



UNIVERSIDADE  
ESTADUAL de LONDRINA

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GUILHERME DE TOLEDO FIGUEIREDO

**MODELAGEM DE NICHOS, RIQUEZA ESPECÍFICA,  
DIVERSIDADE FUNCIONAL E FILOGENÉTICA DE ANFÍBIOS  
EM PAISAGENS FRAGMENTADAS NO SUL DO BRASIL**

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Londrina  
2019



PROGRAMA DE PÓS-GRADUAÇÃO  
CIÊNCIAS BIOLÓGICAS

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Orientador: Prof. Dr. Luiz dos Anjos.

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Londrina, 26 de abril de 2019.

“A Terra é um palco muito pequeno em uma vasta arena cósmica. Pense nas incontáveis crueldades infligidas pelos habitantes de um canto deste pixel aos habitantes praticamente indistinguíveis de algum outro canto. O quanto seus desentendimentos são frequentes, o quanto eles desejam matar uns aos outros, o quanto são ferventes seus ódios. Pense nos rios de sangue derramados por todos aqueles generais e imperadores para que, em glória e triunfo, pudessem se tornar os senhores momentâneos de uma fração deste ponto.”

CARL SAGAN

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Figueiredo, G. de T.; Lourenço-de-Moraes, R.; dos Anjos, L. **Modelagem de nicho, riqueza específica, diversidade funcional e filogenética de anfíbios em paisagens fragmentadas no sul do Brasil** 2019. 131 f. Tese (Doutorado em Ciências Biológicas) – Universidade Estadual de Londrina, Londrina, 2019.

## RESUMO

As maneiras pelas quais as condições ambientais variam no espaço e no tempo e como elas estabelecem limites à distribuição das espécies em seus habitats é uma questão de interesse para a ecologia. Nesta tese é explorado por meio de modelos e trabalhos em campo de como a riqueza, composição, diversidade funcional e filogenética de anfíbios varia em relação aos fatores ambientais em diferentes unidades fitogeográficas no estado do Paraná, Brasil. No primeiro capítulo foi feita uma modelagem de nicho para a espécie *Leptodactylus notoaktites* Heyer, 1978 (ANURA, LEPTODACTYLIDAE) com informações de locais que possui condições bioclimáticas e biogeográficas favoráveis à sua ocorrência. No segundo capítulo o objetivo foi testar se a riqueza e a composição das espécies de anuros estão relacionadas aos microhabitats e ao tipo de paisagem dos corpos d'água amostrados. No terceiro capítulo investigou-se a riqueza, a composição e como a diversidade funcional e filogenética de anfíbios responde aos fatores ambientais nas diferentes unidades fitogeográficas do estado do Paraná.

**Palavras-chave:** ambientes aquáticos; anuros; biogeografia; heterogeneidade ambiental; variáveis ambientais.

Figueiredo, G. de T.; Lourenço-de-Moraes, R.; dos Anjos, L. **Modelagem de nicho, riqueza específica, diversidade funcional e filogenética de anfíbios em paisagens fragmentadas no sul do Brasil** 2019. 131 f. Tese (Doutorado em Ciências Biológicas) – Universidade Estadual de Londrina, Londrina, 2019.

### ABSTRACT

How the environmental conditions changes in space and time and how they influence the species distributions in their habitats is a matter of concern to ecology. In this study were explored through models and fieldwork if the richness, composition, functional and phylogenetic diversity of amphibians changes due to the environmental factors in different phytogeographic units in the state of Paraná, Brazil. In the first chapter, a niche modeling was performed for the species *Leptodactylus notoaktites* Heyer, 1978 (ANURA, LEPTODACTYLIDAE) and described habitat suitability to its occurrence following bioclimatic and biogeographic patterns. In the second chapter, was investigated if the richness and composition of the anurans species are related to the microhabitats availability and the landscape type on the waterbodies sampled. In the third chapter, we studied if the richness, composition, functional, and phylogenetic diversity of amphibians respond to the environmental factors in the different phytogeographic units of the state of Paraná.

**Key words:** biogeography; anurans; environmental variables; aquatic environments; environmental heterogeneity.

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## INTRODUÇÃO GERAL

A relação humana com a natureza e sua história ao longo da evolução dos seres vivos é peculiar e, necessariamente intrínseco a esse fato, há uma necessidade de abordar um tema chamado Ecologia. É cada vez mais evidente que a particularidade do campo da ecologia na ciência é indubitavelmente necessária devido aos impactos que as atividades antrópicas exercem sobre os seres vivos e no planeta terra (Schmitz 2013). Neste sentido, diversos estudos têm sido realizados para entender questões de como as plantas e animais estão distribuídos no espaço e no tempo ao longo do globo levando em consideração a história particular de cada ser vivo. Isto tem inspirado muitos biogeógrafos e ecologistas que procuram respostas a essas questões por meio de modelos de distribuição de espécies (Guisan & Thuiller 2005) que procuram informar essas questões por exemplo por meio de modelos de distribuição de espécies (Guisan & Thuiller 2005).

Os modelos utilizados para estimar a real ou potencial distribuição geográfica das espécies podem ser chamados de SDM (Species Distributions Models) e dentro destes modelos dependendo da abordagem do estudo é utilizado o termo ENM (Ecological Niche Modelling) (Guisan & Thuiller 2005; Phillips et al. 2006; Elith et al. 2006). Desse modo, esses modelos são embasados em caracterizar as condições ambientais que são adequadas para determinada espécie ou comunidade e assim mapear locais que são favoráveis para a ocorrência destes táxons (Pearson 2010). A distribuição espacial dos ambientes que são favoráveis as espécies podem ser mapeadas por diversas técnicas de 02 que podem ser aplicadas em uma variedade de estudos relacionados à biologia da conservação, biogeografia, ecologia e biologia evolutiva (Pearson 2010).

Na ecologia e biogeografia compreender como a riqueza das espécies varia dentro de diferentes escalas da paisagem é uma questão de interesse, assim como quais fatores e de que forma eles atuam na composição das espécies que exploram uma base comum de recursos em uma comunidade (Ricklefs et al. 1994). Por exemplo, os fatores abióticos, responsáveis por todos os componentes físicos e

climáticos, desempenham papel de grande importância sobre a fisiologia de cada espécie, influenciando diretamente na distribuição e, conseqüentemente, na composição das comunidades (Townsend et al. 2009). As comunidades são estruturadas pelos efeitos de vários fatores ambientais, evolutivos, fisiológicos, biológicos e além de suas interações espaciais e temporais. Dessa forma, ambientes complexos como biomas (e.g. Floresta Atlântica e Cerrado), favorecem organismos com histórias de vida particulares.

O estado do Paraná é um dos estados Brasileiros que possuem uma alta diversidade de unidades fitogeográficas. Embora a maior extensão neste estado seja ocupada pela Floresta Atlântica, há pequenas manchas de Cerrado. Essas zonas naturais de paisagem são baseadas na posição de suas escarpas, vales de rios, e cadeias de montanhas e pode ser dividido em duas grandes regiões naturais: o litoral e os planaltos do interior (Maack 2012). Por tais motivos, o estado do Paraná é uma excelente região para ser considerado objeto de estudos biogeográficos e ecológicos. Essa diversidade de unidades fitogeográficas tende a aumentar a diversidade de espécies de diversos grupos animais e vegetais (Maack 2012), o que no caso do presente estudo o grupo em foco são os anfíbios.

No Brasil hoje são reconhecidas 1.080 espécies de anfíbios anuros (Segalla et al., 2016), e este número está em crescimento devida ao potencial número de taxa ainda descritos. Atualmente, os anfíbios são reconhecidos como um dos grupos de animais mais ameaçados de extinção em todo o mundo, e vêm sofrendo uma crise de grandes proporções desde a década de 1980. Cerca de 40% das espécies de anuros correm risco de desaparecer nos próximos anos (IUCN 2019). Desmatamento, poluição das águas continentais, diminuição da camada de ozônio, chuva ácida e introdução de espécies exóticas são alguns dos fatores que mais interferem para a extinção local de espécies deste grupo (Heyer et al. 1988; Collins et al. 2009; Duellman & Trueb 1994).

A fragmentação dos habitats é uma das principais causas que ameaçam a biodiversidade das florestas tropicais, em que as espécies destes fragmentos restantes pequenos e isolados (Ribeiro et al.

2009) são condenadas muitas vezes a sofrerem efeitos potencialmente negativos da deriva genética e, como resultado, a extinção local (Rivera-Ortíz et al. 2015; Toczydlowski, & Waller 2019). Os anfíbios são organismos sensíveis às mudanças no ambiente e devido a restrições fisiológicas ficam limitados aos ambientes úmidos (Blaustein et al., 1994). São diversos os fatores que devem influenciar na sensibilidade de anfíbios à fragmentação florestal (Cushman, 2006), incluindo: baixa capacidade de deslocamento (Bowne & Bowers, 2004); alta vulnerabilidade ao transpor a matriz (Carr et al., 2002); baixa tolerância a certos tipos de habitats (Houlahan & Findlay, 2003); alta vulnerabilidade a patógenos, introdução de espécies exóticas, mudanças climáticas, poluição ambiental e aumento da exposição à radiação ultra-violeta (Blaustein et al. 2000; Bridges & Semlitsch 2000; Stuart et al. 2004).

Além da fragmentação florestal a perda de heterogeneidade ambiental nos ambientes reprodutivos é um fator que afeta negativamente as espécies de anfíbios (Parris 2004). O uso de microhabitats específicos tende a aumentar a riqueza local de anfíbios (Heyer & Berven 1973; Rincón-Franco & Castro, 1998). Sendo assim, a estrutura e disponibilidade da vegetação é um importante fator que afeta a riqueza e composição de espécies de anfíbios em diferentes escalas espaciais como por exemplo escalas locais (Vasconcelos et al. 2009) e regionais (Parris 2004). No entanto, a estrutura da vegetação varia de acordo com a paisagem em que está inserida, das quais por exemplo podem ser uma paisagem urbana, rural ou paisagem florestal (Medley et al. 1995). A transformação das paisagens florestais naturais em áreas destinadas à agricultura e pastagens levam a uma mudança na diversidade de espécies (Ndriantsoa et al. 2017) e o processo de urbanização afeta a composição de anfíbios nos ambientes reprodutivos devido a alterações e perdas dessas formações florestais (Hamer e McDonnell, 2008).

Atualmente os bancos de dados que permitem estudos com objetivos focados na obtenção de informações de padrões biogeográficos, de diversidade e composição das espécies são os museus de história natural (Graham et al. 2004) e banco de dados on-line relacionados às coleções científicas (e.g.

Species Link; IUCN; EOL; GBIF). Apesar de haver imprecisões taxonômicas e geográficas nestas bases de dados on-line, os dados quando utilizados em sincronia com a bibliografia e em escalas mais amplas podem fornecer valiosas informações (Diniz-Filho et al. 2008; Campos et al. 2017; Guedes et al. 2018; Vasconcelos et al. 2018). Além da utilização de base de dados on-line e revisões bibliográficas, o acréscimo de informações coletadas em campo tornam-se importantes para a obtenção de uma base de dados mais íntegra para biologia da conservação, ecologia, macroecologia e biogeografia (Nogueira et al. 2011; Valdujo et al. 2012).

Nas últimas décadas estudos biogeográficos têm sido apoiados pelo avanço da tecnologia aplicada aos métodos estatísticos e computação (Phillips et al. 2006; Thuiller et al. 2012). Além disso, estudos recentes que utilizam modelos contendo mapas de distribuição geográfica, com indicações de áreas prioritárias para a conservação e projeção dos efeitos de mudanças climáticas na diversidade de espécies têm aumentado (Campos et al. 2017; Lourenço-de-Moraes et al. 2018; Vasconcelos et al. 2018). As métricas de diversidade tradicionais utilizadas em um passado recente como a de Shannon e Simpson (Magurran 2004) que consideram somente a riqueza e a contribuição relativa das espécies vem sendo substituídas por medidas como a diversidade funcional ( $DF_{unc}$ ) e diversidade filogenética ( $DF_{ilo}$ ) (Petchey & Gaston 2006; Cianciaruso et al. 2009). A utilização dessas novas métricas de diversidade têm aumentado no âmbito científico porque são mais eficientes em prever os processos envolvidos no funcionamento e estrutura das comunidades (Cianciaruso et al. 2009).

$DF_{ilo}$  é uma medida da biodiversidade que leva em consideração a história evolutiva e as relações filogenéticas entre as espécies (Faith 1992; Magurran 2004). A abordagem principal desta medida é que a diversidade das espécies em determinado local é maior em uma comunidade em que as espécies são mais distantes filogeneticamente (Cianciaruso et al. 2009). Já a  $DF_{unc}$  pode ser entendida como um valor que varia de acordo com os traços funcionais das espécies e que têm influência sobre o funcionamento das comunidades (Cianciaruso et al. 2009). Metodologias que englobam a biogeografia,

informações sobre os atributos funcionais das espécies e as relações filogenéticas têm sido consideradas ótimas premissas para responder questões envolvendo a estruturação das comunidades em diferentes paisagens (Mouquet et. al 2012).

Nesse estudo o habitat, a riqueza, a composição específica, a  $DF_{unc}$  e a  $DF_{ilo}$  de anfíbios foram analisadas em diferentes paisagens no estado do Paraná. A base de dados para as análises foi construída a partir de diferentes fontes: (1) IUCN Red List of Threatened Species™ spatial data, em que os mapas são desenvolvidos como parte de uma avaliação abrangente da biodiversidade global; (2) revisão de literatura com artigos publicados em periódicos científicos e (3) dados coletados em campo. A tese está organizada em três capítulos:

(1) neste primeiro capítulo foi feito uma modelagem de nicho para a espécie *Leptodactylus notoaktites* com informações de locais que possui condições bioclimáticas e biogeográficas favoráveis à sua ocorrência, informações sobre sua vocalização e atualização de sua ocorrência nas diferentes unidades fitogeográficas;

(2) no segundo capítulo o objetivo foi testar se a riqueza e a composição das espécies de anuros que estão relacionadas aos microhabitats e ao tipo de paisagem dos corpos d'água amostrados na Floresta Estacional Semidecidual e assim assume-se que um número maior de microhabitats aumenta a heterogeneidade ambiental e isso, por sua vez, afeta a riqueza de espécies de anfíbios;

(3) no terceiro capítulo investigou-se a riqueza, a composição e como a  $DF_{unc}$  e a  $DF_{ilo}$  de anfíbios responde aos fatores ambientais nas diferentes unidades fitogeográficas do estado do Paraná.

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**ECOLOGICAL NICHE MODELING AND AN UPDATED GEOGRAPHICAL  
DISTRIBUTION MAP OF *LEPTODACTYLUS NOTOAKTITES* HEYER, 1978 (ANURA,  
LEPTODACTYLIDAE) WITH NEW OCCURRENCE RECORDS**

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Running Title: *Ecological Niche Modeling of Leptodactyls notoaktites*

Word count: 6318

**ABSTRACT**

*Leptodactylus notoaktites* Heyer, 1978 is a Neotropical frog that can be found in open areas, forest edges, and inside forest clearings in southern Brazil. In this study, we present an updated distribution map of this species for a variety of vegetation types in the Atlantic Rain Forest and report a new occurrence record in the Alto Paraná Atlantic Forests. We also performed an Ecological Niche Modeling (ENM) which combined environmental variables with occurrence records to predict environmentally suitable areas for this species. Our study was based on published data, specimens collected in the field, and specimens from Brazilian herpetological collections. The ENM predicted high environmental suitability ranging from São Paulo, Paraná, and Rio Grande do Sul states, mainly in Serra do Mar Coastal Forest areas, while the lowest values were in the states of Rio de Janeiro, Minas Gerais, Mato Grosso do Sul and some inland regions in the states of São Paulo and Paraná. Based on the distribution of *L. notoaktites*, we suggest that field efforts should be extended to inland regions of the Atlantic Rain Forest. In fact, species restricted to coastal regions of the Atlantic Rain Forest could have larger ranges than expected if data from such inland regions was available.

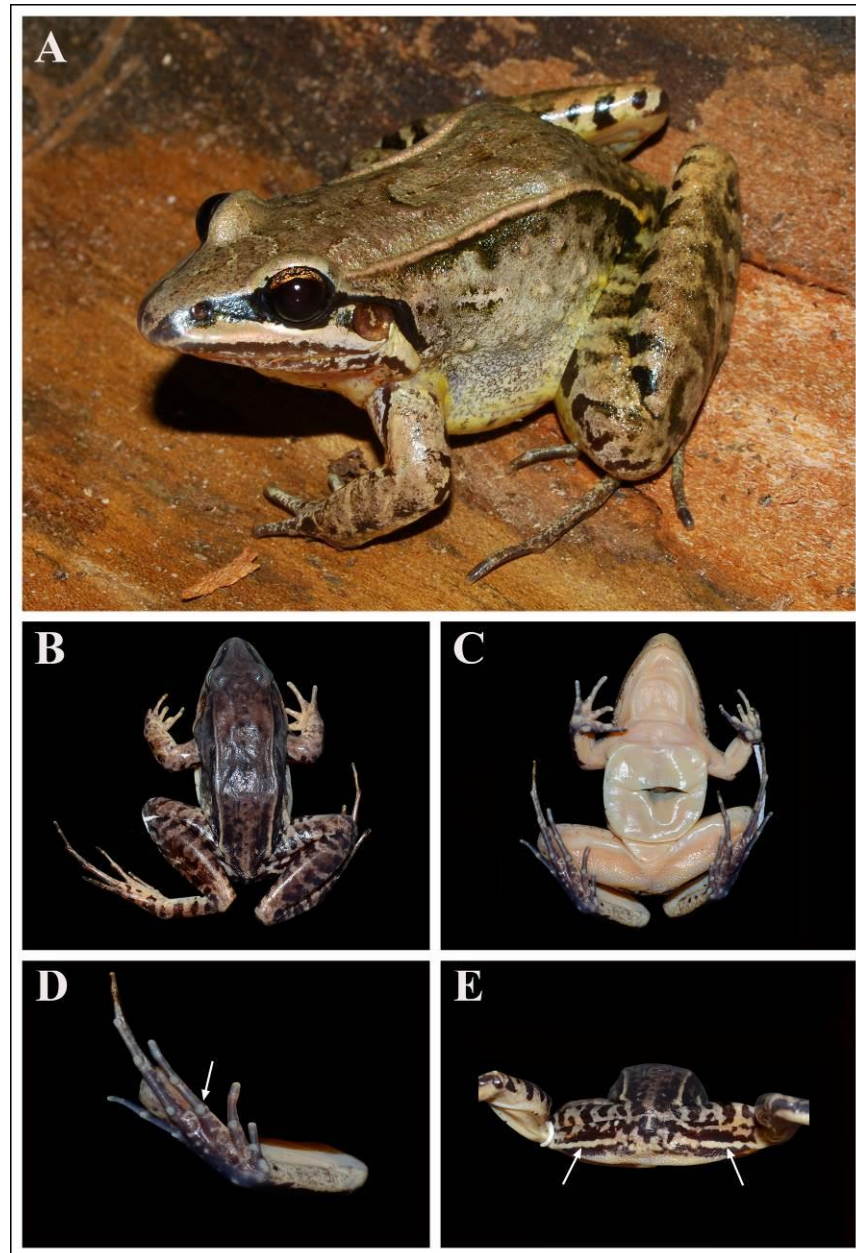
**Keywords:** Atlantic Rain Forest; anurans; Paraná state; advertisement call; environmental variables

## INTRODUCTION

*Leptodactylus notoaktites* Heyer, 1978 belongs to the *L. mystaceus* species complex, which is placed in the *L. fuscus* species group (de Sá *et al.* 2014). This species is a medium-sized frog (mean SVL = 47.4 mm) characterized by an upper shank and tibial barred pattern, two distinct dorsolateral folds, posterior thigh with light stripe, some individuals have white tubercles on the sole of the foot (Figure 1), and only individuals with a light mid-dorsal stripe have six dorsolateral folds (Heyer 1978). The species presents a white lip and black rostral range from the snout to the eardrum (Forlani *et al.* 2010). As for most species in the *L. fuscus* group, *L. notoaktites* lays its eggs in foam nests built in underground burrows, and as the pond floods the exotrophic tadpoles develop (Haddad & Prado 2005).

*Leptodactylus notoaktites* is found in open habitats along rivers, forest edges, and clearings inside forests. Such clearings inside forests are natural or the result of anthropogenic disturbance (Skuk & Heyer 2004). Currently, the species is distributed in the states of São Paulo, Paraná, and Santa Catarina, mainly along the coast up to 900 m a.s.l., which includes several protected areas (Skuk & Heyer 2004; de Sá *et al.* 2014). Despite being classified by the Red List of Endangered Species of International Union for Conservation of Nature as Least Concern and the fact that the species has a stable population trend, potential threats to this species include habitat loss due to deforestation, advance of agricultural areas, and infrastructure development for tourism, which makes additional research on the species geographical distribution necessary (Skuk & Heyer 2004).

Ecological niche models (ENMs) are important tools when evaluating species range, as they provide robust predictions of distributions or suitable environmental regions for species. Given their strong dependence on environmental variables (Duellman & Trueb 1994), ENMs seem particularly valid for anurans. ENMs use environmental variable associations and known species occurrences regions to generate models that define abiotic conditions where survival and reproduction are possible for the populations (Guisan & Thuiller 2005). Furthermore, these models can be used to define cryptic species (Raxworthy *et al.* 2007), predict species with potential invasion success (Peterson 2003; Peterson *et al.* 2006), maintain rare or endangered species (Engler *et al.* 2004), determine impacts of climate changes (Wiens *et al.* 2009), determine priority conservation areas (Chen 2009), and model the spread of crop pests (Venette *et al.* 2010).



