 | AY549357 | CFBHT03250 | AY844452 |
| <i>Hypsiboas sibleszi</i> | AY843667.1 | AY843667.1 | * | AY844455.1 |
| <i>Ischnocnema guentheri C3</i> | CFBHT19403 | CFBHT19402 | * | CFBHT19114 |
| <i>Ischnocnema guentheri C1</i> | CFBHT19304 | CFBHT19304 | CFBHT19304 | CFBHT19304 |
| <i>Ischnocnema guentheri C4</i> | CFBHT19389 | CFBHT19389 | * | CFBHT19389 |
| <i>Ischnocnema henseli</i> | CFBHT19436 | CFBHT19436 | CFBHT19436 | CFBHT19272 |

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|-------------------------------------|------------|------------|------------------|------------|
| <i>Ischnocnema holti</i> | JX267306.1 | JX267306.1 | A819 | A819 |
| <i>Ischnocnema juipoca</i> | DQ283093.1 | DQ283093.1 | CFBHT02292/CC060 | CC060 |
| <i>Ischnocnema parva</i> | EF493532.1 | EF493532.1 | CFBHT13646/CC081 | CC081 |
| <i>Itapotihyla langsdorffii</i> | AY843706.1 | AY843706.1 | KF001942.1 | AY844482.1 |
| <i>Leptodactylus chaquensis</i> | EF613179.1 | EF632055.1 | KU494518/A396 | A396 |
| <i>Leptodactylus flavopictus</i> | TGA192 | TGA192 | TGA192 | TGA192 |
| <i>Leptodactylus furnarius</i> | KC470108.1 | KC477257.1 | TGA1148 | TGA1148 |
| <i>Leptodactylus fuscus</i> | DQ283404.1 | DQ283404.1 | JQ627304.1 | AY323770.1 |
| <i>Leptodactylus labyrinthicus</i> | AY947874.1 | AY947861.1 | KU494534 | A576 |
| <i>Leptodactylus latrans</i> | KM091490.1 | KM091606.1 | KC603989.1 | * |
| <i>Leptodactylus mystaceus</i> | FRS001 | FRSG001 | FRSG001 | KC604029.1 |
| <i>Leptodactylus mystacinus</i> | KC470105.1 | KC477256.1 | TG065 | AY323771.1 |
| <i>Leptodactylus notoaktites</i> | KM091504.1 | KM091604.1 | CFBH | CFBHT19312 |
| <i>Leptodactylus plaumanni</i> | CFBHT19161 | CFBHT19161 | CFBHT19161 | CFBHT19161 |
| <i>Leptodactylus podicipinus</i> | EF613175.1 | EF632051.1 | KU494556 | TG061 |
| <i>Leptodactylus rhodomystax</i> | AY947869.1 | AY947855.1 | CFBHT12226 | * |
| <i>Limnomedusa macroglossa</i> | AY843689.1 | AY843689.1 | KC593345.1 | AY844471.1 |
| <i>Lysapsus laevis</i> | AY843696.1 | AY843696.1 | CFBH | AY844476.1 |
| <i>Lysapsus limellum</i> | AY843697.1 | AY843697.1 | IIBPH_441 | AY844477.1 |
| <i>Macrogenioglottus alipioi</i> | KC593360.1 | KC593360.1 | KU494591 | KC593355.1 |
| <i>Megaelosia aff. boticariana</i> | MEG12 | MEG12 | MEG12 | MEG12 |
| <i>Megaelosia boticariana</i> | MLLA15 | MLL015 | KJ961566 | MLL015 |
| <i>Megaelosia goeldii</i> | DQ283072.1 | DQ283072.1 | CFBHT09330 | DQ503346.1 |
| <i>Melanophryniscus devincenzii</i> | JX961678.1 | JX961678.1 | CFBHT11467 | CFBHT12302 |
| <i>Melanophryniscus moreirae</i> | A117 | MLLA117 | KU494596/A117 | A117 |
| <i>Myersiella microps</i> | A16 | TGA1186 | KU494602 | * |
| <i>Odontophrynus americanus</i> | AY843704.1 | AY843704.1 | JX203939.1 | * |
| <i>Odontophrynus carvalhoi</i> | KF214100.1 | FJ685687.1 | TG456 | FJ685707.1 |
| <i>Odontophrynus cultripes</i> | KF214101.1 | FJ685688.1 | CFBHT04406 | FJ685708.1 |
| <i>Osteocephalus taurinus</i> | AY326041.1 | AY326041.1 | CFBHT13831 | EU034135.1 |
| <i>Paratelmatobius cardosoi</i> | EU224404.1 | A1089 | MLLA1089 | A1089 |