**Figure 1.** Vouchered adult male alive (a) of *Leptodactylus notoaktite* from the Parque Estadual Mata São Francisco, between Cornélio Procópio and Santa Mariana municipalities, state of Paraná, Brazil. Photos of the preserved specimen as follows: (b) dorsal and (c) ventral views; (d) detail of the sole of foot showing presence of white tubercles (white arrow) and (e) detail of posterior thigh with light stripe (white arrows). Photo (a) by Luis Fernando Storti; (b to e) by Guilherme de Toledo Figueiredo.

Currently, there are several ways to generate ENMs from a set of environmental variables (*e.g.*, BIOCLIM, MARS, GARP, Maxent). However, in some particular situations uncertainties in model predictions can arise (Araújo & New 2007). Most of such limitations are due to the fact that ENMs use only presence and/or absence data, which can create a narrower true distribution than expected (Sinclair *et al.* 2010; Vasconcelos *et al.* 2012). If ENMs could include information on biotic interactions or restrictions then dispersal models would become more realistic. To avoid this limitation, it is possible to establish a priori criteria to build the ENMs. Priori criteria include: (I) potentially or abiotically suitable occurrence areas of an organism in a future climate change scenario, and (II) areas with suitable environmental conditions for organisms with little geographical distribution information (Vasconcelos & Nascimento 2016; Vasconcelos *et al.* 2017).

Herein, we provide an updated distribution map and new occurrence record of *L. notoaktites* with comments on its distribution across several types of vegetation in the Atlantic Rain Forest, based on published data, a specimen collected by the authors, and specimens housed in Brazilian herpetological collections. With this information, we generated a map that indicates areas with potentially suitable environmental conditions for *L. notoaktites* using an ENM approach.

## **MATERIAL AND METHODS**

### *Species data collection*

A single calling male of *L. notoaktites* (MZUEL-1575) was collected on 23 October 2011 at the Parque Estadual Mata São Francisco (PEMSF), a forest remnant of 865 ha located between the municipalities of Cornélio Procópio and Santa Mariana, state of Paraná, Brazil. To confirm its identity, we recorded the specimen advertisement call (air temperature 26°C) at night in an underground burrow at the forest's edge. Calls were recorded using a SONY ICD PX-820 digital recorder coupled with a Yoga HT-81 microphone and analyzed with a sampling frequency of 44 kHz and sample size of 16 bits. We analyzed the calls with Raven Pro 1.5 for Macintosh (Cornell 2014) and constructed audiospectrograms in R software using the package “seewave” (Sueur *et al.* 2008; R Development Core Team) with the following parameters: FFT window width = 256, Frame = 100, Overlap = 75, and flat top filter. Terminology used in the call description follows Köhler *et al.* (2017). For morphological and acoustic comparisons with all calls from the *L. mystaceus* species complex (Table 1), we used

data from Heyer *et al.* (1996) and de Sá *et al.* (2014). Four advertisement calls of the collected male (MZUEL-1575) in this study were deposited at FNJV (Fonoteca Neotropical "Jacques Vielliard" da Universidade Estadual de Campinas) sound collection (FNJV-33018 to 33021). Another individual was analyzed at the Museu de Zoologia of Universidade Estadual de Londrina (MZUEL-1333), collected by M.Z. de L. on 15 April 2009 at Parque Ecológico Klabin, in the municipality of Telêmaco Borba and mentioned in the unpublished thesis of Machado (2004).

All data based on previously published data (see Table 1) was obtained by searching in the database of "Scientific Electronic Library Online" (Scielo; [www.scielo.org](http://www.scielo.org)) and "Google Scholar" ([www.scholar.google.com](http://www.scholar.google.com)) on March 2016 with the following search terms: "*Leptodactylus notoaktites*" and "*Leptodactylus notoaktites* distribution". We collected data from the SpeciesLink Database (Cria 2016) and analyzed specimens housed in the following Brazilian herpetological collections: CFBH (Coleção Célio Fernando Baptista Haddad, Universidade Estadual Paulista, Rio Claro, São Paulo); DZSJRP- Amphibia-adults (Coleção de Anfíbios DZSJRP, Universidade Estadual Paulista, São José do Rio Preto, São Paulo); MCP- Anfíbios (Coleção de Anfíbios, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul); SinBiota (Sistema de Informação do programa Biota /FAPESP, Fundação de Amparo à Pesquisa do Estado de São Paulo, Campinas, São Paulo), ZUEC-AMP (Coleção de Anfíbios do Museu de Zoologia da UNICAMP, Campinas, São Paulo), UFMG-AMP (Coleção de Anfíbios do Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais); FNJV (Fonoteca Neotropical "Jacques Vielliard", Universidade Estadual de Campinas, Campinas, São Paulo); and MZUEL (Coleção de Herpetofauna do Museu de Zoologia da Universidade Estadual de Londrina, Londrina, Paraná). We only considered records those identified with the term "*Leptodactylus notoaktites*". Records containing taxonomic inaccuracies (*e.g.*, cf., aff. and gr.) were not considered. Vegetation types in the distribution map of *L. notoaktites* followed the ecoregions of Olson *et al.* (2001).

#### *Environmental data collection*

We used variables associated with the biological conditions necessary for the occurrence of the taxon: (i) 19 bioclimatic variables available at WorldClim, which are already well documented in the literature as important bioclimatic variables for amphibian distributions (*e.g.*, Duellman & Trueb 1994); (ii) altitude, slope

and aspect. Altitude is highly correlated with temperature, and humidity (Hoersch *et al.* 2002) and with solar radiation that plays an important role on moisture for habitat selection by amphibians (Wyman 1988). Slope and aspect were related to a proxy for the amount of solar radiation on the ground surface (Blank & Blaustein 2012); (iii) vegetation type (biome characteristics), which is related to species habitat (Toledo *et al.* 2012); and (iv) percent of tree cover (MODIS), which affects amphibian species composition and distribution (Figueiredo *et al.* unpublished data; Skelly *et al.* 1999). Bioclimatic variables were obtained from WorldClim (WorldClim database version 2.0, <http://www.worldclim.org/>) and were interpolated to 30 arc-sec resolution (Fick & Hijmans 2017) with WGS84 projection; altitude, slope, aspect, and vegetation type from National Aeronautics and Space Administration - NASA (<http://www2.jpl.nasa.gov/srtm/>; Amaral *et al.* 2013); and percent of tree cover from Global Landcover Facility (<http://glcf.umd.edu/data/>; Amaral *et al.* 2013).

#### *Model building and evaluations*

We used the 35 records (see Table 1) to generate the ENM based on the machine-learning modeling method Maxent (maximum entropy algorithm; Phillips *et al.* 2006), which estimates areas with potentially suitable environmental conditions for *L. notoakitites*. In this approach the niche suitability ranges from zero to one; the closer to one, the greater environmental suitability for the species. To avoid over-prediction and low specificity for species distribution (*i.e.*, Amazonian Rainforest), we cropped the bioclimatic layers to span from latitude -18 to -35 and longitude -38 to -58 (values in decimal degrees). To avoid model over-parameterization, we removed multicollinearity variables ( $r > 0.8$ ) and variables with low contribution to the model ( $<1\%$ ) using a Jackknife test of variable importance determined by their biological relevance for *L. notoakitites*. We used this approach because the test excluded one variable at a time when running the model (Baldwin 2009) and, therefore, provided information about the activity of each variable in the model, showing how important each variable was in explaining the species distribution and how much unique information each variable contained. This approach can also present highly correlated variables, thereby allowing us to determine if the rate of contribution values are constrained due to these multicollinear variables (Phillips 2017). Nine of the 19 original bioclimatic variables, vegetation type, and percent of tree cover were used in the final model: Bio2 (Mean Diurnal Range); Bio4 (Temperature Seasonality); Bio5 (Max temperature warmest month); Bio9 (Mean temperature driest quarter); Bio14 (Precipitation of Driest Month); Bio15 (Precipitation Seasonality); Bio17

(Precipitation driest quarter); Bio18 (Precipitation of Warmest Quarter) and Bio19 (Precipitation coldest quarter). The evaluation and performance of species distribution model was tested using the threshold-independent receiver operation characteristic (ROC) analysis. The area under the ROC curve (AUC) ranged from 0 to 1. After tenfold cross validation we used the model test of the AUC scores (Elith *et al.* 2006, Philips *et al.* 2006). AUC values greater than 0.9 are considered very good; AUC between 0.7 and 0.9 are good; and AUC values less than 0.7 are uninformative (Swets 1998). All the analyses were performed in the R platform vs. 3.3.2 (R Core Team 2016), MaxEnt software v. 3.4.1 (Phillips *et al.* 2006) and implemented in ArcGIS 10.5 for desktop (Esri 2016).

## RESULTS

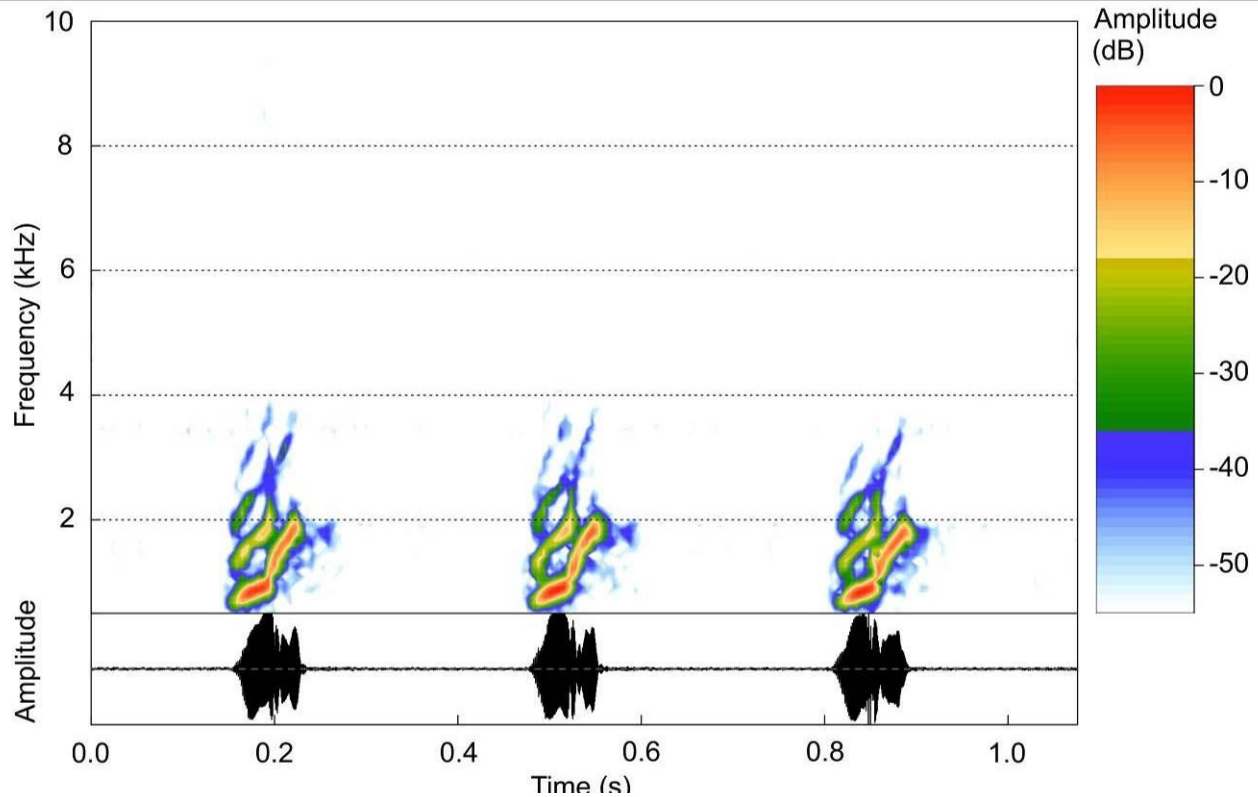
A total of 35 records were compiled: field collection (1); SpeciesLink database records (17) and literature records (17). Based on these records we found the distribution of of *L. notoaktites* in three states of Brazil (Table 1 and Figure 2): São Paulo (16 records), followed by Paraná (12), and Santa Catarina (7). Most of the records are found in the coastal range of these states. Regarding the vegetation type where the records are located we listed six types (Figure 2): Alto Paraná Atlantic Forests (7); Araucaria Moist Forest (7); Grasslands (5); Serra do Mar Coastal Forests (14); and Southern Atlantic Mangroves (2).

**Table 1.** Known localities for *Leptodactylus notoaktites* in southern and southeastern Brazil. Id = Identification number included in Figure 2. States: SP= São Paulo; PR = Paraná; SC= Santa Catarina. Source type: F = Field; LT = Literature; SL = Records from SpeciesLink database.

Municipalities (Locality)	Id	State	Latitude	Longitude	Source Type	Reference
Apiiaí; Iporanga (Parque Estadual Turístico do Alto Ribeira PETAR)	28	SP	24.4952°S	48.6471°W	LT	Araújo <i>et al.</i> (2010)
Barra do Turvo (Parque Estadual Jacupiranga)	26	SP	24.7561°S	48.5042°W	LT	Aguiar-de-Domenico (2008)
Campinas (Barão Geraldo, estrada da Rhodia)	16	SP	22.9000°S	47.0603°W	SL	PresentStudy (ZUEC-AMP 20973)
Capão Bonito (Fazenda Intervalles)	32	SP	24.0000°S	48.3400°W	SL	PresentStudy (ZUEC-AMP 8525)
Corumbataí (Sítio Santa Amélia)	18	SP	22.2200°S	47.6254°W	SL	PresentStudy (DZSJRP 5663)
Eldorado (Pousada Recanto das Águas)	14	SP	24.5200°S	48.1000°W	SL	PresentStudy (CFBH 10665)
Guaraqueçaba (Reserva Natural Salto Morato)	25	SP	25.1686°S	48.2984°W	LT	Garey & Hartmann (2012)

Municipalities (Locality)	Id	State	Latitude	Longitude	Source Type	Reference
Iguape (Estação Ecológica da Juréia - Itatins)	12	SP	24.7000°S	47.5500°W	SL	PresentStudy (ZUEC-AMP 16904)
Cananéia (Parque Estadual Ilha do Cardoso - Charco do Haras)	11	SP	25.1380°S	47.9670°W	SL	PresentStudy (FNJV 12960)
Iporanga (Parque Estadual da Caverna do Diabo)	27	SP	24.5330°S	48.7000°W	LT	Aguiar-de-Domenico (2008) PresentStudy (SINBIOTA C10166T86634)
Registro (Vale do Ribeira)	15	SP	24.4875°S	47.8432°W	SL	
Ribeirão Branco (Fazenda do João Zaquieu e Mathedi)	13	SP	24.2200°S	48.7650°W	SL	PresentStudy (CFBH 6896)
Ribeirão Grande (Parque Estadual Intervalles)	29	SP	24.3232°S	48.2800°W	LT	Bertoluci & Rodrigues (2002)
Ribeirão Grande (Fazenda Intermontes)	31	SP	24.0900°S	48.3600°W	LT	Tacioli (2012)
Rio Claro (Horto Florestal)	17	SP	22.4100°S	47.5600°W	SL	PresentStudy (CFBH 4335)
Tapiraí; São Miguel Arcanjo; Capão Bonito (Parque Estadual Carlos Botelho)	30	SP	24.1414°S	47.9740°W	LT	Forlani <i>et al.</i> (2010)
Antonina (Reserva Natural Rio da Cachoeira)	10	PR	25.4280°S	48.7110°W	SL	PresentStudy (CFBH 12361)
Cornélio Procópio; Santa Mariana (Parque Estadual Mata São Francisco)	1	PR	23.1590°S	50.5660°W	F	PresentStudy (MZUEL 1575)
Curitiba (Parques Municipais de Curitiba)	35	PR	25.4160°S	49.2500°W	LT	Crivellari <i>et al.</i> (2014)
Fazenda Rio Grande (Fazenda Gralha Azul)	21	PR	25.9280°S	49.1980°W	LT	Conte & Rossa-Feres (2007)
Guaratuba (Colônia Castelhanos)	22	PR	25.8820°S	48.5740°W	LT	Cunha <i>et al.</i> (2010)
Morretes (Condomínio Rio Sagrado)	23	PR	25.5650°S	48.7990°W	LT	Armstrong & Conte (2010)
Piraí do Sul (Flona de Piraí do Sul)	34	PR	24.5660°S	49.9160°W	LT	Foerster (2014)
Piraquara (Barragem Piraquara)	9	PR	25.4410°S	49.0630°W	SL	PresentStudy (CFBH 11037)
Ponta Grossa; Carambeí; Castro (Campos Gerais)	33	PR	25.0500°S	49.9500°W	LT	Crivellari <i>et al.</i> (2014)
Rio Negro (Parque Municipal São Luiz de Tolosa)	19	PR	26.0847°S	49.8060°W	LT	Santos & Conte (2014)
São José dos Pinhais (Serro e Gemido)	24	PR	25.4105°S	49.0300°W	LT	Conte & Rossa-Feres (2006)
Telêmaco Borba (Fazenda Monte Alegre)	2	PR	24.2857°S	50.4170°W	LT	Machado (2004); (MZUEL 1333)
Benedito Novo (Reserva Biológica Sassafrás)	3	SC	26.7826°S	49.3640°W	SL	PresentStudy (UFMG-AMP 3091)
Blumenau (Parque das Nascentes)	4	SC	26.9193°S	49.0660°W	SL	PresentStudy (PUCRS-MCP 8681)
Brusque (RPPN Chácara Edith)	5	SC	27.0982°S	48.9170°W	SL	PresentStudy (PUCRS-MCP 7639)
Corupá (RPPN Emílio Fiorentino Battistella)	7	SC	26.4250°S	49.2430°W	SL	PresentStudy (CFBH 28718)
Jaraguá do Sul (Vila Nova - Vila Lenzi)	6	SC	26.4860°S	49.0660°W	SL	PresentStudy (PUCRS-MCP 1482)
Joinville (Serra Dona Francisca)	20	SC	26.1950°S	49.0350°W	LT	Mariotto (2014)
São Bento do Sul (Rio Vermelho - Natal)	8	SC	26.2500°S	49.3780°W	SL	PresentStudy (UFMG-AMP 9866)

The advertisement call of the collected specimen of *L. notoaktites* was composed of a single, harmonic, unpulsed note, with a duration of 0.074–0.091 s ( $0.080 \pm 0.005$  s; Figure 3). The call had ascending frequency modulation, with a dominant frequency of 689–1205 Hz ( $1029 \pm 214$  Hz; Figure 3). Hence, the advertisement call analyzed confirms the identity of the specimen collected in the PEMSF as *L. notoaktites*.



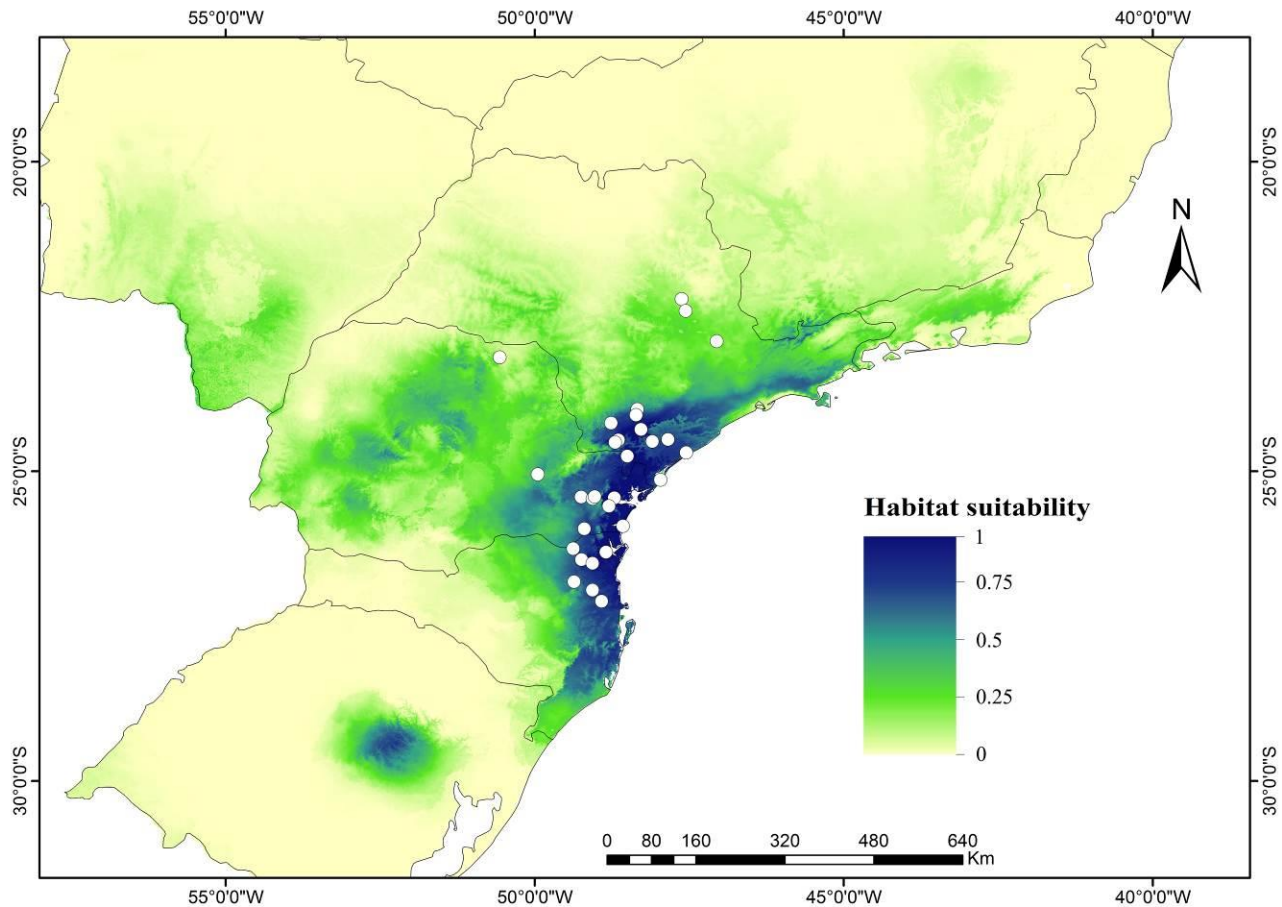
**Figure 3.** Audiospectrogram and oscilogram of the advertisement call of *Leptodactylus notoaktites* recorded from a single male at Parque Estadual Mata São Francisco, Paraná state, Brazil.

**Table 2.** Acoustic parameter comparisons of the advertisement call for the *Leptodactylus mystaceus* species complex. \*Species with frequency modulations in the beginning/end of call.

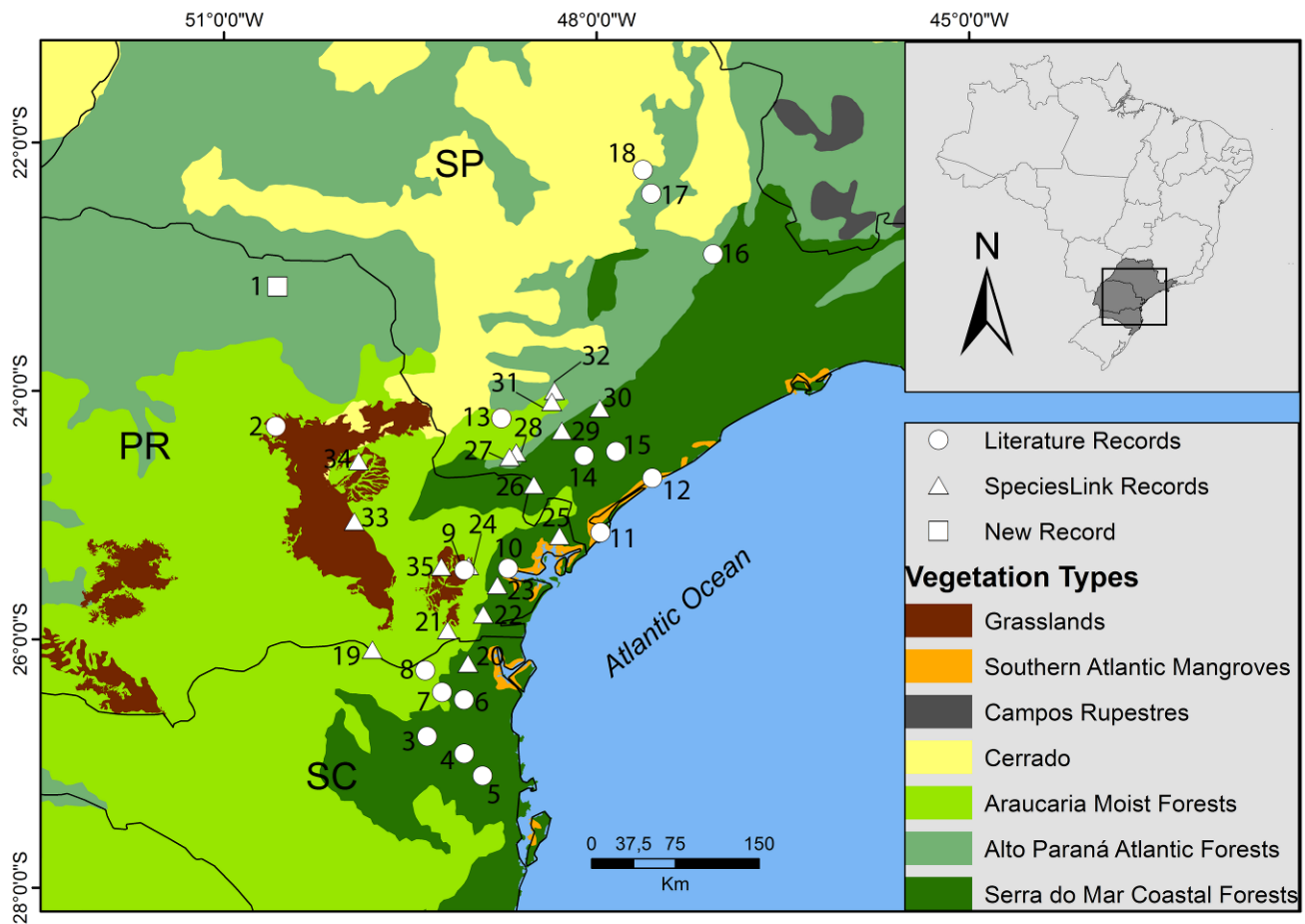
Species	Dominant Frequency (kHz)	Call Duration (s)	Pulses	Call rate	Harmonic	Reference
<i>Leptodactylus notoaktites</i>	689–1.205 Hz/1.550–1.722 Hz*	0.074–0.091s	No	0.6/s	Yes	Present Study
<i>Leptodactylus cupreus</i>	2800–3058 Hz	0.16s	No	–	Yes	Caramaschi <i>et al.</i> 2008
<i>Leptodactylus didymus</i>	510–1.510 Hz	0.09–0.32s	Yes	1.4–3.1/s	Yes	Heyer <i>et al.</i> 1996; Köhler & Lötters 2002
<i>Leptodactylus elenae</i>	700–870 Hz/1.370–1.500 Hz*	–	No	64–120/min	Yes/No	Barrio 1965, Heyer & Heyer 2002

Species	Dominant Frequency (kHz)	Call Duration (s)	Pulses	Call rate	Harmonic	Reference
<i>Leptodactylus mystaceus</i>	700–1.400 Hz	0.2s	Yes	1.8/s	No	Heyer 1978; Heyer <i>et al.</i> 1996
<i>Leptodactylus spixi</i>	1.500–1.722/1.981–2.067 Hz*	0.12 ± 10s	No	80–97/mi n	Yes	Bilate <i>et al.</i> 2007“2006”

AUC test for the replicate runs of the ENMs generated for *L. notoaktites* (Figure 4) was  $0.99 \pm 0.004$ , indicating a very good model. The environmental suitability was explained primarily by Temperature Seasonality (bio4; 34.3%), Precipitation of Warmest Quarter (bio18; 23.8%) and Precipitation of Driest Month (bio14; 13.8%). Altitude, slope, and aspect were discarded due to their poor contribution to the model (<1%). The highest environmental suitability in the model (>0.5; represented by the water blue and blue colors in Figure 4) ranged from São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul states, whereas the most probable areas of occurrence were along the coastal range of São Paulo, Paraná, and Santa Catarina states. The lowest



**Figure 4.** Predicted distribution of *Leptodactylus notoaktites* based on environmental suitability. Occurrence localities can be found in Table 1; Figure 2.



**Figure 2.** Geographical distribution map of *Leptodactylus notoaktites* in the southern and southeastern Brazil with indication of vegetation types. States: SP = São Paulo, PR = Paraná, SC = Santa Catarina. Records: White dots = literature records, white squares = SpeciesLink records, and white triangles = new records. Localities: 1. Parque Estadual Mata São Francisco; 2. Telêmaco Borba; 3. Benedito Novo; 4. Blumenau; 5. Brusque; 6. Jaraguá do Sul; 7. Corupá; 8. São Bento do Sul; 9. Piraquara; 10. Antonina; 11. Parque Estadual Ilha do Cardoso; 12. Iguape; 13. Ribeirão Branco; 14. Eldorado; 15. Registro; 16. Campinas; 17. Rio Claro; 18. Corumbataí; 19. Rio negro; 20. Serra Dona Francisca; 21. Fazenda Rio Grande; 22. Guaratuba; 23. Morretes; 24. São José dos Pinhais; 25. Reserva Natural Salto Morato; 26. Barra do turvo; 27. Iporanga; 28. PETAR; 29. Parque Estadual Intervalles; 30. Parque Estadual Carlos Botelho; 31. Ribeirão Grande; 32. Capão Bonito; 33. Campos Gerais; 34. Pirai do Sul; 35. Curitiba.

values ( $<0.3$ ; green color in Figure 4) were in the states of Rio de Janeiro, Minas Gerais, Mato Grosso do Sul, and some inland regions of São Paulo and Paraná states.

## DISCUSSION

Regarding the geographic distribution of *L. notoaktites* (see Figure 3), our record in the PEMSF, Paraná state, considerably expanded the distribution of the species to the north of the state, and represents a new record for the Alto Paraná Atlantic Forests, an Atlantic Rain Forest vegetation type. The Atlantic Rain Forest was one of the largest rainforests of the Americas with high environmental heterogeneity (Ribeiro *et al.* 2009), being considered a hotspot for conservation (Myers *et al.* 2000). The PEMSF is located approximately 258 km in a straight-line from the type locality of the species in the municipality of Iporanga, São Paulo state, which consists of Serra do Mar Coastal Forests, and is about 140 km in a straight-line from the nearest record in the Telêmaco Borba municipality, located in the Araucaria Moist Forest (Machado 2004).

Other species similar to *L. notoaktites*, which include species from the *L. mystaceus* complex (*L. cupreus*, *L. didymus*, *L. elenae*, *L. mystaceus* and *L. spixi*), have distinct geographical distribution. *Leptodactylus mystaceus*, which occurs in Amazonia, and Minas Gerais and São Paulo states in mesic enclaves of northeastern Brazil, as well as northern portions of the Atlantic Rain Forests of Brazil (de Sá *et al.* 2014), is the only species of the *L. mystaceus* complex that occurs in the nearby region of our new record of *L. notoaktites* in the PEMS (see Affonso *et al.* 2011). Nevertheless, when comparing the morphology of both species it is clear that only individuals of *L. notoaktites* with a light mid-dorsal stripe also have a pair of dorsal folds, while *L. mystaceus* lacks both a pair of dorsal folds and a light mid-dorsal stripe (de Sá *et al.* 2014). Besides, there are several acoustic differences between the species. For instance, the advertisement call of *L. mystaceus* presents no harmonic structure, while the advertisement call of *L. notoaktites* is harmonically structured (de Sá *et al.* 2014; present study). Therefore, bioacoustic studies can be useful for understanding ecological processes under climate change (Møller 2010) or anthropic actions in fragile systems (deforestation and fragmentation of habitats) (Tucker *et al.* 2014), fostering new perspectives in ecology and conservation fields described as Ecoacoustics (Sueur *et al.* 2015).

Most of the records of *L. notoaktites* are along the coastal region of Brazil, mainly in the Serra do Mar Coastal Forests (*e.g.*, Reserva Natural Salto Morato, municipality of Guaraqueçaba, Paraná state; Parque Estadual Intervales, municipalities of Ribeirão Grande, Guapiara, Sete Barras, Eldorado, and Iporanga, São Paulo state) and Araucaria Moist Forest (*e.g.*, São José dos Pinhais, municipality of Piraí do Sul, Paraná state) (Table 1 and Figure 2). The other records are in the Alto Paraná Atlantic Forest (*e.g.*, Parque Estadual Mata São Francisco, municipality of Rio Claro, Paraná state) and Grasslands (In the Phytogeographic unit of Campos Gerais, municipalities of Ponta Grossa, Carambeí, Castro, Paraná state), where there are ecotone with Grasslands and Araucaria Moist Forest (Table 1 and Figure 2). Some individuals were recorded in Southern Atlantic Mangroves (Estação Ecológica da Juréia – Itatins, municipality of Iguape; Parque Estadual Ilha do Cardoso, municipality of Cananéia and Reserva Natural Rio da Cachoeira, municipality of Antonina, São Paulo state). This species also occurs in ecotones with Alto Paraná Atlantic Forests and Cerrado domain vegetation (*e.g.*, municipality of Corumbataí, São Paulo state) and perhaps may even occur in the Cerrado, since the model predicted suitable environments there (see Figure 4).

Records of *L. notoaktites* outside of its occurrence region predicted by ENM may be the result of the lack of field efforts in some regions. This gap in records could limit more robust predictions by the model. This may occur because the geographical range of any species is based on several complex interactions between intrinsic factors of the species (*e.g.*, life history, dispersal abilities, environmental requirements) and extrinsic factors (*e.g.*, variations in space and time), which limit the known distributions (Brown *et al.* 1996). However, many other factors could be affecting their distribution, such as species colonizing regions outside of their known occurrence and breeding in areas that are significantly different from their former pristine breeding habitats as result of deforestation (Rubbo & Kiesecker 2005). Another possibility is that the species has historically occurred in these environments (in another age) and may now be isolated in refugia (Reside *et al.* 2014). Refugia are habitats where populations of species can retreat to, persist in, and even expand their geographical range over ecological and evolutionary time scales of millennia (Keppel *et al.* 2012).

Herein we show that the distribution of *L. notoaktites* is not restricted to the Brazilian Coast and that it can also occur in inland forests of the Atlantic Rain Forest. Nevertheless, this species still does not have a large distribution (*e.g.*, *L. mystaceus*), which reinforces the need to maintain suitable habitats for this species for

conservation and management actions. For biological conservation, ENMs have been mainly applied to discover biodiversity, study species invasion, conservation efforts, and climate changes effects (Rangel & Loyola 2012). Thus, studies that model species distributions along ecosystems, especially for those species with restricted distribution, can serve as a tool for decision-making and indicate priority sites to be preserved. These regions must have suitable conditions for the survival/persistence of rare and/or endangered species or even species with restricted distribution. Furthermore, we highlight that the gaps in field efforts in certain regions, as in the northern Paraná state, should be considered to estimate the range of *L. notoaktites*. As found for this species, it is possible that several anuran species with known ranges restricted to the Serra do Mar Coastal Forests and Southern Atlantic Mangroves of Brazil could actually have larger distributions. In fact, the anurans diversity in Alto Paraná Atlantic Forests and Araucaria Moist Forests may be higher than expected.

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## CAPÍTULO II



**INFLUENCE OF MICROHABITAT ON THE RICHNESS OF ANURAN SPECIES:  
A CASE STUDY OF DIFFERENT LANDSCAPES IN THE ATLANTIC FOREST  
OF SOUTHERN BRAZIL**

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Keyword:	Amphibians, Biodiversity, Anthropic actions, Fragmentation
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A CASE STUDY OF DIFFERENT LANDSCAPES IN THE ATLANTIC FOREST  
OF SOUTHERN BRAZIL**

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**Abstract**

Environmental heterogeneity is a factor which can help explain the higher richness of local species. The objective of this study was to test if richness and composition of anurans species are related to available microhabitats and type of landscape of sampled sites. We assume that a higher number of microhabitats increase environmental heterogeneity and this, in turn, affects species richness of amphibians. We performed the study in the Mesophytic Semideciduous Forest, a vegetation type within Atlantic Forest Domain. Between October 2010 and February 2011, we sampled 23 water bodies located in the agricultural, forest, and urban landscapes. The species richness was determined using survey at breeding sites methodology, and the availability of microhabitats was estimated visually. Thirty-four anuran species belonging to 12 families were recorded. The species richness in water bodies ranged from two to 13 species. The highest species richness was recorded in environments with a higher number of microhabitats, while the species composition in water bodies was partially grouped according to the predominant landscape type that is agricultural, forest, forest edge or urban. Our results suggest that species use specific environments (e.g. landscapes, habitat and microhabitat) for their reproductive activities.

## INTRODUCTION

Environmental heterogeneity is one of the leading factors that contribute to higher species richness in an environment in a variety of organisms (e.g. Tews et al. 2004; Townsend et al. 2009), including anurans (Keller et al. 2009; Vasconcelos et al. 2009). Higher local richness can be achieved through species specialization, which drives the occupancy of specific microhabitats of a given area (Santos et al. 2007; Vasconcelos et al. 2009; Silva et al. 2012; Santos and Conte 2014), promoting high functional and phylogenetic diversity (Campos et al. 2017).

The advance of urbanization and agricultural frontiers has adverse effects on the occurrence of species due to the habitat loss, fragmentation and degradation, which results in a dramatic decrease in the availability of microhabitats (Knutson et al. 1999; Cushman 2006; Cruz-Elizalde et al. 2016; Garey and Provete 2016; Lourenço-de-Moraes et al. 2018). The Atlantic Forest Domain (AFD), for example, is one of the most threatened tropical forests because of fragmentation (Ribeiro et al. 2009). The AFD also harbors the highest rates of endemism and species diversity of anurans, with 529 species reported (Haddad et al. 2013). However, owing to the annual addition of new species descriptions (e.g. Malagoli et al. 2017; Monteiro et al. 2018), richness is expected to increase in the AFD. Mesophytic semideciduous forest (MSF), for example, a vegetation type within the AFD (Oliveira-Filho and Fontes 2000) and the focus of this study, has a considerable richness with 111 species of amphibians, five of which are endemic (Garcia et al. 2007).

Amphibians anurans require specific habitats throughout their life cycle, such as water bodies (e.g. ponds, dams, swamps, marshes, and streams) suitable for breeding and development of tadpoles (Duellman and Trueb 1994; Wells 2007; Provete et al. 2014). Soon after, they depend upon terrestrial environments for the growth and dispersal of

juveniles (Knutson et al. 1999; Price et al. 2004; Wells 2007). In fact, this dependence of both aquatic and terrestrial environments, their sensitive skins, and eggs (due to the absence of a shell) have led those organisms to be considered as excellent bioindicators of environmental change (Blaustein and Wake 1990; Wells 2007; Toledo et al. 2009). Indeed, many microhabitats are used by anurans, especially during the breeding season, including marginal vegetation (e.g. grasses, herbaceous vegetation and tree vegetation) and inside of aquatic environments (e.g. shrubs and aquatic plants) (Bernarde and Anjos 1999; Bertoluci and Rodrigues 2002; Conte and Machado 2005; Conte and Rossa-Feres 2006, 2007; Santos et al. 2007; Vasconcelos et al. 2009; Santos and Conte 2014).

Although several studies have shown that a relationship exists between anuran species richness and environmental heterogeneity (Parris 2004; Menin et al. 2005; Vasconcelos et al. 2009; Keller et al. 2009; Silva et al. 2011a; Silva et al. 2012; Santos and Conte 2014), responses to this association are related to the spatial scale. On a local scale, species occurrence in water bodies is driven, for example, by the duration of water availability (Vasconcelos et al. 2009), by the water depth (Burne and Griffin 2005; Gonçalves et al. 2015) and by the vegetation type within and around the water body (Keller et al. 2009; Gonçalves et al. 2015). On the regional scale, factors driving species occurrence are, for example, distance to forest fragments (Silva et al. 2011a, c; Gonçalves et al. 2015) and the geographical distance between water bodies (Burne and Griffin 2005; Santos and Conte 2016).

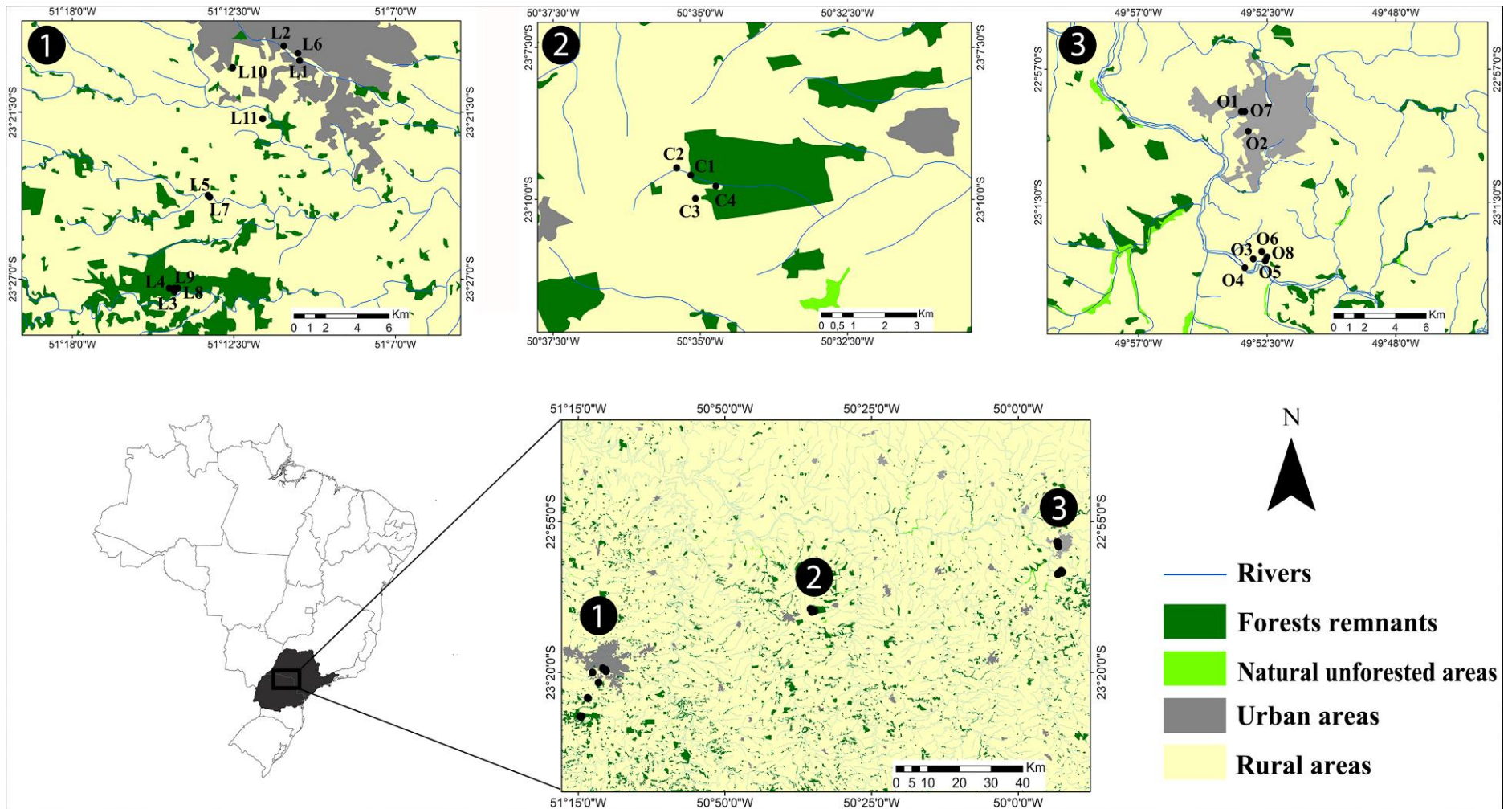
Because of the differences between the spatial scales, studies on amphibians that combine both of those approaches, local environmental heterogeneity, and landscape type, seem relevant (Knutson et al. 1999; Vasconcelos et al. 2009; Silva et al. 2012; Oda et al. 2017). For instance, such studies provide better information for selecting priorities for

conservation and management (Santos et al. 2012; Campos et al. 2017) and particularly to the MSF, the most threatened and fragmented ecosystem of the AFD (Viana and Tabanez 1996). In this study, we investigated the variations in species richness, abundance, and composition of aquatic-breeding anurans in water bodies with a different number of microhabitats within urban, agricultural and forested landscapes. We controlled our samplings through the available microhabitats in each landscape type and tested the following hypotheses: (1) urban and agricultural landscapes have less species richness than forest and forest edge landscapes; (2) species richness of anurans is positively related to the number of microhabitats available in water bodies; (3) the species composition varies according to the landscape type.