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| <i>Paratelmatoobius aff. cardosoi</i> | * | CFBHT19235 | CFBHT19235 | CFBHT19235 |
| <i>Paratelmatoobius gaigeae</i> | EU224397.1 | EU224397.1 | CFBHT17963 | A832 |
| <i>Paratelmatoobius poecilogaster</i> | EU224401.1 | EU224401.1 | KU494620 | A002 |
| <i>Phasmahyla cochranæ</i> | AY843715.1 | AY843715.1 | KU494622 | GQ366076.1 |
| <i>Phasmahyla exilis</i> | GQ366231.1 | GQ366231.1 | CFBHT01448 | GQ366077.1 |
| <i>Phasmahyla guttata</i> | AY843716.1 | AY843716.1 | CFBHT04006 | AY844489.1 |
| <i>Phrynomedusa dryade</i> | CFBHT19380 | CFBHT19380 | KU494625 | CFBHT19380 |
| <i>Phyllobates bicolor</i> | AY326031.1 | AY326031.1 | DQ502884.1 | * |
| <i>Phyllodytes luteolus</i> | AY843721.1 | AY843721.1 | KU494628 | AY844494.1 |
| <i>Phyllomedusa azurea</i> | GQ366250.1 | GQ366250.1 | CFBHT16066 | GQ366084.1 |
| <i>Phyllomedusa burmeisteri</i> | GQ366255.1 | GQ366255.1 | CFBHT13365 | GQ366087.1 |
| <i>Phyllomedusa camba</i> | GQ366259.1 | GQ366259.1 | CFBHT01046 | GQ366088.1 |
| <i>Phyllomedusa distincta</i> | GQ366263.1 | GQ366263.1 | KU494630 | A089 |
| <i>Phyllomedusa hypochondrialis</i> | AY843724.1 | AY843724.1 | KP149222.1 | KC520753.1 |
| <i>Phyllomedusa megacephala</i> | GQ366269.1 | GQ366269.1 | CFBHT02385 | GQ366090.1 |
| <i>Phyllomedusa nordestina</i> | GQ366272.1 | GQ366272.1 | CFBHT08114 | GQ366091.1 |
| <i>Phyllomedusa rohdei</i> | GQ366238.1 | GQ366238.1 | CFBHT02565 | GQ366079.1 |
| <i>Phyllomedusa sauvagii</i> | GQ366283.1 | GQ366283.1 | CFBHT04524 | GQ366093.1 |
| <i>Phyllomedusa tetraploidea</i> | AY843727.1 | AY843727.1 | CFBHT01122 | GQ366096.1 |
| <i>Phyllomedusa tomopterna</i> | AY326045.1 | AY326045.1 | CFBHT00156 | * |
| <i>Physalaemus atlanticus</i> | KC692081.1 | KC692081.1 | KU494635 | MLLA144 |
| <i>Physalaemus bokermanni</i> | CFBHT19362 | CFBHT19362 | CFBHT03487 | CFBHT19362 |
| <i>Physalaemus centralis</i> | KP146062.1 | KP146062.1 | JQ627308.1 | * |
| <i>Physalaemus cuvieri</i> | FRSG051 | KC692083.1 | FRSG051 | AY844499.1 |
| <i>Physalaemus lateristriga</i> | CFBHT19207 | CFBHT19207 | CFBHT19207 | CFBHT19207 |
| <i>Physalaemus maculiventris</i> | KP145929.1 | KP146091. | CFBHT16000 | * |
| <i>Physalaemus marmoratus</i> | KP146083.1 | CFBHT19043 | CFBHT07783 | CFBHT19043 |
| <i>Physalaemus nattereri</i> | FRS045 | FRS045 | KC603984.1 | * |
| <i>Physalaemus olfersii</i> | KP146078.1 | KP146078.1 | KU494653 | CFBHT19390 |
| <i>Physalaemus spiniger</i> | KC692086.1 | KC692086.1 | CFBHT08488 | CFBHT19432 |

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| <i>Pleurodema brachyops</i> | AY843733.1 | AY843733.1 | KP149118.1 | AY844503.1 |
| <i>Pleurodema diplolister</i> | JQ937185.1 | JQ937185.1 | KC603986.1 | * |
| <i>Pristimantis fenestratus</i> | EF493703.1 | EF493703.1 | KU494666 | A361 |
| <i>Pristimantis ramagii</i> | JX267319.1 | JX267319.1 | KU494509 | A548 |
| <i>Proceratophrys appendiculata</i> | KF214130.1 | KM390778.1 | KU494669 | KF214189.1 |
| <i>Proceratophrys avelinoi</i> | KP295643.1 | KP295643.1 | CFBHT02100 | FJ685711.1 |
| <i>Proceratophrys bigibbosa</i> | KF214103.1 | FJ685692.1 | CFBHT09664 | FJ685712.1 |
| <i>Proceratophrys boiei</i> | AY143350.1 | JN814641.1 | KC603982.1 | JN814738.1 |
| <i>Proceratophrys cristiceps</i> | KF214106.1 | FJ685695.1 | KU494682 | FJ685715.1 |
| <i>Proceratophrys laticeps</i> | KF214109.1 | FJ685698.1 | CFBHT05932 | FJ685718.1 |
| <i>Proceratophrys melanopogon</i> | KF214120.1 | KF214142.1 | KU494688 | KF214181.1 |
| <i>Proceratophrys schirchi</i> | KF214112.1 | FJ685701.1 | CFBHT12397 | FJ685721.1 |
| <i>Pseudis minuta</i> | AY843739.1 | AY843739.1 | CFBHT15519 | AY844505.1 |
| <i>Pseudis paradoxa</i> | AY843740.1 | AY843740.1 | KP149190.1 | AY844506.1 |
| <i>Pseudis platensis</i> | CFBHT19075 | CFBHT19075 | CFBHT15155 | CFBHT19075 |
| <i>Pseudopaludicola falcipes</i> | AY843741.1 | AY843741.1 | KC520684.1 | * |
| <i>Pseudopaludicola mineira</i> | KJ147025.1 | KJ147025.1 | KU494698 | CFBHT15571 |
| <i>Pseudopaludicola mystacalis</i> | KJ146982.1 | KJ146982.1 | KU494700 | A087 |
| <i>Pseudopaludicola saltica</i> | KJ146995.1 | KJ146995.1 | CFBHT01467 | A500 |
| <i>Rhinella crucifer</i> | AY680260.1 | AY680260.1 | KU494707 | * |
| <i>Rhinella fernandezae</i> | KP685204.1 | KP685204.1 | CFBHE00337 | * |
| <i>Rhinella granulosa</i> | KP685208.1 | KP685208.1 | CFBHT15697 | * |
| <i>Rhinella henseli</i> | KP685183.1 | KP685183.1 | CFBHT11856 | * |
| <i>Rhinella hoogmoedi</i> | MLLA694 | MLL-A694 | CFBHT11562 | MLLA694 |
| <i>Rhinella icterica</i> | DQ158462.1 | DQ158462 | KU494719 | * |
| <i>Rhinella margaritifera</i> | AY819331.1 | MLL-A699 | KP149211.1 | HM563975.1 |
| <i>Rhinella mirandaribeiroi</i> | KP685228.1 | KP685228.1 | CFBHT12441 | * |
| <i>Rhinella ocellata</i> | DQ158479.1 | DQ158479.1 | CFBHT13966 | * |
| <i>Rhinella ornata</i> | CFBHT19168 | CFBHT19168 | KU494733 | CFBHT19168 |
| <i>Rhinella pygmaea</i> | KP685229.1 | KP685229 | CFBHT15163 | * |
| <i>Rhinella schneideri</i> | DQ158480.1 | DQ158480.1 | CFBHT15848 | * |