## **MATERIALS AND METHODS**

### **STUDY REGION**

Assemblages of anurans were studied in the region encompassed by the northern part of the state of Paraná and the southeast part of the state of São Paulo (Figure 1), a region dominated initially by MSF vegetation type within the AFD (Maack 1981). The MSF is a seasonal forest with a dry period and lower temperatures, and another period with higher rainfall and highest temperatures (Veloso et al. 1991). The climate of the region is classified according to Köppen-Geiger's as a humid subtropical climate (Cfa) (Peel et al. 2007), with average annual temperature ranging between 22 °C – 25 °C and an annual rainfall of 1,612.5 mm (INPE 2015).



**Figure 1.** Sampled water bodies located in the Mesophytic Semideciduous Forest within Atlantic Forest Domain, between October 2010 and February 2011: northern of the state of Paraná and southeast of the state of São Paulo, southern Brazil.

## STUDY SITES

Water bodies were studied within the urban, agricultural, forest and forest edge landscapes (Table I). In the northern area of the state of Paraná, sampling took place in the municipality of Londrina located in the Tibagi river basin at an average elevation of 700 meters a.s.l. The Parque Estadual Mata dos Godoy (PEMG ~ 675 ha), a protected area in that municipality, which was also included in the study given that the PEMG and other fragments connected to it form the largest forested area in northern Paraná (Anjos 1998). Also in northern Paraná, another protected area was sampled, the Parque Estadual Mata São

**Table I.** Municipalities, water body code, landscape types, and coordinates of the studied water bodies between October 2010 and February 2011 in northern of the state of Paraná, southern Brazil. The output format of coordinates is in Geographic Coordinate System and datum WGS84.

Municipality	Water body code	Landscape type	Coordinates	
<b>Ourinhos</b>	O1	Urban landscape	22°58'26.40"S	49°53'16.80"W
	O2	Agricultural landscape	22°59'06.00"S	49°53'09.60"W
	O3	Agricultural landscape	23°03'10.80"S	49°52'40.80"W
	O4	Agricultural landscape	23°03'43.20"S	49°53'16.80"W
	O5	Agricultural landscape	23°03'21.60"S	49°52'30.00"W
	O6	Agricultural landscape	23°03'25.20"S	49°52'58.80"W
	O7	Urban landscape	22°58'26.40"S	49°53'24.00"W
	O8	Agricultural landscape	23°03'28.80"S	49°52'33.60"W
<b>Londrina</b>	L1	Urban landscape	23°19'33.60"S	51°10'19.20"W
	L2	Urban landscape	23°19'19.20"S	51°10'48.00"W
	L3	Forest landscape	23°27'18.00"S	51°14'31.20"W
	L4	Forest landscape	23°27'25.20"S	51°14'31.20"W
	L5	Agricultural landscape	23°24'14.40"S	51°13'22.80"W
	L6	Urban landscape	23°19'48.00"S	51°10'15.60"W
	L7	Agricultural landscape	23°24'18.00"S	51°13'19.20"W
	L8	Forest landscape	23°27'18.00"S	51°14'24.00"W
	L9	Forest landscape	23°27'18.00"S	51°14'42.00"W
	L10	Urban landscape	23°20'02.40"S	51°12'32.40"W
	L11	Agricultural landscape	23°21'43.20"S	51°11'31.20"W
<b>Cornélio Procópio</b>	C1	Forest edge landscape	23°09'36.00"S	50°35'09.60"W
	C2	Forest edge landscape	23°09'28.80"S	50°35'24.00"W
	C3	Forest edge landscape	23°09'46.79"S	50°34'44.10"W
	C4	Forest edge landscape	23°09'59.14"S	50°35'04.85"W

Francisco (PEMSF ~ 832,5 ha) which is located between municipalities of Cornélio Procópio and Santa Mariana in the Rio das Cinzas river basin at an average elevation of 543 meters a.s.l. In the state of São Paulo only the municipality of Ourinhos, located in the Paranapanema river basin at elevation 492 meters a.s.l. was sampled

## DATA COLLECTION

### *Anuran surveys*

For each water body, the samplings were carried out during the hottest and wettest seasons of the year, which coincide with the breeding season of the anurans species. In this period (October to March) species detection is increased due to abiotic factors such as precipitation and temperature, which are relevant for breeding of most species (e.g. Bernarde & Anjos 1999; Eterovick & Sazima 2000; Conte & Rossa-Feres 2007; Garey & Silva 2010). A total of 23 water bodies were sampled: eleven in Londrina; four in the PEMSF; and eight in Ourinhos. These samples represented the different landscapes as follows: agricultural landscape with nine samples; urban landscape with six samples; forest landscape with four samples; and forest edge landscape with four samples (see Figure 1; Table I).

Water bodies were sampled between October 2010 and February 2011 to determine the species richness and abundance of anurans. For this, a breeding site survey methodology was used (Scott and Woodward 1994). Each water body within both open and forested areas had covered environments, where all visual and acoustic contacts of the species were recorded. Each environment was sampled on a single day, during a six-hour period from 18:00h to 00:00h and with the same sampling effort (hours/water body),

totaling 120 field hours. Between 2010 and 2017, we performed occasional field visits to the study region and used data from the PEMSF species list (Storti 2012), which were added to the list of species richness overall (see Table II).

One individual of each species was manually collected when possible, euthanized with xylocaine 5%, fixed with formalin 10%, and preserved in alcohol 70%. All the animal handling and collecting procedures follow resolution 301 of the Federal Council of Biology. Collecting permits were provided by Ministry of the Environment, Chico Mendes Institute for Biodiversity Conservation (SISBIO 2920-1 and 12120-1). Specimens were deposited in the Museum of the State University of Londrina (MZUEL) (Appendix I).

#### *Landscape and microhabitat description*

The landscapes were classified as follows: agricultural landscape (AL: areas at a distance of more than 500 m from forest remnants, with the size of these above 200 ha); forest edge landscape (FEL: less than 50 m from the interior of the remnants, with the size of these above 200 ha and less 500 m from the edge of the remnant; or riparian forest with 200 ha of area overall); forest landscape (FL: located more than 50 m from the edge to the interior of the remnants, with the size of these above 200 ha); and urban landscape (UL: area with houses, buildings, people transit and vehicles). Characterization of the environments involved collecting data on the vegetation on the banks of water bodies and in the water, taking into consideration the microhabitats reported in Conte and Machado (2005); Conte and Rossa-Feres (2007); Santos et al. (2007), plus new microhabitats that were added in the present study (Table III). For the soil and marginal vegetation, we recorded the following: arboreal vegetation (AV), bulrushes (BR), grasses (GR), non-vegetation (NV), shrub vegetation (SV) and trunks (TR). For the type of soil and vegetation

in the water, we recorded the following: arboreal vegetation (AV), bulrushes (BR), grasses (GR), non-vegetation (NV), shrub vegetation (SV), and water hyacinths (WH).

**Table III.** Microhabitats of the studied water bodies in southern Brazil. Microhabitats were: arboreal vegetation (AV), bulrushes (BR), grasses (GR), no vegetation (NV), shrub vegetation (SV), trunks (TR), and water hyacinths (WH). L = Londrina; O = Ourinhos; C = Cornélio Procópio.

Water body	Microhabitats Availability		Total
	Inside	Edge	
O1	NV, GR	NV, GR	4
O2	NV, GR	NV	3
O3	NV, GR	NV, GR	4
O4	GR, SV	NV	3
O5	NV, GR, BR, SV, AV, TR	NV, GR, BR, SV, AV, WH	12
O6	NV, GR, SV	GR, SV	5
O7	NV, GR, AV	NV, GR	5
O8	NV, GR, BR, SV, AV, TR	NV, GR, BR, SV, AV, WH	12
L1	NV, GR, SV, AV	NV, GR, SV, AV	8
L2	NV, GR, AV, TR	NV, GR	6
L3	GR, SV, AV, TR	NV, GR, BR, SV, AV	10
L4	GR, SV, AV, TR	NV, GR, BR, SV, AV	10
L5	GR, SV, AV, TR	NV, GR, BR, SV, AV	10
L6	NV, GR, TR	NV, GR	5
L7	GR, SV, AV	GR, SV, AV, TR	7
L8	GR, SV, AV, TR	NV, GR, BR, SV, AV	10
L9	GR, SV, AV, TR	NV, GR, BR, SV, AV	10
L10	NV, GR, SV, TR	NV, GR	6
L11	NV, GR, TR	NV, GR	5
C1	NV, GR, BR, SV, AV, TR	NV, GR, BR, SV, AV	11
C2	NV, GR, BR, SV, AV, TR	NV, GR, BR, SV, AV	11
C3	GR, BR, SV, AV, TR	GR, BR, AV	8
C4	NV, GR, BR, SV, AV, TR	NV, GR, BR, SV, AV, WH	12

## DATA ANALYSIS

### *Species richness vs microhabitat*

We investigated the relationship between species richness and the availability of microhabitats in the environment with simple linear regression analysis and, subsequently,

a correlation graph was constructed. Through the characterization of the environments, it was possible to quantify the number of available microhabitats, making it a predictive variable, and to consider the anuran species richness in each sampled environment as a response variable. To achieve the assumption of data normality, the response variable was  $\log_{10}$  transformed. Afterward, we perform a Shapiro-Wilk test to check if the data had a normal distribution indeed. Significant values were considered when  $p < 0.05$ . The analysis was performed using the software R statistic version 3.4.2 (R core team 2017).

#### *Species composition vs breeding environment*

To identify patterns in the composition of anuran communities in the breeding environments concerning the landscape type where water bodies were located (agricultural, forest, forest edge and urban), a cluster analysis (UPGMA) was performed with the index of Bray-Curtis similarity (Clarke 1993), in which the abundance of each species was considered in each water body. Subsequently, to test the significance of the generated groups, we used a multivariate ANOSIM similarity analysis with 999 permutations. ANOSIM is a robust analysis when comparing two or more groups based on distance matrices, which are converted into ranks and compared within and between groups (Clarke 1993). To visualize the pairwise test resultant from the ANOSIM, we generated a second hierarchical cluster analysis (UPGMA, Euclidean distance) which ranges from a distance of zero (highest similarity) to one (lowest similarity). To estimate the contribution of each species to the dissimilarity of the observed groups, a percentage similarity analysis (SIMPER) with a cumulative contribution of 90% was performed (Clarke and Warwick 1994). The analyses were performed using the software Primer-E version 6 (Clarke and Gorley 2006).

## RESULTS

We recorded 2.695 individuals, 12 families and 34 anuran species (Table II): Brachycephalidae (1), Bufonidae (2), Centrolenidae (1), Craugastoridae (1), Cycloramphidae (1), Hylidae (15), Hylodidae (1), Leptodactylidae (8), Microhylidae (1), Odontophrynidae (1), Phyllomedusidae (1), and Ranidae (1). All species recorded are classified by the IUCN Red List of Threatened Species (IUCN 2016) as Least Concern (LC), with the exception of *Crossodactylus cf. schmidti*, which is classified as Near Threatened (NT). The municipality of Ourinhos (ORS) presented the lowest species richness with 20 species, followed by the municipality of Cornélio Procópio (CP) with 26 species and municipality of Londrina (LDN), the highest, with 29 species. Richness in water bodies ranged from 2 – 13 species. The range of species richness, however, varied according to the landscape type: the highest species richness was recorded in the FEL, between 8 – 13 species, followed by 2 – 9 species in the AL, 4 – 8 species in the FL, and 3 – 5 in the UL (see Table II and Figure 3).

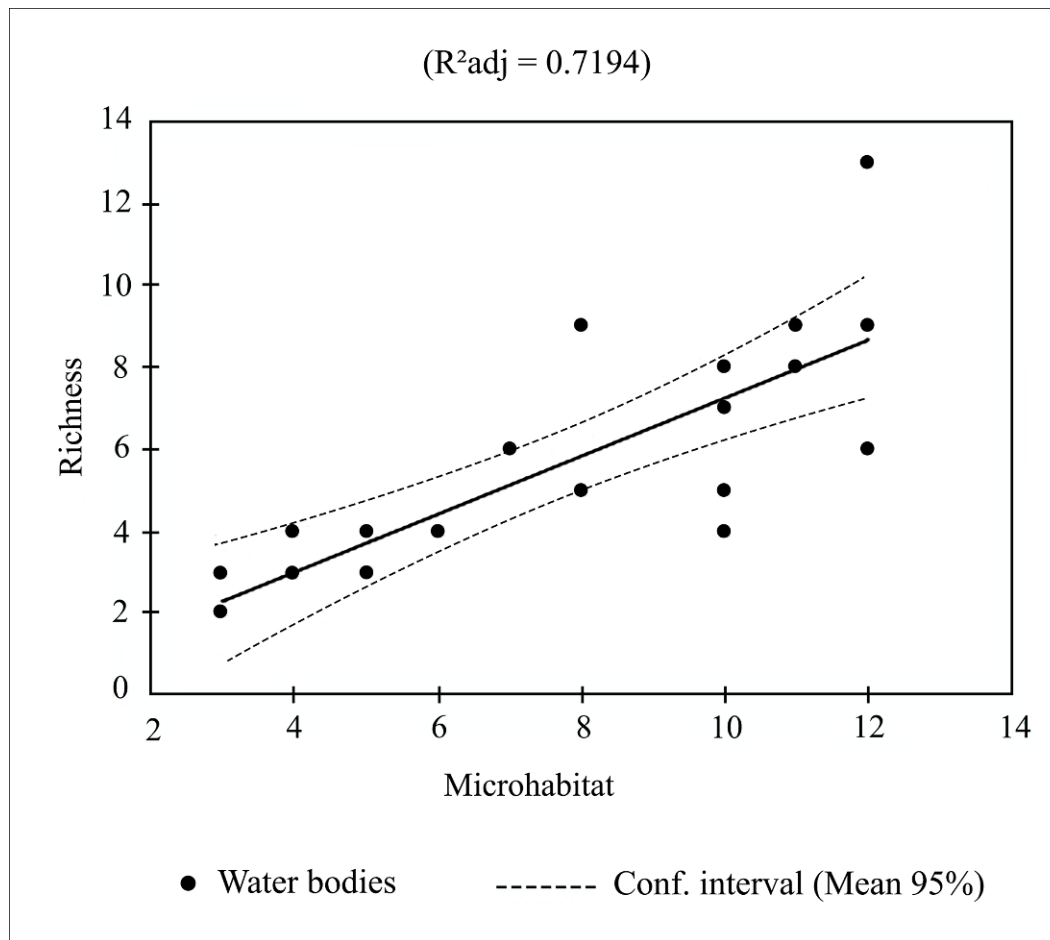
Our fitted model of the linear regression (Figure 2) was normally distributed with  $\log_{10}$  transformation (Shapiro-Wilk  $W$  test  $W = 0.954$ ,  $p = 0.3612$ ). The species richness of all water bodies studied can be partially explained by the variability of microhabitats ( $df = 21$ ,  $F = 57.42$ ,  $p < 0.0001$ ,  $R^2_{adj} = 0.7194$ ).

The composition of the anuran communities grouped partially according to the landscape types (ANOSIM:  $R = 0.451$ ;  $p < 0.001$ ) (see Figure 3). Thus, based on Figure 3 (left side), we can highlight a trend in the cluster arrangement, which is associated with the composition of anurans species in water bodies related to the type of landscape:

**Table II.** Species recorded in the Mesophytic Semideciduous Forest in the Ourinhos (ORS), Cornélio Procópio (CP) and Londrina (LDN) municipalities in 23 water bodies, southern Brazil. \*Species which were found in the study region but were not recorded in water bodies between October 2010 and February 2011.

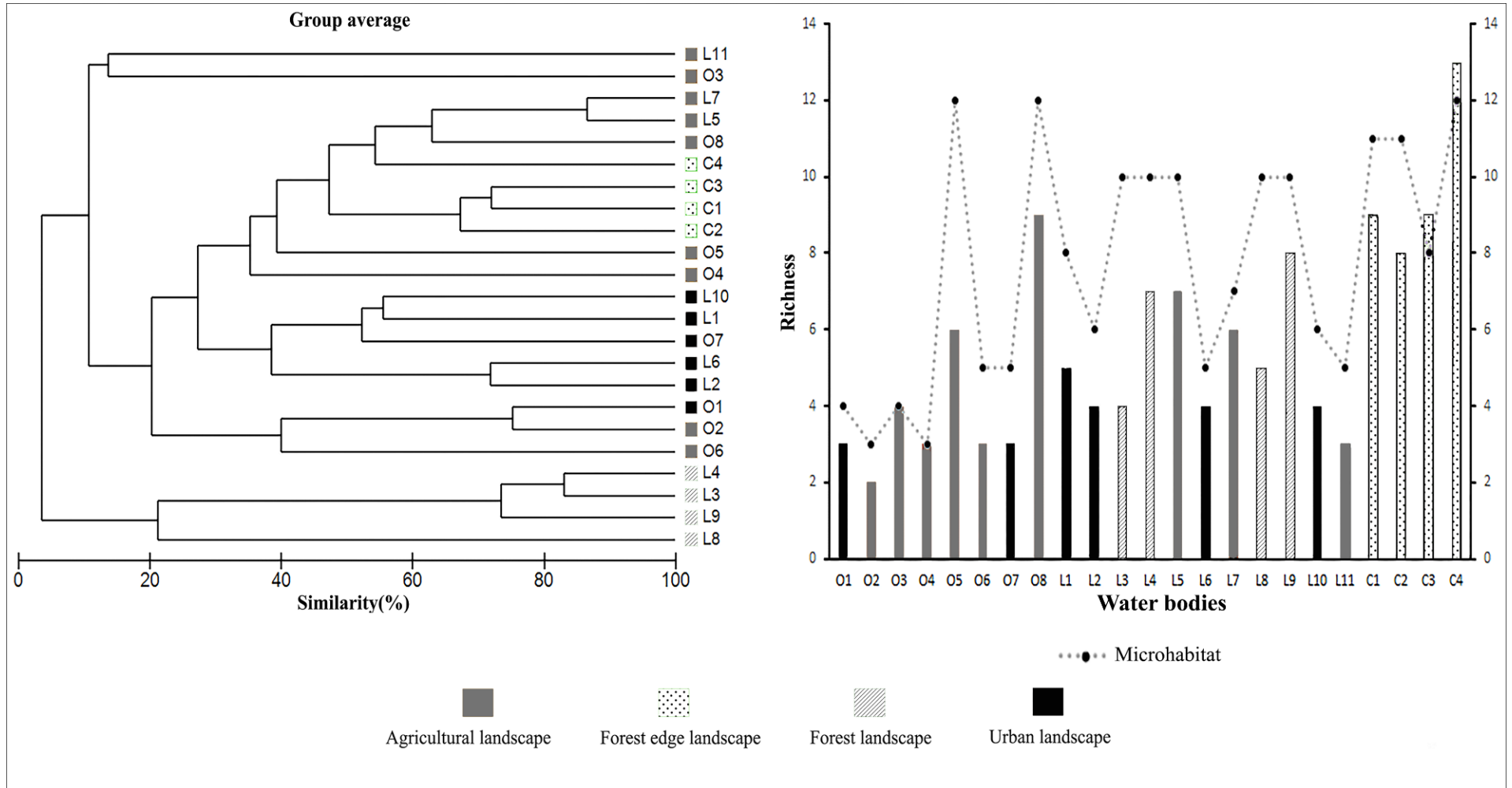
Family/Specie	Sampled sites			Landscape			
	ORS	CP	LDN	UL	AL	FEL	FL
<b>Brachycephalidae</b>							
<i>Ischnocnema cf. henselii</i>		•*	•				•
<b>Bufo</b>							
<i>Rhinella ornata</i> (Spix, 1824)		•	•			•	•
<i>Rhinella schneideri</i> (Werner, 1894)	•	•	•	•	•	•	•
<b>Centrolenidae</b>							
<i>Vitreorana uranoscopa</i> (Müller, 1924)		•*	•				•
<b>Craugastoridae</b>							
<i>Haddadus binotatus</i> (Spix, 1824)			•				•
<b>Cycloramphidae</b>							
<i>Proceratophrys avelinoi</i> Mercadal de Barrio & Barrio, 1993			•			•	•
<b>Hylidae</b>							
<i>Aplastodiscus perviridis</i> Lutz, 1950			•			•	•
<i>Boana albopunctata</i> (Spix, 1824)	•	•	•		•	•	
<i>Boana faber</i> (Wied-Neuwied, 1821)	•	•	•		•	•	
<i>Boana prasina</i> (Burmeister, 1856)		•*	•			•	•
<i>Boana raniceps</i> Cope, 1862	•	•	•		•	•	
<i>Dendropsophus anceps</i> (Lutz, 1929)	•*				•	•	
<i>Dendropsophus minutus</i> (Peters, 1872)	•	•	•	•	•	•	
<i>Dendropsophus nanus</i> (Boulenger, 1889)	•	•	•	•	•	•	
<i>Dendropsophus sanborni</i> (Schmidt, 1944)	•*				•	•	
<i>Ololygon berthae</i> (Barrio, 1972)		•*	•*		•	•	
<i>Ololygon rizibilis</i> (Bokermann, 1964)		•	•*			•	•
<i>Scinax fuscmarginatus</i> (Lutz, 1925)	•				•	•	
<i>Scinax fuscovarius</i> (Lutz, 1925)	•	•	•	•	•	•	
<i>Scinax perereca</i> Pombal, Haddad & Kasahara, 1995		•	•			•	•
<i>Trachycephalus typhonius</i> (Linnaeus, 1758)		•*	•*			•	•
<b>Hylodidae</b>							
<i>Crossodactylus cf. schmidti</i> Gallardo, 1961		•*	•*				•
<b>Leptodactylidae</b>							
<i>Leptodactylus fuscus</i> (Schneider, 1799)	•	•	•	•	•	•	
<i>Leptodactylus latrans</i> (Steffen, 1815)	•	•	•	•	•	•	
<i>Leptodactylus mystacinus</i> (Burmeister, 1861)	•*	•*	•	•	•	•	
<i>Leptodactylus notoaktites</i> Heyer, 1978		•*				•	•
<i>Leptodactylus podicipinus</i> (Cope, 1862)	•	•	•		•	•	
<i>Leptodactylus labyrinthicus</i> (Spix, 1824)	•*	•*	•*		•	•	•
<i>Physalaemus cuvieri</i> Fitzinger, 1826	•	•	•	•	•	•	
<i>Physalaemus nattereri</i> (Steindachner, 1863)	•				•		
<b>Microhylidae</b>							
<i>Elachistocleis bicolor</i> (Guérin-Ménéville, 1838)	•*	•	•	•	•	•	
<b>Odontophrynidae</b>							
<i>Odontophrynus americanus</i> (Duméril & Bibron, 1841)	•*	•	•*		•	•	
<b>Phyllomedusidae</b>							
<i>Phyllomedusa tetraploidea</i> Pombal & Haddad, 1992	•*	•*	•			•	•
<b>Ranidae</b>							
<i>Lithobates catesbeianus</i> (Shaw, 1802)			•*	•	•		
<b>Total richness</b>	20	26	29	10	23	28	14

(group 1) water bodies in agricultural landscape (e.g. L7, L5, L11, and O3); (group 2) water bodies in forest landscape (e.g. L3, L4, and L9); (group 3) water bodies in urban landscape (e.g. L1, L10, and O7); and (group 4) water bodies in forest edge landscape (e.g. C1, C2, and C3).



**Figure 2.** Linear regression between microhabitats and richness of anuran species in the 23 water bodies located in the agricultural landscape, forest landscape, forest edge landscape, and urban landscape.

Species which had occurrence exclusively in the AL and FEL were: *Boana albopunctata*, *Boana faber*, *Boana raniceps*, *Dendropsophus anceps*, *Dendropsophus sanborni*, *Ololygon berthae*, *Scinax fuscomarginatus*, *Leptodactylus podicipinus*, and *Odontophrynus americanus*. Species which had occurrence exclusively in the FL were:

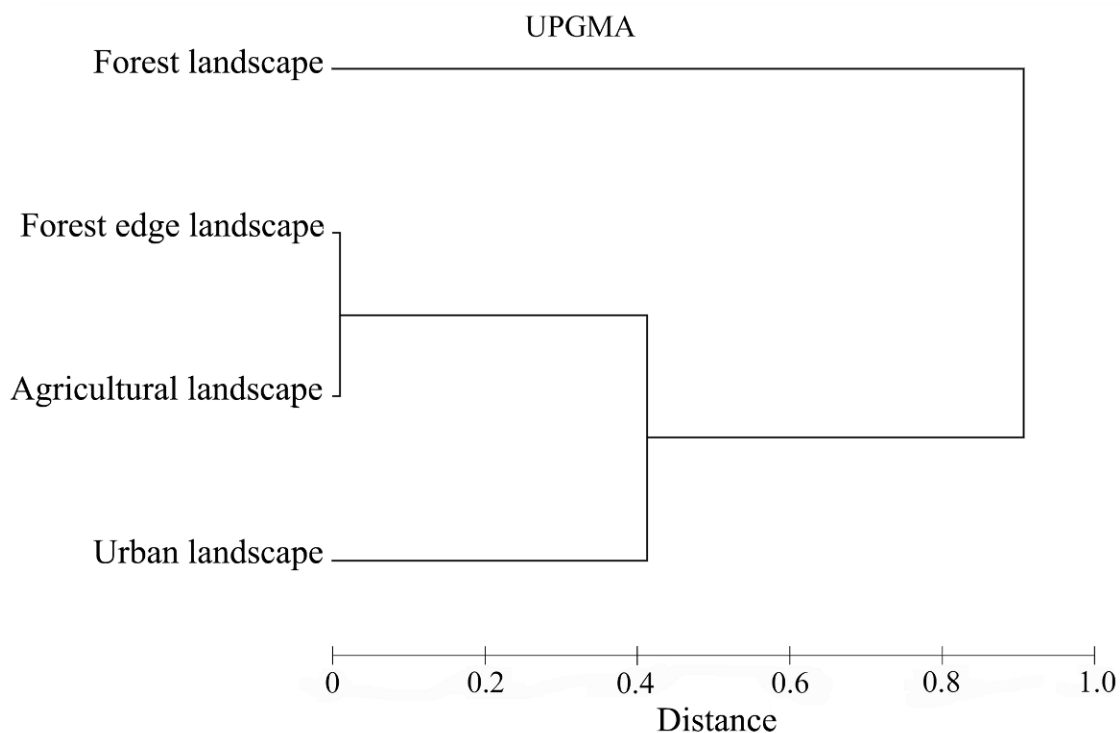


**Figure 3.** On the left side, dendrogram generated using the Bray-Curtis Similarity Index among water bodies sampled across the landscapes types relating to the abundance of anuran species in Mesophytic semideciduous forest areas between October 2010 and February 2011. On the right side, numbers of species (bars)/microhabitats (interrupted line) in each water body. Patterns of the bars represent the landscape types: agricultural landscape, forest edge landscape, forest landscape, and urban landscape. Codes of water bodies as presented in Table 1.

*Crossodactylus* cf. *schmidti*, *Haddadus binotatus*, *Ischnocnema* cf. *henselii*, and *Vitreorana uranoscopa*. Species which, had occurrence exclusively in the FEL and FL were:

*Aplastodiscus perviridis*, *Boana prasina*, *Leptodactylus notoaktites*, *Oloolygon rizibilis*, *Phyllomedusa tetraploidea*, *Proceratophrys avelinoi*, *Rhinella ornata*, *Scinax perereca*, and *Trachycephalus typhoni*. The only species which occurred in AL, FEL, and FL but did not found in UL was *Leptodactylus labyrinthicus*. The species of anurans recorded in UL occurring in three or more landscapes, were: *Rhinella schneideri*, *Dendropsophus minutus*, *Dendropsophus nanus*, *Scinax fuscovarius*, *Physalaemus cuvieri*, *Leptodactylus fuscus*, *Leptodactylus latrans*, *Leptodactylus mystacinus*, and *Elachistocleis bicolor* with the exception of *Lithobates catesbeianus*, which was the only species exclusive to AL and UL.

The cluster generated by the pairwise test from ANOSIM (Figure 4) demonstrates



**Figure 4.** Hierarchical cluster analysis (UPGMA, Euclidean distance) resulting from the pairwise test of ANOSIM comparing the four landscapes types: agricultural landscape, forest landscape, forest edge landscape and urban landscape.

that the FL was the most distinctive landscape in species composition, FEL and AL were the most similar landscapes and UL was intermediary between these last ones. The most distinct landscapes were UL × FL. The pairwise test from SIMPER for all groups of landscape types compared with each other had an average dissimilarity of approximately 85% ( $85.3 \pm 11.9$ ). Twenty species contributed to this dissimilarity in the composition of the species among the four landscapes where water bodies were located (Appendix II).

## DISCUSSION

We found that (1) the highest species richness was in the FEL and not in the FL, (2) the number of microhabitats is positively correlated with species richness, and (3) the species composition is strongly affected by the landscape type.

The anurans richness recorded in this study represented 30% of the known species for the MSF ecosystem (Garcia et al. 2007). In the region of Londrina 27 species were recorded (Bernarde and Anjos 1999; Machado et al. 1999; Machado and Bernarde 2002), all of which were also recorded in the present study. We included two new species documented for the region, *Ololygon berthae* and *Ololygon rizibilis* (see distribution map in Nascimento et al. 2016 and Figueiredo et al. 2014 respectively). Compared with other studies performed in the MSF, our study demonstrated higher local species richness than others (see Table IV), except municipalities of Gália and Avilândia in São Paulo (34 species; Brassaloti et al. 2010) that had the same species richness.

Our results corroborate studies in which environmental heterogeneity partly explains variations in the richness and composition of the anuran communities (Parris 2004; Vasconcelos and Rossa-Feres 2005; Vasconcelos and Rossa-Feres 2008; Vasconcelos et al. 2009; Oda et al. 2016; Oda et al. 2017). In fact, the number of

microhabitats used by males as vocalization sites tends to influence the anurans species richness and composition (Afonso and Eterovick 2007; Vasconcelos and Rossa-Feres 2008; Pirani et al. 2013).

<b>Municipality</b>	<b>State</b>	<b>Richness</b>	<b>Reference</b>
Avilândia and Gália	SP	34	Brassaloti et al. 2010
Araçatuba	SP	23	Oda et al. 2017
Guararapes	SP	26	Bernarde and Kokubum 1999
Icém	SP	12	Silva and Rossa-Feres 2007
Nova Itapirema	SP	27	Vasconcelos and Rossa-Feres 2005
Pontal do Paranapanema	SP	21	Vasconcelos et al. 2009
Rio Claro	SP	24	Zina et al. 2007
Rio Claro	SP	21	Toledo and Haddad 2003
Santa Fé do Sul	SP	20	Santos et al. 2007
Teodoro Sampaio	SP	28	Santos et al. 2009
Diamante do Norte	PR	19	Oda et al. 2016
Maringá	PR	21	Afonso et al. 2014
Porto Rico	PR	18	Afonso et al. 2013
São Pedro do Ivaí	PR	14	Santos and Conte 2016
Fênix	PR	15	Santos and Conte 2016
Três Barras do Paraná	PR	23	Bernarde and Machado 2001/2000
Tuneiras do Oeste and Cianorte	PR	22	Afonso and Gomes 2013
Londrina	PR	24	Bernarde and Anjos 1999
Londrina	PR	24	Machado et al. 1999
Londrina	PR	27	Machado and Bernarde 2000
Ourinhos/Londrina and Cornélio Procópio	SP/PR	34	Present study
Chavantes/Ribeirão Claro and Jacarezinho	SP/PR	25	Nazaretti and Conte 2015

**Table IV.** List of municipalities that had the anurofauna studied in the Mesophytic semideciduous forest in the states of São Paulo (SP) and Paraná (PR) considered in this study.

Water bodies with higher microhabitat numbers encompass habitat heterogeneity: water bodies with a higher number of microhabitats available (e.g. presence of arboreal vegetation, shrub vegetation, and bulrushes) provide calling sites for arboreal species (e.g. *Boana* spp., *Phyllomedusa* spp.), as well as shelter for larvae and adults from predators, which facilitate the increase of species richness (Vasconcelos et al. 2009; Oda et al. 2016).

Otherwise, water bodies with lower microhabitats number (e.g. only soil and grasses) in the surroundings reduces the species richness since they offer calling sites only for terrestrial species (e.g. *Leptodactylus* spp.) and those species which reproduce in herbaceous vegetation (e.g. *Dendropsophus nanus*) (Santos and Rossa-Feres 2007; Vasconcelos and Rossa-Feres 2008; Vasconcelos et al. 2009; Silva et al. 2011b). Indeed, the decrease in microhabitat availability limits the possibility of spatial partitioning (Cardoso et al. 1989).

We did not find the highest richness of anurans in the FL but in the FEL probably related to the reproductive mode of species. The specific ecological characteristics related to their reproductive modes (open or forested areas) provide the establishment of species (Haddad and Prado 2005; Cruz-Elizalde et al. 2016; Oda et al. 2016). Amphibians anurans associated with forest use water bodies connected to the forest edge for their reproduction (Silva et al. 2011b). However, species related to open areas also access the forest edge for breeding (Ferreira et al. 2016; Ferrante et al. 2017). Thus, the edge of forest remnants in the MSF maintain higher species richness, since those associated to the forest and those associated to the open areas use the FEL to breeding (Becker et al. 2007; Ferreira et al. 2016; Ferrante et al. 2017; present study).

In fact, MSF exhibits just a few species restricted to the forest interior (Bernarde and Anjos 1999; Santos et al. 2009; Garey and Silva 2010; Santos and Conte 2016; Lourenço-de-Moraes et al. 2018). Most of the species exclusive to forest interior have specialized reproductive mode, such as leaf-litter breeders with direct development (mode 23: Haddad and Prado 2005) (e.g. *Ischnocnema* cf. *henselii* and *Haddadus binotatus*), and others, dependent on streams inside the forest for breeding (e.g. *Vitreorana uranoscopa*; *Crossodactylus* cf. *schmidtii*) (mode 23 and mode 3 respectively: Haddad and Prado 2005).

Probably, because of the specialized breeding of anurans, the FL species composition was the most distinct among the studied landscapes (see Figure 3 and 4). Moreover, the restrict species of FL (Bernarde and Anjos 1999; Machado and Bernarde 2002) could be considered the most sensitive to forest fragmentation in the present study.

Water bodies that were constructed for use in crop irrigation or fish production (Hartel and Wehrden 2013) allow some anurans species to use these artificial habitats for their reproduction (Babitt and Tanner 2000; Vasconcelos and Rossa-Feres 2005; Colombo et al. 2008). Most of the recent studies using anurans as a model in the agricultural landscapes suggest that the species richness is strongly associated with forest cover (Ferreira et al. 2016; Collins and Fahrig 2017; Ferrante et al. 2017; Gangenova et al. 2018) and negatively influenced by the mean crop field size (Collins and Fahrig 2017). In this way, farmland with smaller mean field sizes should benefit all tolerant anurans species, due to available food sources and for providing more effortless movement between the refuge habitats and breeding environments (Collins and Fahrig 2017). Furthermore, in the agricultural landscape, water bodies configuration related to the microhabitat availability changes according to the matrix type where it is located, which has a bearing on species richness and composition (Ferrante et al. 2017).

Among the studied water bodies, those in the agricultural landscapes of Ourinhos are located in the ecotone region between the Cerrado and the MSF and this explains the recording of typical Cerrado species, such as *Physalaemus nattereri* (Aquino et al. 2004; Santos et al. 2009). These species are opportunistic and are benefited by some anthropic activities, which could explain their extended geographical distributions (Haddad and Sazima 1992).

We highlight the record of the invasive species *Lithobates catesbeianus* native to North America which has been introduced to the region of Londrina for commercial purposes (Machado and Bernarde 2002). In occasional visits to areas of Londrina, we recorded a water body with more than 25 individuals of *L. catesbeianus* breeding. This is a concerning situation because this species is a generalist predator (Toledo et al. 2007) which compete for prey (Leivas et al. 2012), transmit pathogens (Schloegel et al. 2010), and have several negative impacts on native species of anurans (Both et al. 2011).

As several studies have shown, our results suggest that some species of amphibians tolerate environments altered by anthropic actions, such as urban areas (e.g. *Dendropsophus nanus*, *Elachistocleis bicolor*, *Leptodactylus latrans*, *Physalaemus cuvieri*, and *Scinax fuscovarius*), while others mentioned above are dependent on microhabitats and/or environmental conditions only found in forested areas (Moraes et al. 2007; Haddad et al. 2013) and some are sensitive to the edge effect (Lourenço-de-Moraes et al. 2014; Ferreira et al. 2016). Anurans are negatively influenced by the use of habitats in urban areas as a result of a variety of factors, such as pollution (air, water, and noise), fragmentation, loss and isolation of habitat (see review in Hammer and McDonnell 2008), artificial lighting (Perry et al. 2008) and roads and traffic (Rytwinski and Fahrig 2015).

The availability of microenvironments to anurans in these urban areas is also another factor which negatively affects species richness (Gagné and Fahrig 2007). In this way, in the UL, we commonly found water bodies with the lowest vegetation structure available and could record only species with reproductive mode 1 (eggs and exotrophic tadpoles in still water) and those modes resistant to desiccation, such as modes 11 and 30 (eggs embedded in foam nest; sensu Haddad and Prado 2005). However, forest remnants near or inserted in urban areas, even with a variety of microenvironments available, are

negatively affected as to richness and composition of amphibian species (Lourenço-de-Moraes et al. 2018).

The physiological dependence of anurans of both water and terrestrial habitats have highlighted their importance as good bioindicators (Blaustein and Wake 1990; Wells 2007; Toledo et al. 2009). However, we found only four species which could be considered as a true indicator, in the studied case indicator of the FL, which are: *Crossodactylus* cf. *schmidti*, *Haddadus binotatus*, *Ischnocnema* cf. *henselii*, and *Vitreorana uranoscopa*. Others nine species could be regarded as moderate indicators of FL since they can use both FL and FEL as breeding habitat: *Aplastodiscus perviridis*, *Boana prasina*, *Leptodactylus notoaktites*, *Ololygon rizibilis*, *Phyllomedusa tetraploidea*, *Proceratophrys avelinoi*, *Rhinella ornata*, *Scinax perereca*, and *Trachycephalus typhonius*. So, the use of anurans as indicators of the forest should be restricted to a relatively small proportion of the recorded species (38%). The other species are mainly tolerant to different levels of environmental disturbances.

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**Appendix I.** Voucher specimens collected during the study in the Mesophytic Semideciduous Forest, southern Brazil.

*Aplastodiscus perviridis* – MZUEL1426; *Boana albopunctata* – MZUEL1428; *Boana faber* – MZUEL 1419; *Boana prasina* – MZUEL1793; *Boana raniceps* – MZUEL1793; *Crossodactylus* cf. *schmidti* – MZUEL1801; *Dendropsophus minutus* – MZUEL; *Dendropsophus nanus* – MZUEL 1425; *Elachistocleis bicolor* – MZUEL1502; *Haddadus binotatus* – MZUEL1800; *Ischnocnema* cf. *henselii* – MZUEL1583; *Leptodactylus fuscus* – MZUEL1456; *Leptodactylus labyrinthicus* – MZUEL 1802; *Leptodactylus latrans* – MZUEL1450; *Leptodactylus mystacinus* – MZUEL 1691; *Leptodactylus notoaktites* – MZUEL1575; *Leptodactylus podicipinus* – MZUEL1511; *Odontophrynus americanus* – MZUEL1544; *Ololygon berthae* – MZUEL1552; *Ololygon rizibilis* – MZUEL1523; *Phyllomedusa tetraploidea* – MZUEL1423; *Physalaemus cuvieri* – MZUEL1461; *Physalaemus nattereri* – MZUEL1442; *Proceratophrys avelinoi* – MZUEL1421; *Rhinella ornata* – MZUEL1790; *Rhinella schneideri* – MZUEL1688; *Scinax fuscovarius* – MZUEL1446; *Scinax perereca* – MZUEL1565; *Trachycephalus typhonius* – MZUEL1385; *Vitreorana uranoscopa* – MZUEL1420.

**Appendix II.** Anuran species contribution to the average dissimilarity between the four landscapes sampled: **AL:** agricultural landscape; **FL:** forest landscape; **FEL:** forest edge landscape; **UL:** urban landscape. **Av. Diss:** Average Dissimilarity; **Contrib. %:** Contribution (%); **Cum. %** Cumulative Percentage.