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|--------------------------------------|------------|------------|------------|------------|
| <i>Scinax argyreonatus</i> | CFBHT19364 | CFBHT19364 | CFBHT11948 | CFBHT19364 |
| <i>Scinax crospedospilus</i> | MLL444 | MLL-A444 | CFBHT00880 | A444 |
| <i>Scinax fuscomarginatus</i> | A218 | KJ004144.1 | KJ004218.1 | A218 |
| <i>Scinax fuscovarius</i> | AY843758.1 | AY843758.1 | JQ627325.1 | AY844519.1 |
| <i>Scinax hayii</i> | CFBHT19110 | CFBHT19110 | CFBHT19110 | CFBHT19110 |
| <i>Scinax imbegue</i> | CFBHT19417 | CFBHT19417 | CFBHT19417 | CFBHT19417 |
| <i>Scinax littoralis</i> | A273 | CFBHT19172 | CFBHT10715 | A273 |
| <i>Scinax nasicus</i> | AY843759.1 | AY843759.1 | KJ004280.1 | AY844520.1 |
| <i>Scinax obtriangulatus</i> | FRS970 | FRS970 | FRS970 | FRS970 |
| <i>Scinax perereca (aff. hayii)</i> | CFBHT19288 | CFBHT19288 | CFBHT19288 | CFBHT19288 |
| <i>Scinax perpusillus</i> | CFBHT19376 | CFBHT19418 | CFBHT19095 | CFBHT19095 |
| <i>Scinax rizibilis</i> | CFBHT19316 | CFBHT19316 | CFBHT11701 | CFBHT19316 |
| <i>Scinax ruber</i> | AY326034.1 | AY326034.1 | KP149255.1 | AY844521.1 |
| <i>Scinax similis</i> | CFBHT19029 | CFBHT19029 | CFBHT08252 | CFBHT19029 |
| <i>Scinax squalirostris</i> | AY843760.1 | AY843760.1 | KJ004283.1 | AY844522.1 |
| <i>Scinax tymbamirim</i> | FRS963 | FRS963 | FRS963 | FRS963 |
| <i>Scythrophrys sawayae</i> | DQ283099.1 | DQ283099.1 | CFBHT03240 | A1006 |
| <i>Sphaenorhynchus caramaschii</i> | A123 | KP096220.1 | KU494796 | A123 |
| <i>Sphaenorhynchus dorisae</i> | AY843766.1 | AY843766.1 | CFBHT05102 | AY844526.1 |
| <i>Sphaenorhynchus lacteus</i> | AY549367.1 | AY549367.1 | CFBHT05471 | AY844527.1 |
| <i>Stereocyclops incrassatus</i> | MLL079 | MLLA79 | KU494800 | * |
| <i>Thoropa miliaris</i> | DQ283331.1 | DQ283331.1 | DQ502874.1 | * |
| <i>Thoropa taophora</i> | A186 | MLL186 | CFBHT05702 | GQ345288.1 |
| <i>Trachycephalus imitatrix</i> | J4504 | 4504 | J4504 | 4504 |
| <i>Trachycephalus lepidus</i> | FRS1002 | 1002 | FRS1002 | FRS1002 |
| <i>Trachycephalus jordani</i> | AY326042.1 | AY326042.1 | * | EU034150.1 |
| <i>Trachycephalus mesophaeus</i> | FRS1038 | 1038 | FRS1038 | AY844491.1 |
| <i>Trachycephalus nigromaculatus</i> | AY843772.1 | AY843772.1 | CFBHT15355 | CFBHT15355 |
| <i>Trachycephalus typhonius</i> | CFBHT19027 | CFBHT19027 | CFBHT19027 | CFBHT19027 |
| <i>Vitreorana eurygnatha</i> | AY843595.1 | AY843595.1 | CFBHT10700 | AY844383.1 |
| <i>Vitreorana uranoscopa</i> | KF639775.1 | MLLA775 | CFBHT15323 | JX298194.1 |
| <i>Xenohyla truncata</i> | AY843775.1 | AY843775.1 | KU494824 | A071 |

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|----------------------------|------------|------------|----------|----------|
| <i>Zachaenus carvalhoi</i> | MTR12613 | MTR12613 | MTR12613 | MTR12613 |
| <i>Zachaenus parvulus</i> | KC593362.1 | KC593362.1 | KU494825 | A021 |
| <i>Pipa carvalhoi</i> | NC015617 | NC015617 | NC015617 | HM998974 |

Table S5. Reproductive modes associated with the anuran species recorded in this study. Trait_1 = Eggs deposited in water; Trait_2 = Eggs and exotrophic tadpoles in lentic water; Trait_3 = Eggs and exotrophic tadpoles in lotic water; Trait_4 = Eggs and early larval stages in constructed subaquatic chambers; exotrophic tadpoles in streams; Trait_5 = Eggs and early larval stages in natural or constructed basins; subsequent to flooding, exotrophic tadpoles in ponds or streams; Trait_6 = Eggs and early larval stages in subterranean constructed nests; subsequent to flooding, exotrophic tadpoles in ponds or streams; Trait_7 = Eggs and exotrophic tadpoles in water in tree holes or aerial plants; Trait_8 = Eggs and endotrophic tadpoles in water in tree holes or aerial plants; Trait_9 = Eggs in bubble nest; Trait_10 = Bubble nest floating on pond; exotrophic tadpoles in ponds; Trait_11 = Eggs in foam nest (aquatic); Trait_12 = Foam nest floating on pond; exotrophic tadpoles in ponds; Trait_13 = Foam nest floating on water accumulated in constructed basins; exotrophic tadpoles in ponds; Trait_14 = Terrestrial or arboreal eggs (not in water); Trait_15 = Eggs on ground or rock above water; upon hatching, exotrophic tadpoles move to water; Trait_16 = Eggs on humid rocks, in rock crevices, or on tree roots above water; exotrophic semiterrestrial tadpoles living on rocks and rock crevices in a water film or in the water–land interface; Trait_17 = Eggs hatching into endotrophic tadpoles that complete their development in the nest; Trait_18 = Direct development of terrestrial eggs; Trait_19 = Arboreal eggs; Trait_20 = Eggs hatching into exotrophic tadpoles that drop in lentic water; Trait_21 = Eggs hatching into exotrophic tadpoles that drop in lotic water; Trait_22 = Eggs in foam nest (terrestrial or arboreal); Trait_23 = Foam nest with eggs and early larval stages in subterranean constructed nests; subsequent to flooding, exotrophic tadpoles in ponds; Trait_24 = Foam nest in subterranean constructed chambers; endotrophic tadpoles complete development in nest; Trait_25 = Eggs carried by adult; Trait_26 = Eggs carried on dorsum or in dorsal pouch of female; endotrophic tadpoles in bromeliads or bamboo.

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| <i>Dendropsophus_seniculus</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Dendropsophus_werneri</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Dermatonotus_muelleri</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Elachistocleis_bicolor</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Elachistocleis_cesarii</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Fritziana_aff._fissilis</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| <i>Haddadus_binotatus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hylodes_asper</i> | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hylodes_dactylocinus</i> | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hylodes_phyllodes</i> | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hylodes_sazimai</i> | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hypsiboas_albomarginatus</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hypsiboas_albopunctata</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hypsiboas_bandeirantes</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hypsiboas_bischoffi</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hypsiboas_caingua</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hypsiboas_caipora</i> | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hypsiboas_faber</i> | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hypsiboas_lundii</i> | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hypsiboas_pardalis</i> | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hypsiboas_pulchellus</i> | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Hypsiboas_semilineatus</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Ischnocnema_guentheri_C1</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Ischnocnema_guentheri_C3</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Ischnocnema_guentheri_C4</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Ischnocnema_henseli</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Ischnocnema_parva</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Itapotihyla_langsdorffii</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Leptodactylus_chaquensis</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Leptodactylus_flavopictus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Leptodactylus_furnarius</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| <i>Leptodactylus_fuscus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|------------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| <i>Scinax_fuscovarius</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax_hayii</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax_imbegue</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax_obtriangulatus</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax_littoralis</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax_perereca_aff.hayii</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax_perpusillus</i> | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax_rizibilis</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax_similis</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax_squalirostris</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Scinax_tymbamirim</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Sphaenorhynchus_caramaschii</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Thoropa_taophora</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Trachycephalus_imitatrix</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Trachycephalus_mesophaeus</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Trachycephalus_lepidus</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Trachycephalus_typhonius</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Vitreorana_uranoscopa</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |

Table S6. Climatic variables from WorldClim and topography from Google Earth each community.

| | Current climatic | | | | Historical climatic (LGM) | | | | Topography | | |
|-----|------------------|--------|------|-------|---------------------------|-------|-------|--------|------------|------|-------|
| | AMT | TS | AP | PS | AMT | TS | AP | PS | MAEL | MIEL | ElevR |
| EJA | 21.47 | 218.37 | 1386 | 74.57 | 16.41 | 11.15 | 59.10 | 301.25 | 515 | 835 | 320 |
| EAS | 20.66 | 266.64 | 1328 | 50.09 | 17.00 | 10.92 | 58.17 | 310.68 | 520 | 590 | 70 |
| EIT | 19.78 | 226.58 | 1371 | 73.33 | 16.23 | 10.96 | 57.68 | 310.95 | 705 | 750 | 45 |
| PEV | 21.13 | 220.39 | 1395 | 75.44 | 16.41 | 11.15 | 59.10 | 301.25 | 590 | 740 | 150 |
| FEN | 20.28 | 245.91 | 1306 | 74.33 | 16.23 | 10.96 | 57.68 | 310.95 | 550 | 750 | 200 |
| ESB | 20.33 | 245.67 | 1256 | 59.18 | 16.22 | 10.73 | 56.74 | 317.91 | 600 | 680 | 80 |
| ECA | 20.33 | 261.47 | 1295 | 61.91 | 17.56 | 11.19 | 59.60 | 295.34 | 500 | 680 | 180 |
| ECB | 17.68 | 275.61 | 1368 | 51.45 | 16.18 | 9.84 | 51.96 | 330.75 | 30 | 1003 | 973 |
| CUR | 17.21 | 242.60 | 2619 | 40.37 | 16.93 | 9.29 | 51.29 | 315.09 | 15 | 870 | 855 |
| EEJ | 19.43 | 260.22 | 2195 | 45.63 | 17.00 | 9.19 | 51.36 | 315.93 | 0 | 1300 | 1300 |
| PEJ | 17.72 | 252.50 | 1823 | 51.53 | 16.10 | 9.94 | 52.21 | 327.94 | 870 | 1030 | 160 |
| PET | 20.55 | 327.93 | 1409 | 41.08 | 15.54 | 9.87 | 51.86 | 338.46 | 80 | 1146 | 1066 |
| SIV | 18.76 | 231.17 | 2085 | 54.83 | 17.04 | 8.81 | 50.38 | 303.28 | 740 | 1620 | 880 |
| SEB | 19.02 | 236.53 | 2375 | 40.79 | 18.19 | 7.71 | 48.86 | 284.12 | 0 | 1200 | 1200 |