	Species	Av.Diss	Contrib%	Cum.%		Species	Av.Diss	Contrib%	Cum.%
UL x AL	<i>Dendropsophus nanus</i>	12,07	15,89	15,89	UL x FEL	<i>Dendropsophus nanus</i>	14,8	19,02	19,02
	<i>Boana albopunctata</i>	9,75	12,84	28,73		<i>Leptodactylus podicipinus</i>	13,29	17,08	36,1
	<i>Leptodactylus fuscus</i>	8,88	11,7	40,43		<i>Boana faber</i>	8,52	10,95	47,05
	<i>Physalaemus cuvieri</i>	8,09	10,65	51,08		<i>Boana albopunctata</i>	7,27	9,34	56,39
	<i>Dendropsophus minutus</i>	6,83	9	60,08		<i>Scinax perereca</i>	6,23	8	64,39
	<i>Boana raniceps</i>	4,79	6,31	66,39		<i>Physalaemus cuvieri</i>	4,66	5,99	70,38
	<i>Leptodactylus mystacinus</i>	4,34	5,72	72,11		<i>Dendropsophus minutus</i>	3,82	4,91	75,29
	<i>Boana faber</i>	3,65	4,81	76,92		<i>Leptodactylus fuscus</i>	2,83	3,64	78,93
	<i>Scinax fuscovarius</i>	3,59	4,72	81,64		<i>Scinax fuscovarius</i>	2,74	3,53	82,45
	<i>Rhinella schneideri</i>	3,16	4,17	85,81		<i>Oloolygon rizibilis</i>	2,53	3,25	85,71
	<i>Elachistocleis bicolor</i>	3	3,95	89,76		<i>Elachistocleis bicolor</i>	2,43	3,12	88,82
	<i>Leptodactylus latrans</i>	2,76	3,63	93,39		<i>Boana raniceps</i>	2,34	3	91,83
UL x FL	<i>Dendropsophus nanus</i>	15,32	15,75	15,75	AL x FEL	<i>Dendropsophus nanus</i>	15,24	21,64	21,64
	<i>Proceratophrys avelinoi</i>	10,26	10,55	26,3		<i>Leptodactylus podicipinus</i>	11,96	16,99	38,63
	<i>Leptodactylus fuscus</i>	8,91	9,16	35,46		<i>Boana faber</i>	6,11	8,68	47,3
	<i>Vitreorana uranoscopa</i>	7,19	7,4	42,86		<i>Scinax perereca</i>	5,67	8,05	55,35
	<i>Boana prasina</i>	6,84	7,04	49,9		<i>Boana albopunctata</i>	5,45	7,73	63,08
	<i>Physalaemus cuvieri</i>	6,67	6,86	56,76		<i>Dendropsophus minutus</i>	4,09	5,8	68,89
	<i>Aplastodiscus perviridis</i>	5,76	5,92	62,68		<i>Physalaemus cuvieri</i>	3,84	5,45	74,34
	<i>Ischnocnema cf. henselii</i>	5,48	5,64	68,32		<i>Boana raniceps</i>	3,41	4,85	79,19
	<i>Haddadus binotatus</i>	4,76	4,9	73,21		<i>Leptodactylus fuscus</i>	2,72	3,86	83,05
	<i>Scinax perereca</i>	4,76	4,89	78,1		<i>Scinax fuscovarius</i>	2,48	3,53	86,57
	<i>Leptodactylus mystacinus</i>	4,67	4,8	82,91		<i>Oloolygon rizibilis</i>	2,29	3,26	89,83
	<i>Rhinella schneideri</i>	3,05	3,13	86,04		<i>Elachistocleis bicolor</i>	2,27	3,22	93,05
AL x FL	<i>Boana albopunctata</i>	9,31	9,55	9,55	FL x FEL	<i>Dendropsophus nanus</i>	20,8	22,4	22,4
	<i>Proceratophrys avelinoi</i>	8,95	9,18	18,73		<i>Leptodactylus podicipinus</i>	12,91	13,9	36,3
	<i>Dendropsophus nanus</i>	8,34	8,55	27,28		<i>Boana faber</i>	7,17	7,72	44,02
	<i>Physalaemus cuvieri</i>	6,56	6,73	34,01		<i>Boana albopunctata</i>	7,05	7,59	51,61
	<i>Dendropsophus minutus</i>	6,51	6,68	40,68		<i>Physalaemus cuvieri</i>	6,4	6,89	58,5
	<i>Vitreorana uranoscopa</i>	6,28	6,44	47,12		<i>Scinax perereca</i>	4,98	5,36	63,86
	<i>Boana prasina</i>	6	6,16	53,27		<i>Proceratophrys avelinoi</i>	4,54	4,89	68,75
	<i>Leptodactylus fuscus</i>	5,11	5,24	58,51		<i>Boana prasina</i>	2,9	3,13	71,87
	<i>Aplastodiscus perviridis</i>	5,02	5,15	63,66		<i>Vitreorana uranoscopa</i>	2,89	3,11	74,98
	<i>Ischnocnema cf. henselii</i>	4,78	4,9	68,56		<i>Dendropsophus minutus</i>	2,75	2,96	77,94
	<i>Boana raniceps</i>	4,54	4,66	73,22		<i>Aplastodiscus perviridis</i>	2,55	2,75	80,69
	<i>Haddadus binotatus</i>	4,16	4,27	77,49		<i>Oloolygon rizibilis</i>	2,45	2,64	83,33
	<i>Scinax perereca</i>	4,15	4,25	81,74		<i>Ischnocnema cf. henselii</i>	2,35	2,53	85,86
	<i>Boana faber</i>	3,9	4	85,74		<i>Boana raniceps</i>	2,28	2,45	88,31

### CAPÍTULO III

## **SOLAR RADIATION IS AN IMPORTANT FACTOR DRIVING AMPHIBIAN SPECIES DIVERSITY AND COMPOSITION AT PHYTOGEOGRAPHICAL SCALE IN SOUTHERN BRAZIL**

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**Abstract**

The species distributions throughout the terrestrial and aquatic ecosystems have been a challenge in herpetological studies, but also an opportunity for ecologists stimulate studies on ecology, biogeography, and macroecology. Here we investigate the richness, species composition, functional diversity, and phylogenetic diversity of amphibians in the state of Paraná which encompass five phytogeographic units. A data set was built based on available information from IUCN spatial data, published literature and unpublished fieldwork to assess species composition of amphibians in the state of Paraná. We used PCoA to investigate the composition of species across the phytogeographic units. We performed generalized linear models (GLMs) to investigate the relative effects of environmental variables on variation of species functional and phylogenetic diversity and tested the power of each one with PERMANOVA. The highest values of richness, functional diversity and phylogenetic diversity were found in Serra do Mar coastal forest, Grasslands, and Atlantic Moist forest and the lowest in the Alto Parana Atlantic forest and Cerrado. Solar radiation, the percentage of forest cover, altitude, annual precipitation, and the vertical distance to the nearest drainage were those environmental factors that had the highest contribution to the fitted model for functional and phylogenetic diversity. Among these environmental variables, solar radiation was the one that most influenced negatively the variations of functional and phylogenetic diversity.

## 1- INTRODUCTION

Species distribution has been a challenge for ecologists such as describe which areas are of biogeographical interest and their relationships with the assemblages that inhabit these regions. However, because many databases of species distributions are now available online [e.g. IUCN spatial data; (EOL) Encyclopedia of Life's Open Data] and thus, integrated with the explanation of statistical methods, studies on this matter have been recently stimulated and researchers are using these databases as a tool to answer specific questions within ecology and biogeography (e.g. Vasconcelos et al. 2014, 2018; Campos et al. 2017; Lourenço-de-Moraes et al. 2019). Hence, the majority of biogeographic regions tend to have an exclusive composition and richness of species, mainly resulted of multiple factors such as physical geography, environmental variables, and biotic relationships within assemblages (Ricklefs 1987; White & Hurlbert 2010; Lessard et al. 2016).

Several measures of biodiversity rising in the last two decades, given an increase in interest within ecology for this approach (Cianciaruso et al., 2009). Alternatively, the use of richness as biodiversity measures has been replaced by the combined interest on variations of functional diversity (FD) and phylogenetic diversity (PD), which include information on the functional role of the species in the ecosystems and their phylogenetic relationships (Ricotta et al., 2005; Petchey & Gaston 2006). The underlying processes that rule the species richness are therefore integrated with the ecosystem functioning and these alternative diversity measures can respond better questions that is insufficient by the traditional measures of diversity (Cianciaruso et al. 2009; Wiens & Graham 2005).

To explain functional diversity, many ecologists opted for use trait-based approach, leading to an increase of studies based only on organism level to broad-scale

patterns within ecosystems (e.g. Safi et al. 2011; Campos et al. 2017; Rapacciuolo et al. 2018; Loureço-de-Moraes et al. 2018). In any ecosystem, a trait is defined as a morphological, physiological or phenological that can be measurable at the individual level (Violle et al. 2007). All the information external to individual level can be environmental factors or other levels of organization such as population, community, and ecosystem (Violle et al. 2007).

The state of Paraná, southern Brazil, has five phytogeographic units, of which four are inserted within (AF) and one is just only a few patches of the Cerrado domain (Roderjan et al. 2002). Such variety of phytogeographic regions is probably due to the location of Paraná state, in a transition zone between tropical and subtropical areas. This climatic transition zone influences particularly precipitation and temperature, which varied from the north to the south of the state (Caviglione et al. 2000).

Environmental gradients including altitude, precipitation, and temperature are important drivers of amphibians richness and composition in the ecosystems (Duellman & Trueb 1994) including Atlantic Forest (Vasconcelos et al. 2010). In fact, it is known that the abiotic factors have influence in the variation of functional diversity and functional composition of species in an ecosystem (Laureto et al. 2015). However, the influence of solar radiation, drainage density, vertical distance to the nearest drainage, percent tree cover on the variation of FD and PD for amphibians was not investigated until now (see Leão-Pires et al. 2018; Lescano et al. 2018).

In this study, the richness and composition of anuran species were investigated among the five phytogeographical units of the state of Paraná. We expected to find the highest richness in the Serra do Mar Coastal forests due to higher regularity of rainfall during the year and the higher spectrum of altitude (2000m a.s.l.). The lowest richness is expected to find in the Alto Parana Atlantic forests due to an annual dry season,

which not favor amphibians, and lower spectrum of altitude (200-500m a.s.l.). Because of the large contact zone, we expected to find a higher similarity between APAF, AMF, and CE. We expected the lowest similarity between APAF and SMCF because are separated geographically by a mountain chain, called "Serra do Mar". We also investigated the variation of FD and PD among the five phytogeographic units within the state of Paraná. We predicted that the measures of FD and PD will follow a similar pattern of richness, with the highest values in the wets phytogeographic units (SMCF and GRASS) instead of the inland dry units (APAF and CE). Finally, we evaluated which environmental factors are more closely associated with FD and PD in each phytogeographical units: altitude, annual precipitation, annual temperature, slope, aspect, distance to the nearest vertical drainage, drainage density, and vapor pressure.

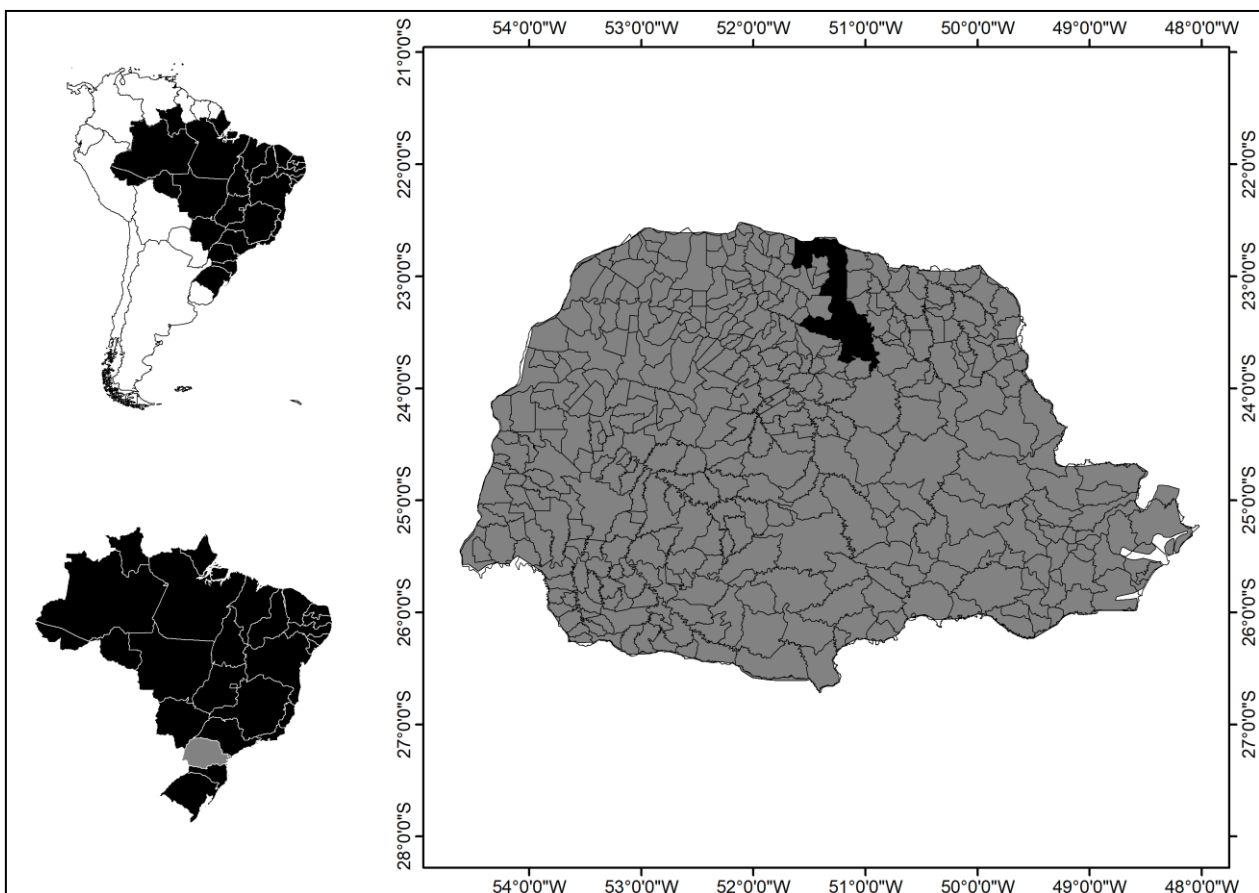
## **2- MATERIALS AND METHODS**

### **2.1.1 *Study area***

We studied the distribution of amphibians in the state of Paraná, Brazil (Figure 1). The state of Paraná is located in southern Brazil and has an extension of 468 km north-south and 647km east-west. Due to its great rivers and orthographic lineaments, the state of Paraná has clear boundaries and, within this region, natural zones of a landscape. The division of these zones is based on the position of escarpments, valleys of water-dividing rivers, as well as on the exclusive physiographic characteristics of the landscape within the natural limits (Maack, 2012). Thus, the most evident feature in the surface aspect of the state of Paraná is the division into two great natural regions: the coastline and the inland plains.

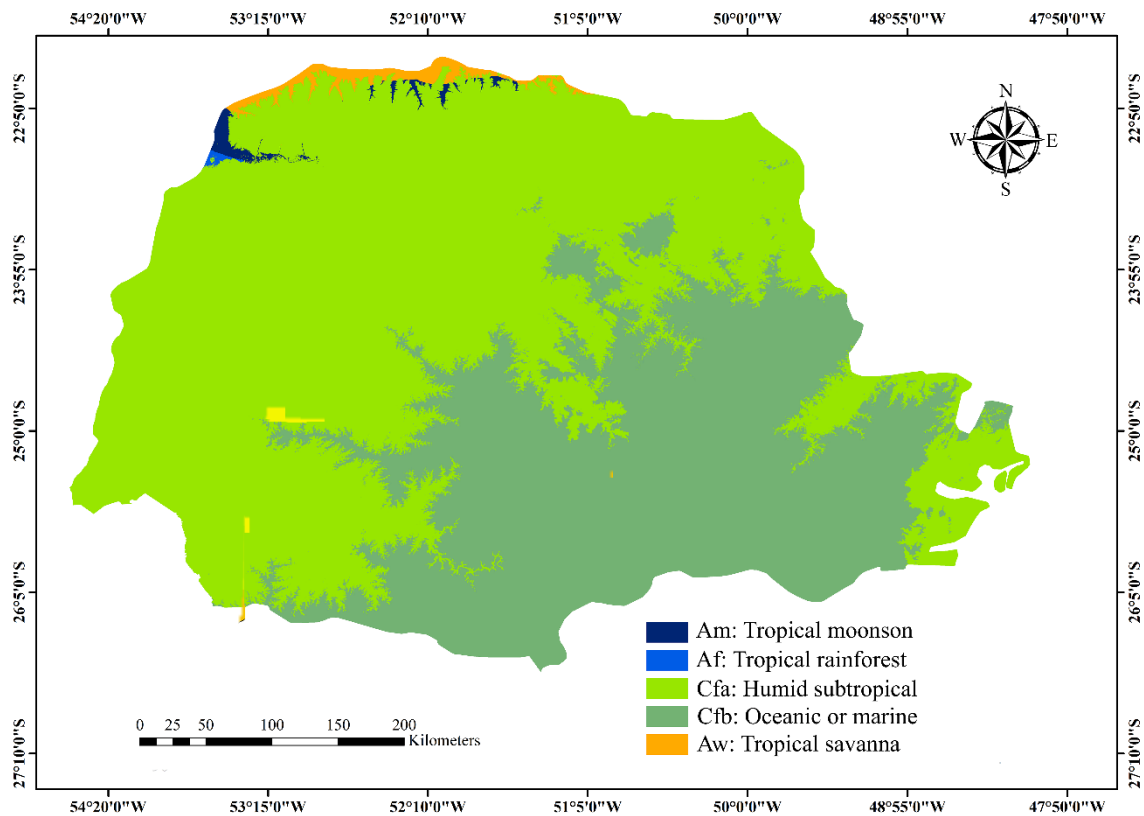
The coast of the state of Paraná is very narrow and it works with a zone of transition between the ocean, and therefore, it is considered an inland State. The coastal

and the plateaus of the inland regions are separated by a mountain chain that follows the great rift escarpment of the crystalline complex. This escarpment at certain areas forms isolated mountains because it is split by transverse rifts into raised and lowered blocks. This mountain chain favors the diversification of plants and animals through the two zones due to climatic transition and physical geography (Maack 2012).



**Figure 1.** Map of the studied area in grey. Filled in black the municipalities where the fieldwork was performed, in the state of Paraná, southern Brazil.

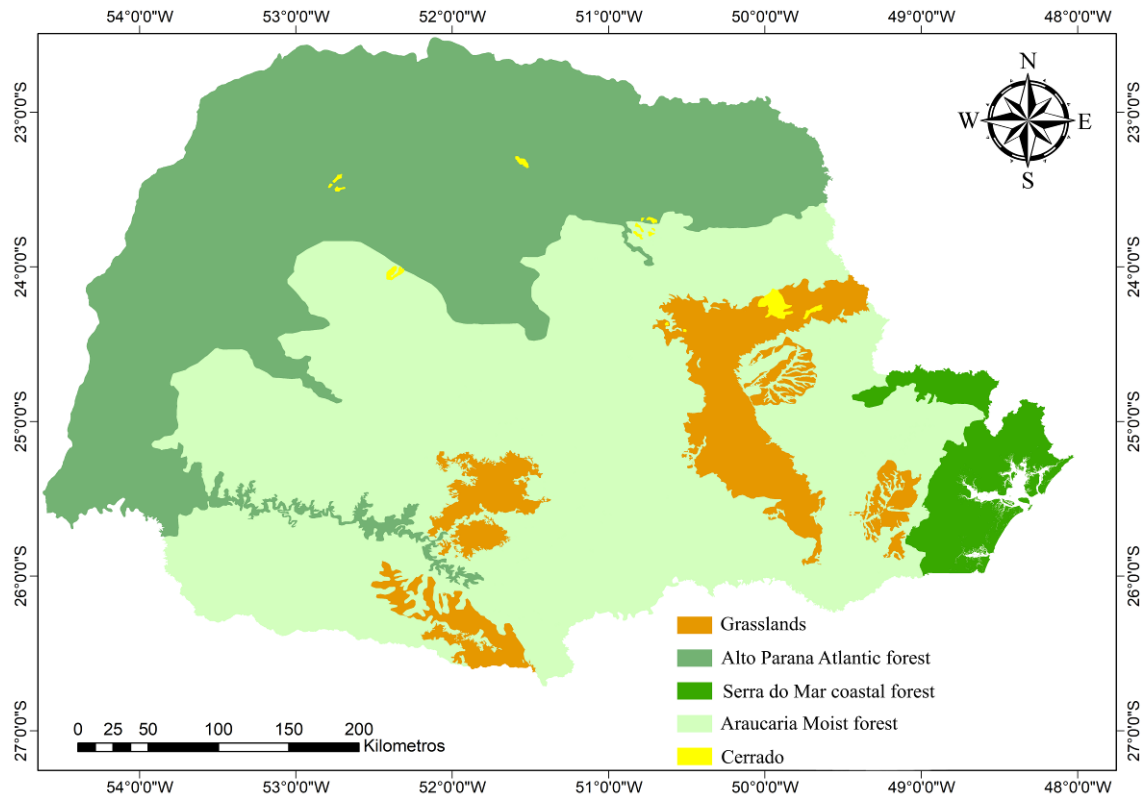
### 2.1.2 Climate



**Figure 2.** Climatic map of the state of Paraná, southern Brazil.

In the state of Paraná, the climate classification modified from Köppen (1936) for Alvarez et al. (2013) show that the predominant climate is of type C (Humid subtropical zone) and some small parts of type A (Tropical zone) (Table 2). As such, the climate of Paraná can be divided as follows: Af (without dry season) that occurs in a small northwest portion of the state; Am (monsoon) climate with small parts of the northwest and north of the state; Aw (with dry winter) a small line in the northwest of the state; Cfb (with temperate summer) is scattered over the higher elevation lands of plateaus and mountain ranges from small parts of the coast to the interior of the state; and Cfa (with hot summer) spread throughout the north, northwest, west, and southwest of the entire state of Paraná.

### 2.1.3 Phytogeographic units



**Figure 3.** Map of the five phytogeographic units of the state of Paraná.

The state of Paraná can be divided into five phytogeographic units (Roderjan et al., 2002) (Figure 3). (1) In the eastern part of the state lies the natural geographical barrier of the Serra do Mar with a maximum elevation of 1887m a.s.l. A mountain chain, the "Serra do Mar", isolates the phytogeographic unit "Serra do Mar Coastal Forests" from the others, characterized by being influenced by hot and wet air masses coming from the Atlantic Ocean, causing rains well-distributed throughout the year. Still, in the western part of the Paraná, in the small portions of coastal was located the Southern Atlantic Mangroves (Roderjan et al., 2002). (2) To the west of Serra do Mar, with a maximum elevation of 1200m a.s.l, is the Araucaria Moist Forests. This vegetation type does not suffer direct ocean influence but maintains well-distributed

rains throughout the year and is strongly influenced by cold air masses leading to low temperatures and occurrence of frost in winter (Roderjan et al., 2002). (3) In the northern and western parts of Paraná, with a maximum elevation of 800 m a.s.l., is located the Alto Parana Atlantic Forests characterized by distinct seasons, one dry and cold and another hot and wet throughout the year (Veloso et al., 1991). (4) Grasslands is distributed throughout the southern, southeastern and northeastern parts of Paraná and characterized by well-distributed rainfall throughout the year with low winter temperatures (Roderjan et al., 2002; see Figure 2). (5) The Savanna or Cerrado characterized by the semiarid climatic condition occupy small relicts distributed in the northern, northwest and northeast part of the state.

## ***2.2 Composition of database:***

We built the data set used in the present study based on the distributions of amphibian's species available in the state of Paraná (Figure 1) which encompass three sets of data: (1) published studies, (2) data compiled from the International Union for Conservation of Nature (IUCN) database, and (3) fieldwork.

### ***2.2.1 Published studies***

All data acquired based on previously published studies (see Appendix II) were obtained by searching in the database of “Scientific Electronic Library Online” (SciELO; [www.scielo.org](http://www.scielo.org)) and “Google Scholar” ([www.scholar.google.com](http://www.scholar.google.com)) on June 2017 with the following search terms: “Amphibians of the state of Paraná”, “anurans of the state of Paraná”, “anurans Paraná”, “amphibians Paraná”, “anuran richness state Paraná”, “amphibian richness state Paraná”, “diversity of anurans Paraná”, and “diversity of amphibians Paraná”.

### **2.2.2 International Union for Conservation of Nature (IUCN) spatial data**

Species range maps of amphibians were obtained from the International Union for Conservation of Nature (IUCN) database to determine the full recorded range of species distributions. We discarded 34 species records from the analysis due to the occurrence of narrower distribution or that need a further taxonomic revision in the state of Paraná. However, were included in species list overall (Appendix II) to emphasize some narrowly-ranged species presented for some species that need further investigation about their distributions.

### **2.2.3 Fieldwork**

As a way to complement the study were performed fieldwork in which were sampled water bodies in northern of the Paraná state. The data were implemented to the final database of the state of Paraná species distribution for *posteriori* calculation of species functional and phylogenetic diversity.

The methodology of Survey at breeding sites was used to sample amphibians in 115 water bodies from October-February in the years of 2010-2017(see Scott Jr. and Woodward 1994). Each water body was visited once, from 18h to 00h. Both open and forested areas had covered environments, where all visual and acoustic contact of the species was recorded. Each of aquatic environment was sampled on a single day, during a six-hour period from 18h to 00h and with the same sampling effort (hours/water body), totaling 690 field hours. Amphibians form congregations in the breeding season at specific sites, which encompass nearly all aquatic environments (Crump 1974). Species breeding cycle, which occurs during the hottest and wettest months, was the year period selected for sampling (Sott Jr. and Woodward 1994). Samplings in this season increase species detection due to abiotic factors such as precipitation and

temperature, which are relevant for breeding of most species (e.g. Bernarde & Anjos 1999; Sazima & Eterovick 2000; Conte & Rossa-Feres 2007; Garey & Silva 2010).

### 2.3 Functional Traits

We characterized ecological traits of 147 amphibian species (Table 1) according to Haddad et al. (2013) and AmphiBio (Oliveira et al. 2017), ecological information available in IUCN data base, and literature on the original descriptions of the species.

Ecological traits were:

**Table 1.** Traits of amphibians species utilized in the analysis.

Traits	Types	Description
Activity time	Nocturnal; Diurnal.	Overall diel period as active
Limbs	Tetrapod; Apodal.	If the species have limbs or not.
Body metrics	Size (cm); Body mass (g).	Maximum adult body size.
Diet	Artropods; Vertebrates.	“Food items from the eating habits of adults using qualitative dietary categories. Information is based of specialist guess, direct observation or stomach content examination, as reported in the literature” (Oliveira et al. 2017)
Calling site	Bamboo groove; Marsh lake; Bromelia; Forest floor; Tree crown; Cavern or burrow; Rocky wall; Backwater; Stream; Stream or river; Vegetation; Low vegetation.	Calling site which males use when they are in reproductive season.
Poisonous	Toxic; Unpalatable or bad odour; Not toxic.	Poisonous or not.
Habit	Arboreal; Phytotelmata; Terrestrial; Cryptic; Fossorial; Reophilic; Semiaquatic; Aquatic.	Indicates the way of life and the habitat where the species is generally found.

Habitat	Forested; Open areas.	It refers to the general vegetation cover where the species live.
Seasonality	Wet warm; Wet cold; Dry warm; Dry cold.	Seasonal period as active. Based on the comparison of the precipitation (wet or dry) and temperature (warm or cold) conditions when active in relation to the average climatic conditions over the year.
Breeding strategy	Direct; Larval; Viviparous	Whether the species reproduce via direct, larval development or is viviparous.
Offspring or eggs per clutch	Maximum; Minimum	Number of eggs per clutch.
Egg size	Maximum; Minimum	Size of the eggs.
Reproductive modes	1 to 39	see Haddad & Prado 2005 for details.

All these functional traits have direct or indirect roles in the ecosystem (Duellman & Trueb 1994; Wells 2007; Haddad et al. 2013).

## 2.4 Data analysis

### 2.4.1 Model built and environmental input variables

We built a presence-absence matrix from our database of species 'geographical distributions and merged them with spatial environmental raster using a combination of both ArcGIS 10 software (ESRI, 2016) and R software through 'letsR' package (Vilela and Villalobos 2018). Subsequently were superimposing a grid system with cells of 0.1 latitude/longitude degrees, creating a network with 1782 grid cells. Each grid cell was represented by a gradient of colors (see Figure 6-7) which demonstrate low (yellow) to high (redder) values of FD and PD that range from 0 to 10. All the phytogeographic

units spatial data for the state of Paraná was acquired in the ITCG (Instituto de Terras Cartografia e Geologia do Paraná) that encompasses five phytogeographic units which were: Alto Paraná Atlantic forest (APAF), Araucaria moist forests (AMF), Cerrado or Savannah (CE), Grasslands (GRASS), and Serra do Mar coastal forests (SMF).

Environmental variables were extracted from 10 raster values expressed in an average of each grid within the map of the state of Paraná, which each raster contained information about variables (Appendix III) associated with the biological conditions that have influence in the occurrence of amphibians:

(i) two bioclimatic variables available at WorldClim, which are already well documented in the literature that temperature (AT) and precipitation (AP) is an important variable for amphibian distributions (e.g., Duellman & Trueb 1994);

(ii) altitude (ALT), slope (SLP) and aspect (ASP): altitude is highly correlated with temperature and humidity (Hoersch et al. 2002) and with solar radiation that plays an important role on moisture for habitat selection by amphibians (Wyman 1988). Slope and aspect were related to a proxy for the amount of solar radiation on the ground surface (Blank & Blaustein 2012);

(iv) solar radiation (RAD;  $\text{kJ m}^{-2} \text{day}^{-1}$ ): beyond the characteristics mentioned above, ultraviolet radiation (UVR) can act negatively in amphibians species such as kill directly by desiccation, cause sublethal effects and can interact with contaminants and pathogens (Blaustein et al. 2003);

(v) vapor pressure (VP): the vapor pressure gradient act on amphibian activity due to the divergence in vapor pressure between frog skin and the atmosphere that has influence in evaporation rates (Hillman et al. 2009);

(vi) drainage density (DD) and vertical distance to the nearest drainage (VDND): VDND is indirectly related to the depth of the water table, which in turn indicates the availability of groundwater. Small values of vertical distance (close to zero) indicate regions where the water table is near the surface and hence the soil is close to saturation. High values of vertical distance identify regions with the deep water table, i.e. well-drained areas (Amaral et al. 2013). These variables may it is related to the availability of temporary ponds for amphibians reproduction;

(vii) percent of tree cover (PTC): which affects amphibian species composition and distribution (Figueiredo et al. unpublished data; Skelly et al. 1999).

Bioclimatic variables were obtained from WorldClim (WorldClim database version 2.0, <http://www.worldclim.org/>) and were interpolated to 30 arc-sec resolution (Fick & Hijmans 2017) with WGS84 projection; altitude, slope, aspect, and vegetation type from National Aeronautics and Space Administration - NASA (<http://www2.jpl.nasa.gov/srtm/>; Amaral et al. 2013); and percent of tree cover from Global Landcover Facility (<http://glcf.umd.edu/data/>; Amaral et al. 2013).

#### ***2.4.2 Species richness and composition***

For each grid was calculated the average richness. Afterward, the average and overall richness per phytogeographic units was calculated. To visualize the species composition across the phytogeographic units we performed a principal coordinate analysis (PCoA) (Legendre & Legendre 1998) using occurrence (presence/absence) data originated from the model. Subsequently, we performed a cluster analysis (UPGMA; Krebs 1999) to verify the similarity of species composition among the phytogeographic units. Both analysis, cluster and PCoA, were calculated with Jaccard's index of similarity (Magurran 1988) in R v. 3.4.2 (R Development Core Team, 2017).

### ***2.4.3 Functional and Phylogenetic diversity***

To calculate FD we follow Patchey and Gaston (2006) approach, which first was constructed a species-trait matrix and subsequently was converted into a distance matrix based on Gower index proposed by Pavoine et al. (2009). Subsequently, was built a dendrogram based on a clustering distance matrix (UPGMA) and finally were calculated FD values by the sum of the total amount of dendrogram branches lengths of species community. The study spatial scale may influence the relative importance of functional diversity because other factors can affect the ecosystem process (Loreau 2000; Fridley 2002).

To calculate the (PD) was used the phylogenetic diversity index (Faith 1992) that was quantified by the sum of the branches length of the phylogenetic tree for all the species selected to compose the analyses. We randomized our community data matrix that contains presence and absence of species by the use of a constrained null model called “Independent Swap” to certificate that the FD and PD values were independent of the species richness (Swenson 2014).

We generated the estimated phylogeny according to the protocol proposed by Pyron and Wiens (2011) for amphibians phylogeny and used 12 genes for all the species present in each grid units for the state of Paraná. The phylogenetic relationships were analyzed with Bayesian analyses in software BEAST 1.8 (Drummond and Rambaut 2007). The phylogeny tree was generated based on the data matrix of species with applying an HKY model of sequence evolution for one partition for all genes using a Yule speciation process for 100 million generations. Thus, we checked if the number of generations was sufficiently assessed after convergence using Tracer v1.6 (Drummond and Rambaut 2007) and finally combining the results using both Logcombiner 1.8 and Treeannotator 1.8 (Drummond and Rambaut 2007).

#### ***2.4.4 Model fitting and evaluation***

We fitted generalized linear models (GLMs) to investigate the relative effects of environmental variables on the variation of species functional and phylogenetic diversity. GLMs is a dynamic generalization of linear regression that permits a flexible response of variables that have error distribution models other than a normal distribution. GLMs allows the linear model to be related to the variable response via a link function (e.g. “log”, “inverse”) and the magnitude of the variance of each measurement to be a function of its predicted value (Wiley & Wiley 2019). In other words, GLMs are a broad class of models that encompass both simple regression analysis and analysis of variance (ANOVA) (Wiley & Wiley 2019). We used Gaussian error distribution and the model assumptions were checked by examining the residual deviance as well as the residual plots for significant and uncertain significant predictors. The analyze was made in R 3.4.2 (R Development Core Team, 2017) with “glm” function.

We examined if the independent variables have multicollinearity with the variance inflation factor (VIF) (Zuur et al., 2009) and identified variables with a high VIF value ( $>10$ ), and hence these variables were excluded from the analysis. VIF values near one indicate a little impact of collinearity. Very high VIF values ( $>10$ ) may indicate that the inclusion of highly correlated explanatory variables inflated the variance of the covariance matrix parameter, which results in very standard errors and confidence intervals (Wiley & Wiley 2019). All values for other variables after VIF analysis had values  $< 2.0$ . To determine the optimal model, we started with a model in which the fixed component contained all explanatory variables. Then we generated sub-model sets from the global model using the “dredge” function implemented in the MuMIn package v. 1.42.1 (Barton 2018) that selected the best model based on the

lowest score of Akaike Information Criterion (AICc; Burnham & Anderson 2002) and Akaike weight value (w).

#### ***2.4.5 Functional and Phylogenetic diversity vs Environmental variables***

To test the influence of each environment variables in the variation of functional and phylogenetic diversity throughout the entire area and phytogeographic units of the state of Paraná we performed a permutational analysis of variance (PERMANOVA; Anderson 2001) with 999 permutations. PERMANOVA is a more robust alternative to MANOVA and ordination methods, which shows how much variation is attributed to different variables (Legendre & Anderson 1999). Additionally, we performed a Spearman correlation test to verify if each environmental variable had a positive or negative correlation with FD and PD. To reduce the probability of Type I errors or “false positives” in the correlation test we performed a Bonferroni correction (Rice, 1989).

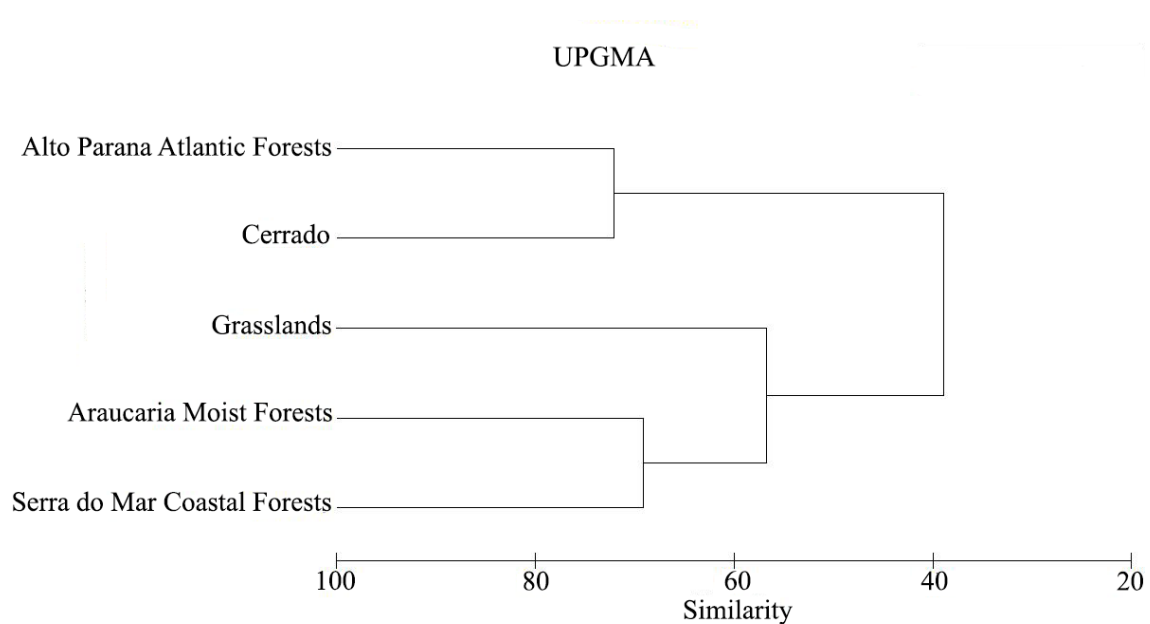
### **3- RESULTS**

One hundred and forty-seven species of amphibians, belonging to 17 families and 41 genus, were recorded in the state of Paraná (Appendix I). The average richness per grid was higher in SMCF than in GRASS, CE and APAF (Table 2). For the entire area of each phytogeographic unit the highest richness was found in the AMF (n = 116), followed by SMCF (n = 109), GRASS (n = 88), APAF (n = 53) and CE (n = 51).

**Table 2.** Richness of amphibians species in different phytogeographic units within the state of Paraná, southern Brazil.

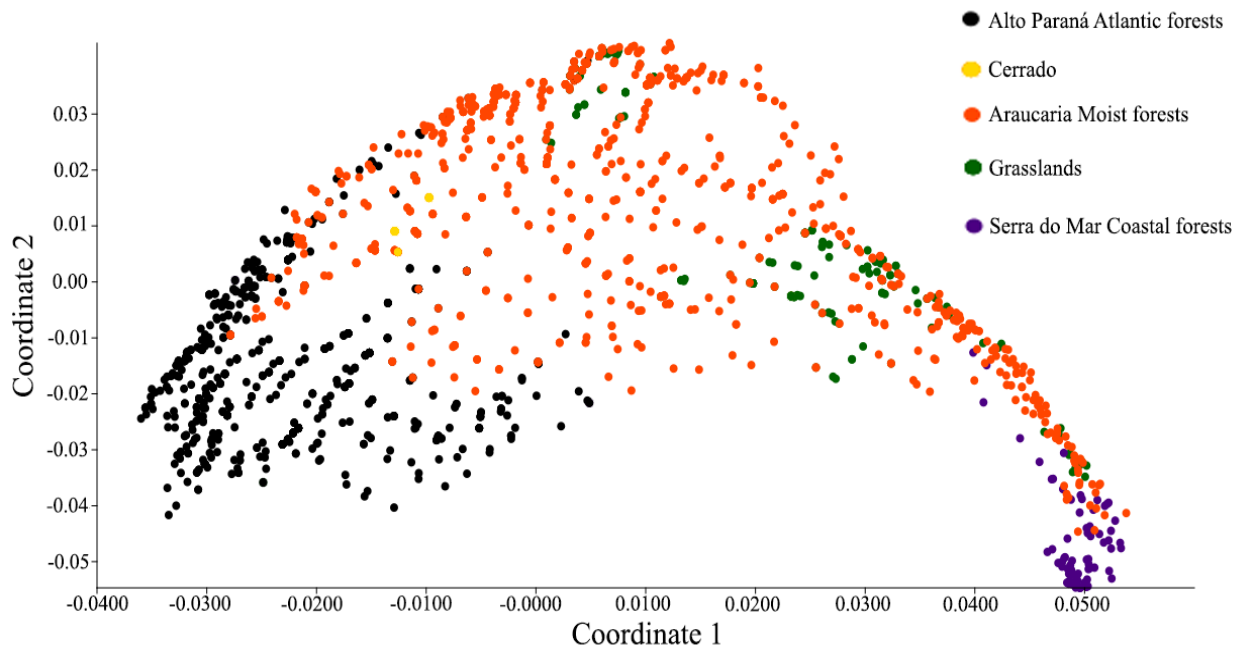
Phytogeographic unit	Mean	Total	Species range
Araucaria Moist forests	36 ± 6.23	116	67 - 27
Alto Paraná Atlantic forests	29 ± 2.67	53	38 - 24
Cerrado	33 ± 4.04	51	38 - 30
Grasslands	39 ± 5.43	88	61 - 33
Serra do Mar coastal forests	54 ± 5.12	109	64 - 45

The similarity in species composition clustered APAF and CE and SMCF, AMF and GRASS (Figure 4): CE and APAF had ~73% of similarity, AMF and SMCF had 70% of similarity and these with GRASS had ~57% of similarity. The ordination of species composition resulted in 180 PCoA axes. The first three axes represented 60% of the species composition gradients. The first two axes represented the composition gradient from Alto Paraná Atlantic forests to Cerrado and Araucaria Moist forests with some portion of Grasslands to Serra do Mar coastal forests amphibian communities (Figure 5).



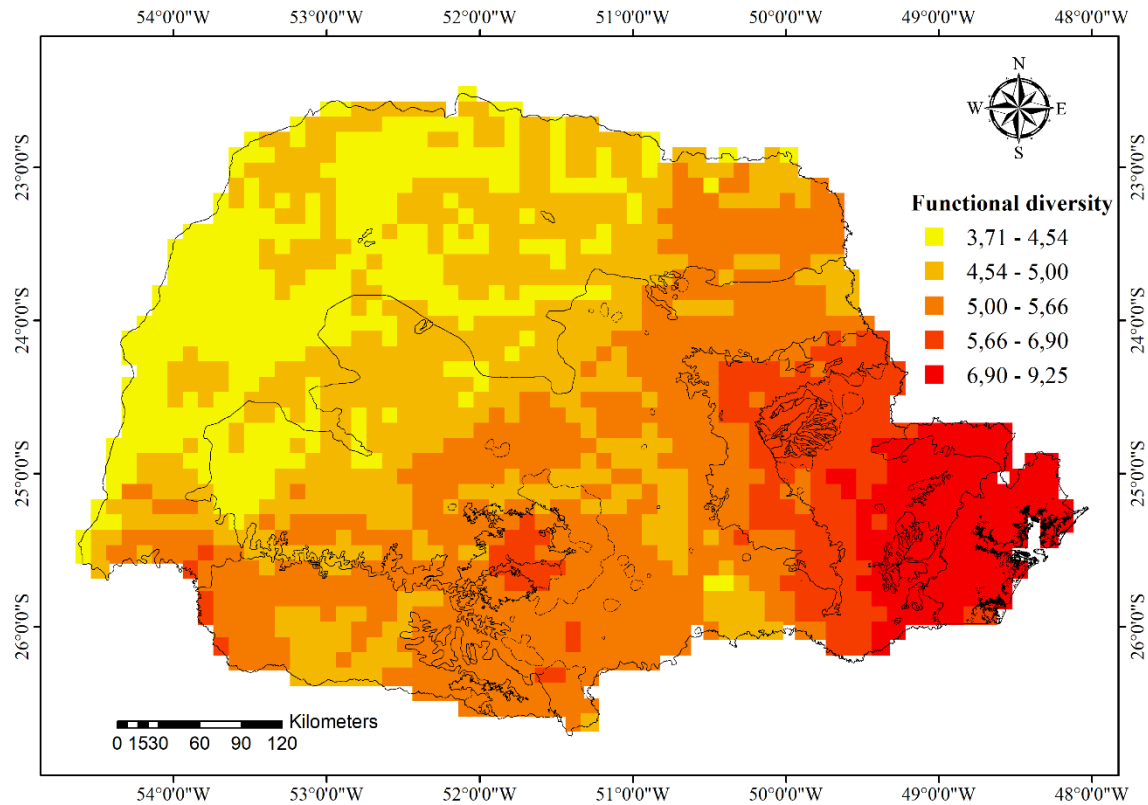
**Figure 4.** Composition of amphibians analysed with application of Jaccard's similarity index in the five different phytogeographical units in the state of Paraná, south of Brazil.