AMT = Annual Mean Temperature; TS = Temperature Seasonality; AP = Annual Precipitation; PS = Precipitation Seasonality; MAEL = Maximum

Elevation; MIEL = Minimum Elevation; ElevR = Elevational Range (difference between MAEL and MIEL).

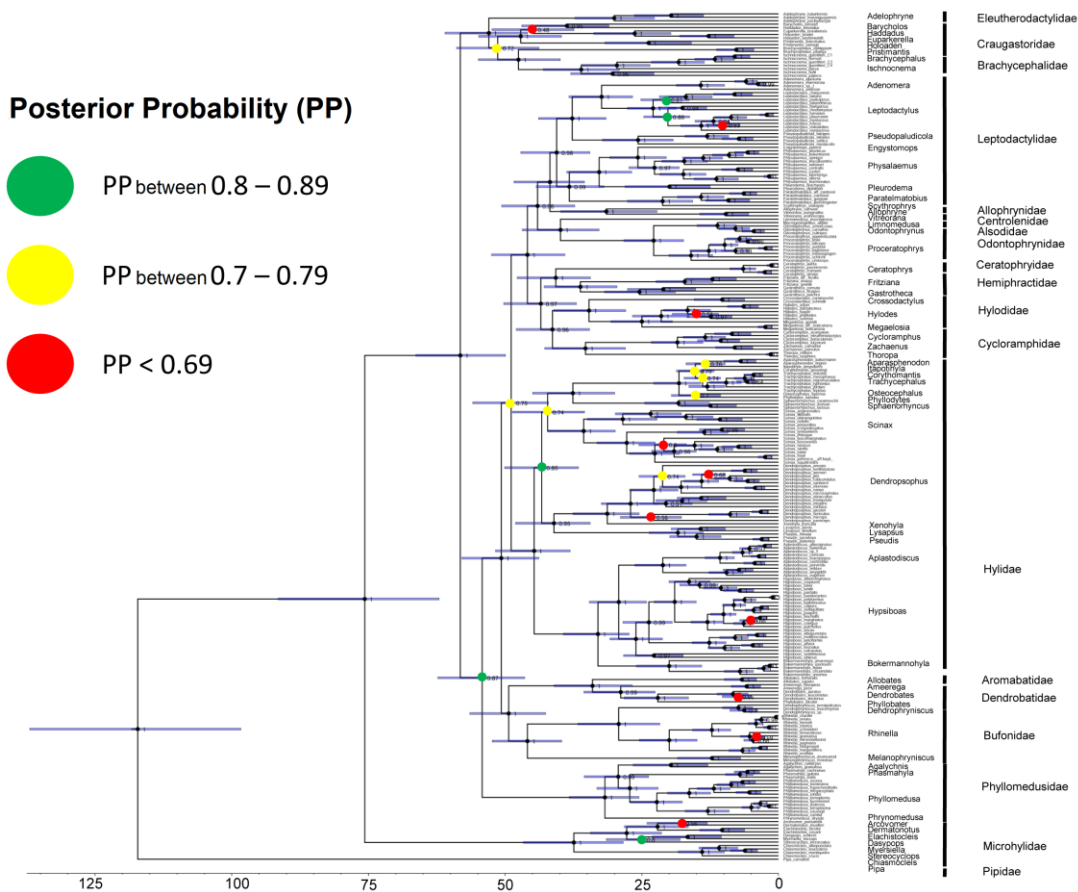


Figure S1. Phylogenetic tree with maximum clade credibility built by the Bayesian method.

Uncertainties in node dating are represented by blue bars (95% higher posterior density). Circles with different colors represent the posterior probability (PP) of the nodes, indicating the reliability of the branches (nodes without colors indicate PP with values greater than 90%). On the right side are described the genera and families of the species present in the phylogeny.

Comparing the phylogeny obtained in this study with literature

In general, the relationships among clades were congruent with the recently published phylogenetic hypotheses (e.g., Frost et al., 2006; Roelants et al., 2007; Pyron & Wiens, 2011; Wiens 2011; Duellman et al., 2016). We use a “Primitive” Frogs (Pipidae family) as an outgroup. For “Advanced” frogs (Neobatrachia), the phylogeny of anuran amphibians obtained in this study present high support, > 0.95 posterior probability, and it is in agreement with recent studies (as for

example, Darst & Cannatella, 2004; Pyron & Wiens, 2011; Feng et al., 2017). On the other hand, differs from traditional works (e.g., Hedges & Maxson, 1993), where most of the nodes in Neobatrachia have bootstrap values of < 50%. Ford & Cannatella (1993) also indicate that relationships within the Neobatrachia generally are poorly supported. Specifically, for the main large families, such as Bufonidae, Hylidae, Leptodactylidae and Microhylidae, our results were congruent with most of the published phylogenies, but with some differences. For example, the relationships among Brachycephalidae, Eleutherodactylidae, and Craugastoridae presented high support in the nodes, but did not support the monophyletic anurans with direct development of the New World (proposed by Hedges et al., 2008 and reviewed by Blackburn & Wake, 2011). Here, we found that *Pristimantis* is within Craugastoridae, and that Brachycephalidae and Craugastoridae are more closely related than Craugastoridae and Strabomantidae (as found by Hedges et al., 2008). Our result is in agreement with Pyron & Wiens (2011), who consider Strabomantidae as non-monophyletic and considered the group a subfamily (Strabomantinae) within the Craugastoridae. Furthermore, the phylogenetic position of the genera and species of these families was similar to that found in other studies (e.g., Heinicke et al., 2009; Heinicke et al., 2018), but here with low support between the nodes (48%) for the genera *Barycholos*, *Holoaden*, *Haddadus* and *Euparkerella* in Craugastoridae. For Hemiphractidae family, these results were similar to those proposed by Blackburn & Duellman (2013) and Castroviejo-Fisher et al. (2015) where *Fritziana* is recovered as the sister of *Gastrotheca*. However, our results suggest that *Fritziana* (and not *Flectonotus* or *Gastrotheca*, as found in Wiens et al., 2007; Duellman et al., 2011) is the most basal lineage within Hemiphractidae. Frost et al. (2006) recognized the members of Odontophrynini as part of their Cycloramphidae. However, here, we found that Cycloramphidae, composed of three genera (*Cycloramphus*, *Zachaenus* and *Thoropa*), is not monophyletic, which is corroborated by other studies (e.g., Heinicke et al., 2009). Heinicke et al. (2009) also proposes that *Odontophrynus* and *Proceratophrys* are closer phylogenetically (Odontophrynidae family). The species of the family Leptodactylidae, in general, presented high support in the nodes, except for a relation

between *Leptodactylus fuscus* and *L. notoaktites* with support of 53%. For the species of the Hylodidae family, our analysis corroborates with Nuin & Val (2005), which provides a phylogenetic analysis of the group, resulting in the topology *Megaelosia* + (*Crossodactylus* + *Hylodes*), with all three genera being monophyletic and presented high support in the nodes, except for a relation between *Hylodes heyeri* and *H. phyllodes* and *H. sazimai* with support of 56%. For species of the family Hylidae, species of the genus *Aplastodiscus*, *Bokermannoyla*, *Dendropsophus*, *Hypsiboas* and *Sphaenorhynchus* form clades similar to those found by Duellman et al. (2016). However, some nodes within the *Dendropsophus* clade and the relationships between *Trachycephalus*, *Aparasphenodon*, *Itapotihyla* and *Corythomantis* have less than 80% support of nodes. For the family Bufonidae, species of the genus *Dendrophryniscus*, *Rhinella*, and *Melanophryniscus* form clades similar to those found by Fouquet et al. (2012) in general, presenting high support in the nodes, except for a relation between *Rhinella granulosa* and *R. mirandaribeiroi* with support of 59%. This analysis also supported the monophyly of the Microhylidae family, with a large group of microhylids separating three groups of species, with a clade with all *Chiasmocleis* together, a clade with *Dasytops*, *Myersiella* and *Stereocyclops* and other clade with *Elachistocleis*, *Arcovomer*, *Dermatonotus* (according to de Sá et al., 2012). In addition, our analyzes also corroborate the findings of de Sá et al. (2012) - who found that Otophryninae is distantly related to all other New World microhylids. For all other groups our analyzes showed congruent with recently published phylogenetic hypotheses (e.g., Frost et al., 2006; Roelants et al., 2007; Pyron & Wiens, 2011; Wiens 2011; Duellman et al., 2016).



Figure S2. Anuran species recorded at the Protected Areas (PAs). a = *Rhinella icterica*, b = *R. hoogmoedi*, c = *R. ornata*, d = *Dendrophryniscus brevipollicatus*, e = *Brachycephalus pitanga*, f = *Ischnocnema* gr. *guentheri*, g = *Ischnocnema* gr. *guentheri*, h = *I. henselii*, i = *I. parva*, j = *Haddadus binotatus*, k = *Proceratophrys melanopogon*, l = *Cycloramphus boraceiensis*, m = *Hylodes asper*, n = *H. phyllodes* and o = *Thoropa taophora* (da Silva et al., 2017).



Figure S3. Anuran species recorded at the Protected Areas (PAs). p = *Megaelosia* cf. *bocainensis*, q = *Adenomera marmorata*, r = *Adenomera* sp., s = *Leptodactylus furnarius*, t = *L. fuscus*, u = *L. latrans*, v = *Physalaemus atlanticus*, w = *P. bokermanni*, x = *P. cuvieri*, y = *P. olfersii*, z = *Paratelmatobius cardosoi*, a1 = *Chiasmocleis leucosticta*, b1 = *Myersiela microps*, c1 = *Fritziana* sp. (aff. *fissilis*), d1 = *Phrynomedusa dryade* (da Silva et al., 2017).



Figure S4. Anuran species recorded at the Protected Areas (PAs). e1 = *Aplastodiscus leucopygius*, f1 = *Bokermannohyla circumdata*, g1 = *B. hylax*, h1 = *B. izecksohni*, i1 = *Dendropsophus berthalutzae*, j1 = *D. elegans*, k1 = *D. microps*, l1 = *D. minutus*, m1 = *D. seniculus*, n1 = *Boana albomarginata*, o1 = *B. albopunctata*, p1 = *B. bandeirantes*, q1 = *B. bischoffi*, r1 = *B. faber* and s1 = *B. pardalis* (da Silva et al., 2017).



Figure S5. Anuran species recorded at the Protected Areas (PAs). t1 = *B. semilineata*, u1 = *Ololygon argyreomata*, v1 = *O. littoralis*, w1 = *O. perpusilla*, x1 = *O. rizibilis*, y1 = *Scinax crospedospilus*, z1 = *S. fuscovarius*, a2 = *S. hayii*, b2 = *S. perereca*, c2 = *S. tymbamirim*, d2 = *Trachycephalus imitatrix*, e2 = *T. mesophaeus* (da Silva et al., 2017).

Anexos

Relatório das atividades acadêmicas desenvolvidas durante meu período de doutoramento (2015 – 2019)

Esta seção tem como objetivo relatar as atividades desenvolvidas pelo doutorando Ronildo Alves Benício no período de 10 de junho de 2015 a 31 de maio de 2019. Neste período passei por toda parte organizacional de um projeto - realizando coletas de dados em campo, tabulação dos dados, abordagens estatísticas, leitura de artigos científicos, interpretação de resultados, discussão com orientador e cientistas da área, apresentando parte dos resultados em congressos nacionais e internacionais, uso responsável de Reserva Técnica, redação de relatórios anuais (por ex., FAPESP) e, até a burocracia da compra de materiais permanentes.

Participei, como membro efetivo, de banca avaliadora de trabalho de conclusão de curso (TCC). Ministrei minicursos sobre ecologia e herpetofauna. Participei, como monitor, de curso de campo na área de Ecologia (<http://ecopan.sites.ufms.br/>) sendo responsável, pelo processo de orientação de projetos em ecologia e conservação, formulação de hipóteses e desenho experimental, análise e interpretação de dados, comunicação científica, oral e escrita, e revisão de manuscritos.

Fui professor temporário (4 horas-aula semanais) na Universidade Estadual Paulista – UNESP, campus de Rio Claro, sendo responsável, durante um semestre (2018.2), pela disciplina Ecologia de comunidades, área na qual atuo e é compatível com este projeto de pesquisa, abordando e revisando teorias e temas relevantes da Ecologia de comunidades que foram importantes para o desenvolvimento desta tese. O conteúdo programático da disciplina abordou: os princípios básicos de ecologia de comunidades; estruturação de comunidades; padrões e processos em ecologia de comunidades; padrões de diversidade; metacomunidades; processos temporais e sucessão; macroecologia de comunidades; influência das interações na estruturação de comunidades; padrões de distribuição de espécies; padrões de diversidade; fragmentação e distúrbios em geral, e conservação da biodiversidade. Os objetivos da disciplina foram identificar os principais problemas

tratados em ecologia de comunidades; ter uma análise conceitual dos princípios básicos da ecologia de comunidades; compreender os principais processos responsáveis pela montagem e dinâmica das comunidades locais; compreender e propor delineamentos amostrais adequados para teste de hipóteses em ecologia de comunidades; e fazer inferências sobre processos e mecanismos agindo sobre os padrões de diversidade. A disciplina contou com aulas expositivas (40 – 50 min); seminários em grupo; discussão sobre o tema da aula e de artigos científicos; lista de exercícios, e contou ainda com uma excursão a campo. O critério de avaliação da aprendizagem contou com: prova do livro didático e aulas expositivas; apresentação de seminários em grupo; avaliação do desempenho do aluno em sala de aula e no campo durante excursão; discussão de artigos científicos; e resolução da lista de exercícios. Ao final do semestre todos os alunos (15 no total) foram aprovados na disciplina. No geral, a avaliação do meu desempenho como docente (segundo avaliação da disciplina respondida pelos alunos) foi excelente.

Por fim, submeti, em parceria com pesquisadores de várias instituições do Brasil (por ex., USP, UNICAMP, UFMS, UFSC, UFPI, UFERSA, UFSCAR, UNIFAP), diversos artigos em periódicos de circulação nacional e internacional.

A seguir, são apresentadas as atividades realizadas – participações em eventos, e os artigos publicados, aceitos e/ou submetidos no período.

Participação em eventos científicos durante meu período de doutoramento (2015 – 2019)

1. **Benício, R.A.** and da Silva, F.R. 2016. *Environmental filters and niche conservatism influencing amphibian community assemblages in the Brazilian Atlantic Forest*. Trabalho apresentado em formato oral na *101th ESA Annual Meeting*, ocorrido de 7 a 12 de agosto de 2016, em Fort Lauderdale, Flórida, EUA.
2. **Benício, R.A.** and Campos-Silva, L.A. 2016. *People have negative stereotypes of aposematic patterns of snakes?* Trabalho apresentado em formato pôster no *II Simpósio Internacional de Ecologia*, ocorrido de 16 a 19 de agosto de 2016, em São Carlos, São Paulo, Brasil.
3. **Benício, R.A.** and Albernaz, A.L.K.M. 2016. *Occupation patterns are affected by the variation in the detection of species*. Trabalho apresentado em formato oral no *II Simpósio Internacional de Ecologia*, ocorrido de 16 a 19 de agosto de 2016, em São Carlos, São Paulo, Brasil.
4. **Benício, R.A.** and da Silva, F.R. 2017. *Influence of regional species pool in the phylogenetic structure of anuran communities in the Brazilian Atlantic Forest*. Trabalho apresentado em formato pôster no *VIII Congresso Brasileiro de Herpetologia*, ocorrido de 14 a 18 de agosto de 2017, em Campo Grande, Mato Grosso do Sul, Brasil.
5. **Benício, R.A.** and da Silva, F.R. 2017. *Notes on an unusual position of calling site of *Dendropsophus minutus* (Anura: Hylidae), northeastern São Paulo, Brazil*. Trabalho apresentado em formato pôster no *VIII Congresso Brasileiro de Herpetologia*, ocorrido de 14 a 18 de agosto de 2017, em Campo Grande, Mato Grosso do Sul, Brasil.
6. **Benício, R.A.** and da Silva, F.R. 2018. *Influência do clima e topografia sobre a estrutura filogenética de comunidades de anuros na Mata Atlântica brasileira*. Trabalho apresentado

em formato oral no *I Workshop Ecologia de Comunidades*, ocorrido de 09 a 14 de abril de 2018, na UNESP – Campus de São José do Rio Preto, São Paulo, Brasil.

7. **Benício, R.A.** 2018. *Herpetologia*. Minicurso teórico e prático realizado pela *Ambiens Jr. - Empresa Júnior de Biologia*, da Universidade Federal de São Carlos, campus de Sorocaba, ocorrido de 11 a 12 de dezembro de 2018, em Sorocaba, São Paulo, Brasil.
8. **Benício, R.A.** 2019. *O mundo encantado dos sapos*. Micro curso apresentado na *XIII Semana da Biologia UFSCar*, ocorrido de 20 a 23 de maio de 2019, em Sorocaba, São Paulo, Brasil.

Artigos adicionais que publiquei durante meu período de doutoramento (2015 – 2019)

1. **Benício, R.A.** and da Silva, F.R. 2017. Notes on an unusual position of calling site of *Dendropsophus minutus* (Peters, 1872) (Anura: Hylidae), northeastern São Paulo, Brazil. *Herpetology Notes* 10: 421–423. <https://www.biotaxa.org/hn/article/view/22695/29162>
2. **Benício, R.A.** and da Silva, F.R. 2017. Amphibians of Vassununga State Park, one of the last remnants of semideciduous Atlantic Forest and Cerrado in northeastern São Paulo state, Brazil. *Biota Neotropica* 17(1):e20160197. <http://dx.doi.org/10.1590/1676-0611-bn-2016-0197>
3. **Benício, R.A.** and Lima, J.D. 2017. Anurans of Amapá National Forest, Eastern Amazonia, Brazil. *Herpetology Notes* 10:627–633. <https://www.biotaxa.org/hn/article/view/31651/30359>
4. **Benício, R.A.** and da Silva, F.R. 2017. Natural History Notes. *Rhinella schneideri* (Cururu Toad). Parasitism. *Herpetological Review* 48:170–171. <https://www.researchgate.net/publication/320765807>
5. **Benício, R.A.** 2018. Phoneutrism inside of one protected area in the State of São Paulo. *Revista da Sociedade Brasileira de Medicina Tropical* 51 (1):118. <http://dx.doi.org/10.1590/0037-8682-0052-2017>
6. **Benício, R.A.** and Martins, M. 2018. Natural History Notes. Defensive behavior of a juvenile *Crotalus durissus* Linnaeus, 1758. *Herpetozoa* 30 (3/4):217–218. <https://www.researchgate.net/publication/323969369>
7. Ceron, K., Moroti, M.T., **Benício, R.A.**, Balboa, Z.P., Marçola, Y., Pereira, L.B., Santana, D.J. 2018. Diet and first report of batracophagy in *Leptodactylus podicipinus* (Anura: Leptodactylidae). *Neotropical Biodiversity* 4(1):69–73. <https://doi.org/10.1080/23766808.2018.1467173>

8. **Benício, R.A.** 2018. Natural History Notes. *Rhaebo guttatus* (Smooth-sided Toad). Ectoparasitism. *Herpetological Review* 49(2):309. <https://www.researchgate.net/publication/326131357>
9. **Benício, R.A.** 2018. Natural History Notes. Notes on habitat use of *Crotalus durissus* (South American Rattlesnake). *Herpetology Notes* 11:645–646.
10. **Benício, R.A.**, Ceron, K., Moroti, M.T., Santana, D.J. 2018. Natural History Notes. A record of a *Thrichomys pachyurus* (Rodentia: Echimyidae) as prey of *Bothrops mattogrossensis* (Serpentes: Viperidae). *Herpetology Notes* 11:647–649.
11. **Benício, R.A.** and Fonseca, M.G. 2019. Women and Science: a portrait of herpetology in the state of Piauí. *Brazilian Journal of Biology* 79(4):1–3. <http://dx.doi.org/10.1590/1519-6984.193049>
12. **Benício, R.A.**, Ortega, Z., Mencia, A., Passos, D.C. 2019. Microhabitat selection of *Ameiva ameiva* (Linnaeus, 1758), in the Brazilian Pantanal. *Herpetozoa* 31(3/4):211–218.
13. **Benício, R.A.** 2019. Uncertain future for women scientists in Brazil. *Nature* 567(7746):31. <http://dx.doi.org/10.1038/d41586-019-00762-1>

Artigos adicionais aceitos e/ou em avaliação durante meu período de doutoramento (2015 – 2019)

1. **Benício, R.A.**, Ortega, Z., Mencía, A., Passos, D.C. Microhabitat selection by *Tropidurus lagunablanca* (Squamata: Tropiduridae) in the Pantanal, Brazil. *Papéis Avulsos de Zoologia* (Submetido em 11/01/2019).
2. **Benício, R.A.** Natural History Notes. Arboreal habitat use by *Adelphobates galactonotus* (Anura: Dendrobatidae): a poison frog endemic of the Brazilian Amazon. *Amphibian and Reptile Conservation* (Submetido em 13/02/2019).
3. **Benício, R.A.**, Carvalho, T., Barbosa, M.D.R., Costa, J.M., Silva, F.C.C., Fonseca, M.G. Worrying news for Brazilian Caatinga: new records and high prevalence of *Batrachochytrium dendrobatidis* in amphibians. *Biodiversity and Conservation* (Submetido em 30/03/2019).
4. **Benício, R.A.**, Ávila, R.W., Fonseca, M.G. Biologia de Anfíbios. In: Fonseca, M.G. and da Silva, R.J. (Eds). Doenças em Anfíbios - Sanidade e Impactos Ecológicos. EDUFPI, Teresina, Piauí (Submetido em 22/04/2019).
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