The positive side of first principal coordinate (Coordinate 1) axis clearly varied from amphibian compositions restricted to SMCF to those that were distributed in the negative side of the axis restricted to APAF across the phytogeographies within the state of Paraná.

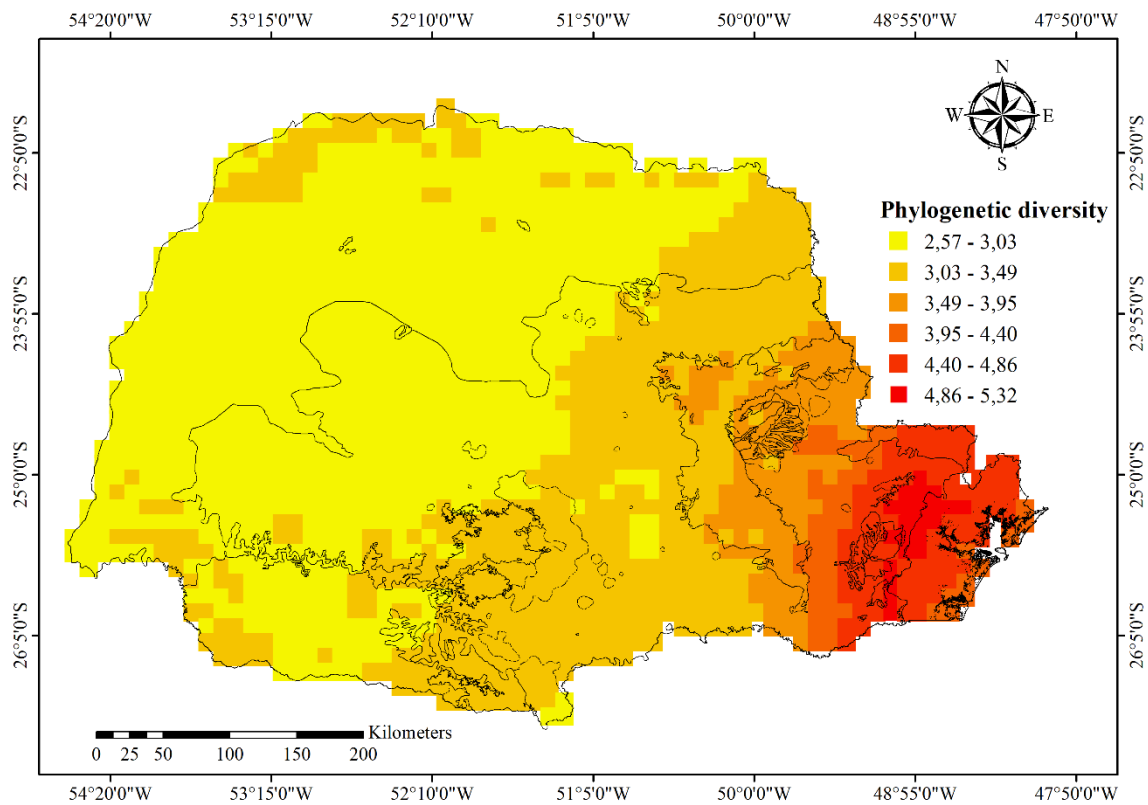


**Figure 5.** Ordination of amphibian species composition across the five phytogeographic units. First two axes of principal coordinate analysis (PCoA) revealing composition gradients across the study areas (Coordinate 1 and Coordinate 2).

The FD values within the grids of the different phytogeographic units in the state of Paraná range from 3.71 to 9.25 (Figure 6) and PD values range from 2.57 to 5.32 (Figure 7). The highest values of FD and PD were registered in the Serra do Mar coastal forests and Grasslands and the lowest values of were related to Cerrado and Alto Paraná Atlantic forests (see Figure 6 -7). For the mean values of PD and FD in all the 1782 grids follows a sequence from highest to the lowest values (Table 3): Serra do Mar coastal forests, Grasslands, Araucaria moist forests, Cerrado and Alto Paraná Atlantic forests.



**Figure 6.** Functional diversity values of amphibian species in the state of Paraná, south of Brazil. For details of phytogeographic units see figure 3.



**Figure 7.** Phylogenetic diversity values of amphibian species in the state of Paraná, south of Brazil. For details of phytogeographic units see figure 3.

Three variables were correlated with each other and two with the highest VIF values were removed from analysis (Table 4). Among all models of predictor variables, in the state of Paraná, the best model that explaining the variation of FD was (ALT + AP + PTC + RAD + SLP + VDND) and the best one for explaining the variation of PD was (ALT + AP + PTC + RAD + VDND) (Table 5 - 6).

**Table 3.** Mean of 1782 grids values of richness, phylogenetic and functional diversity of amphibians originated from the model in five phytogeographical units in the state of Paraná, south of Brazil.

<b>Phytogeographic units</b>	<b>PD mean</b>	<b>FD mean</b>
Serra do Mar coastal forests (SMCF)	4.55±0.33	7.82±0.65
Alto Paraná Atlantic forests (APAF)	2.94±0.13	4.60±0.32
Grasslands (GRASS)	3.48±0.41	5.78 ±0.63
Araucaria Moist forests (AMF)	3.25±0.45	5.30±0.79
Cerrado (CE)	3.23±0.30	5.29±0.36

Permutational multivariate analysis of variance (PERMANOVA) results reveal that the variable with best explication in the variation of FD at the fitted model was:

RAD (F = 2982.1; p < 0.001; r<sup>2</sup> = 0.47) followed by: ALT (F = 581.4; p < 0.001; r<sup>2</sup> = 0.09); PTC (F = 554.5; p < 0.001; r<sup>2</sup> = 0.08); AP (F = 200.3; p < 0.001; r<sup>2</sup> = 0.03); VDND (F = 148.6; p < 0.001; r<sup>2</sup> = 0.02) and SLP (F = 37; p < 0.001; r<sup>2</sup> = 0.005).

The variable with best explication in the variation of PD at the fitted model was:

RAD (F = 3078.7; p < 0.001; r<sup>2</sup> = 0.51); PTC (F = 563.9; p < 0.001; r<sup>2</sup> = 0.09); ALT (F = 388.3; p < 0.001; r<sup>2</sup> = 0.06); VDND (F = 177.1; p < 0.001; r<sup>2</sup> = 0.02) and AP (F = 148.6; p < 0.001; r<sup>2</sup> = 0.02).

**Table 4.** Variance inflation factor (VIF) calculated from 10 variables extracted from rasters with mean values of 1782 grids values of the state of Paraná

<b>Environmental variables</b>	<b>VIF</b>
Altitude (ALT)	19.39
Annual precipitation (AP)	3.13
Annual temperature (AT)	24.41
Aspect (ASP)	1.05
Drainage density (DD)	1.28
Percent of tree cover (PTC)	1.21
Slope (SLP)	1.36
Solar radiation (RAD)	1.98
Vapor pressure (VAP)	36.12
Vertical distance to the nearest drainage (VDND)	1.91

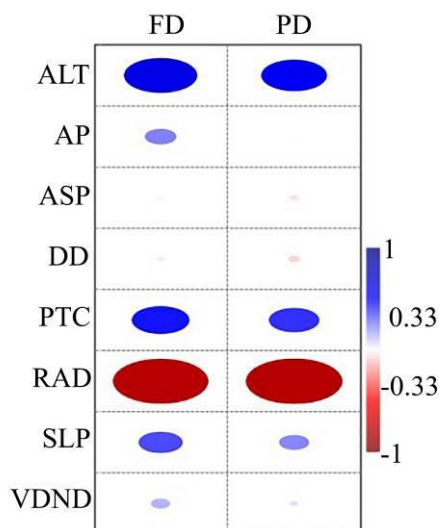
**Table 5.** Top five models resulted from functional and phylogenetic diversity with environmental variables. Altitude (ALT), annual precipitation (AP), aspect (ASP), drainage density (DD), percent tree cover (PTC), solar radiation (RAD), slope (SLP), vertical distance to the nearest drainage (VDND)

<b>Model (FD)</b>	<b>Variables</b>	<b>df</b>	<b>logLik</b>	<b>AICc</b>	<b>ΔAICc</b>	<b>Weight</b>
1	ALT + AP + PTC + RAD + SLP + VDND	8	-1255.831	2527.7	0	0.966
2	ALT + AP + ASP + PTC + RAD + SLP + VDND	7	-1260.196	2534.5	6.71	0.034
3	ALT + AP + DD + PTC + RAD + SLP + VDND	7	-1265.969	2546	18.26	0
4	ALT + AP + ASP + PTC + RAD + VDND	6	-1271.687	2555.4	27.68	0
5	ALT + AP + ASP + DD + PTC + RAD + SLP + VDND	7	-1327.486	2669	141.29	0
<b>Model (PD)</b>	<b>Variables</b>	<b>df</b>	<b>logLik</b>	<b>AICc</b>	<b>ΔAICc</b>	<b>Weight</b>
1	ALT + AP + PTC + RAD + VDND	7	-161.605	337.3	0	0.233
2	ALT + AP + DD + PTC + RAD + VDND	8	-161.014	338.1	0.84	0.153
3	ALT + AP + PTC + RAD + SLP + VDND	8	-161.472	339	1.75	0.097
4	ALT + AP + ASP + PTC + RAD + VDND	8	-161.552	339.2	1.91	0.089
5	ALT + AP + DD + PTC + RAD + SLP + VDND	9	-160.745	339.6	2.32	0.073

**Table 6.** The best model resulting from generalized linear model (GLM) between (FD) and (PD) with environmental variables.

Variables (FD)	Estimate	Std. Error	t value	Pr(> t )
(intercept)	1.95E+01	3.11E-01	62.718	< 2e-16
Altitude	7.55E-04	5.73E-05	-14.24	< 2e-16
Annual precipitation	7.55E-04	6.07E-05	12.437	< 2e-16
Percent tree cover	1.84E-03	4.08E-04	4.507	7.01E-06
Slope	1.03E-02	3.49E-03	2.953	0.00319
Solar radiation	-1.02E-03	1.91E-05	-53.661	< 2e-16
Vertical distance to the nearest drainage	1.28E-03	1.05E-04	12.192	< 2e-16
Variables (PD)	Estimate	Std. Error	t value	Pr(> t )
(intercept)	1.18E+01	1.65E-01	71.367	< 2e-16
Altitude	-5.12E-04	3.10E-05	-16.522	< 2e-16
Annual precipitation	6.87E-05	3.24E-05	2.121	0.034
Percent tree cover	1.04E-03	2.20E-04	4.731	2.41E-06
Solar radiation	-5.68E-04	1.01E-05	-56.234	< 2e-16
Vertical distance to the nearest drainage	7.32E-04	5.50E-05	13.309	< 2e-16

The significant values ( $p < 0.05$ ) of correlation among variables and FD (Figure 8) were: ALT ( $r = 0.59$ ); AP ( $r = 0.24$ ); PTC ( $r = 0.46$ ); RAD ( $r = -0.77$ ); SLP ( $r = 0.34$ ) and VDND ( $r = 0.13$ ). For PD significant values ( $p < 0.05$ ) were: ALT ( $r = 0.52$ ); PTC ( $r = 0.40$ ); RAD ( $r = -0.78$ ) and SLP ( $r = 0.22$ ).



**Figure 8.** Correlation among FD and PD and environmental variables.

## 4 - DISCUSSION

Using a compilation of different sets of data, we comprehensively assessed the taxonomic amphibians composition on different phytogeographic units across the state of Paraná based on available information. The importance of studies performed at different scales for biodiversity and biogeographical trends are valuable for understanding the patterns across spatial scales (Gaston 2000; Chun & Lee 2018). Here, we explored biogeographical patterns and the underlying mechanisms that drivers the functional and phylogenetic diversity of amphibians assemblages in different phytogeographic units within the state of Paraná.

### 4.1 - *Species richness and composition*

Our results show that the richness of 143 species of amphibians anurans registered is similar to a recently published study (Santos-Pereira et al. 2018), which reported 137 species. We documented six new species records to the state of Paraná that were: *Boana punctata*, *Phrynomedusa appendiculata*, *Scinax rossaferesae*, *Leptodactylus chaquensis*, *Physalaemus marmoratus*, and *Pseudis platensis*. Different than expected, we found the highest overall richness in the AMF. Because of the great contact zone, we suggest that species of AMF and SMCF share the most species which occur in the mountain chain of Serra do Mar, whereas others are exclusively species that occur only in inland physiognomies (e.g. *Melanophryniscus vilavelhensis*, *Scinax rossaferesae*), factor that may be related to the species turnover (Werner et al. 2007). Furthermore, there is not has a delimited marking between the areas where the landscape actually changes to another phytogeographic unit, since there is a large ecotone area between the two units. Therefore, the AMF and SMCF have equivalent overall richness. Nevertheless, the average richness per grid analyzed, the SMCF is

considerably higher among other phytogeographic units, demonstrating that the richness in this region per unit area is greater than all others.

The third phytogeographic unit with higher average richness is GRASS, whereas the altitude and slope are significative factors which increase the richness, functional and phylogenetic diversity of amphibians (Vasconcelos et al. 2010; Lourenço-de-Moraes et al. 2018; present study). Thus, GRASS is characterized for have high altitudes, in which even as SMCF are associated with high temperatures and rainfall well distributed along the year, without a biological dry season (Veloso 1991). In contrast, the lowest richness was registered for that phytophysiognomies or the inland forests such as APAF and CE. These inland forests are associated with well-defined seasons, summer with high rainfall and winter with physiological drought, characterized by the presence of the trees with partially shedding leaves during the dry winter (Veloso1991).

The composition of anurans at a regional scale in the different phytogeographic units in our study corroborates the patterns found by Vasconcelos et al. (2014) at broad-scale in the AF. As Vasconcelos et al (2014), species composition in the SMCF its related to small-ranged species associated with climate, structure vegetation and rough topography as well as the east portion of AMF and GRASS adjacent with SMCF share a great number of species (Crivellari et al. 2014; Rossa-Feres et al. 2017; Foerster & Conte 2018). On the other hand, species composition of inland phytogeographic units such as APAF and CE are associated with widely-ranged species, which share a homogeneous topographic and a rigorous climate such as seasonality and high temperatures (Santos et al. 2009; Vasconcelos et al. 2014).

#### **4.2 - *Functional and Phylogenetic diversity***

FD and PD followed the patterns of richness variation. In the SMCF the topography it is notably meaningful in predicting high PD and FD because this region harbours a complex mountain chain that disfavours dispersal rates of amphibians population (Haddad & Prado 2005). In this way, high speciation rates are expected in these areas through geographic isolation (Haddad & Prado 2005). Thus, the entirely SMCF and their adjacent areas with other phylogeographic units such as GRASS, AMF have recognized to harbour high diversity and “rare” species with restricted ranges (Villalobos et al. 2003; Vasconcelos et al. 2014). In contrast, the inland phylogeographic units of the state of Paraná are related to areas with minor topographic variation (Maack 2012). Areas with homogeneous topography (see Appendix II) favor dispersal population, and in fact these areas have low speciation rates (Vasconcelos et al. 2014), and consequently, is expected to have low FD and PD (see Figure 6 – 7).

Our results reinforce the role of environmental conditions in shaping amphibian assemblages when FD and PD is calculated based on a complete set of traits (e.g. reproductive modes, habitat, vocalization site, habit). These results provide evidence that amphibian communities in the state of Paraná show greater dissimilarity in seasonality, habitat-related and reproductive functional traits among eastern AMF, SMCF, and GRASS with APAF and CE. Accordingly, that environmental conditions present in each similar group of phytophysiognomies act to a certain extent as a barrier for these communities (Haddad & Prado 2005; Vasconcelos et al. 2014).

In this sense, the distribution of species within the phylogeographic units which are not similar phylogenetically (see figure 7) probably it is related to the occupancy of different niches and in evolutionary history has undergone a process of evolutionary divergence (Tsianou & Kallimanis 2017). Whereas, functionally similar species that

sharing traits which are not phylogenetically similar it is related to evolutionary convergence (Tsianou & Kallimanis 2017). However, phylogenetic diversity tends to be more preserved along evolutionary history in a comparison of functional traits (e.g., body mass), which are more susceptible to be lost (Fritz & Purvis 2010). Also, speciation process may lead to a different pattern of functional diversity and may promote different species pools due to the evolutionary processes via historical, dispersal limitations, ecological drift and niche selection (Schleuter et al., 2012, Shiono et al., 2015, Spasojevic et al. 2018).

Factually, the phylogenetic and functional patterns can contribute to the concept of community assembly rules, since they can improve our understanding on the potential role of possible evolutionary effects generating FD and PD patterns in different scales through macroevolution. Thus, lower FD and PD should generally decrease variation in trait composition among local communities (i.e., functional biodiversity) and weaken relationships between community composition and the environment lineage-pool (see Spasojevic et al. 2018). In fact, multiple factors could structure the community assembly rules across different phytogeographic units, which are not limited by a small number of processes that might define species co-existence (Spasojevic et al. 2018).

#### ***4.3 - Functional and Phylogenetic diversity vs Environmental variables***

The importance of topography driving the richness, composition, and structure of species assemblages in mountains has been detected in tropical forests, including amphibians (Vasconcelos et al. 2010, 2014) and other vertebrate groups such as birds (Menger et al. 2017) and mammals (Safi et al. 2011). Our results suggest that the ALT and SLP are good predictors in the variation of FD and PD of amphibians across the phytogeographic units in a tropical region (see Table 5; Figure 8). The energy use and

the time dispensed by the species movement in the exploration of resources in an area depend on the terrain slope, which generally is related to a rough topography (Coughenour 1991). Thus, slope irregularity may favour the presence of species with plasticity in functional traits through the exploration of complementary resources, which consequently increase functional and phylogenetic diversity (Diaz et al. 2007). Moreover, the variability in altitude and slope, in fact, allows species to exploit different habitats according to their vagility abilities (García-Llamas et al. 2019), including the variability in the number of reproductive modes to amphibians (Haddad & Prado 2005; Vasconcelos et al. 2010).

To our knowledge, this study is the first to compare the relationship among vertical distance to the nearest drainage and drainage density with FD and PD for amphibians. Our results suggest that the VDND is a good environmental predictor with a positive correlation with FD, whereas the DD had no significant correlation. Higher VDND values mean large gravitational potential (low vertical draining potential) and proximity to the water table, where lack of drainage leads to waterlogging (Nobre et al. 2011). Drainage density is a characteristic of a river network, which accounts for the total length of channels per unit area of a river basin (Moglen et al. 1998).

The relationship among VDND and FD and PD seem to be expected, whereas the most amphibians in tropical zone generally reproduce in floodplains and temporary water bodies (Prado et al. 2005), which the soil probably have higher VDND values and tends to be flooded. In contrast, permanent water bodies such as streams have less species richness, although these species have a specialized reproductive mode and are restricted to this environment (*e.g.* genera *Crossodactylus*, *Vitreorana*, sensu Haddad & Prado 2005). Moreover, rivers and streams have high predation pressure on eggs and

tadpoles (Joly & Morand 1994; Prado et al. 2005). Accordingly, this pressure becomes a factor that limits the reproductive activity of many species (Haddad & Prado 2005).

Changes in some environmental variables are related to a homogenization of species composition, such as the conversion of forests in open areas destined to agriculture and pastures (Cruz-Elizalde et al. 2016; Nowakowski et al. 2018). The forest cover loss favours some species which have a pre-adaptation to anthropic activities (i.e. still water in cattle pastures) while others are threatened and may disappear (McKinney & Lockwood 1999; Nowakowski et al. 2017a). The evolutionary histories of species reflect how much they are sensitive to habitat conversion, a factor that is bound and determined by species traits and explained by the perspective of phylogenetic approach (Todd et al. 2017; Nowakowski et al. 2018). Thus, changes in species composition along evolutionary history, between natural and converted habitats, is a tradeoff, whereas that species which can exploit human-altered habitats gain in abundance, while sensitive species decrease until local extinction (Kurz et al. 2014; Nowakowski et al. 2017a). Accordingly, the forest cover loss implies in a non-random structuring of species assemblages leading to a homogenization of amphibians compositions and decrease in phylogenetic and functional diversity (Nowakowski et al. 2018). Hence, this corroborates with the fact that the local and ecological components in explaining the presence of open and forested habitat species throughout the distribution of some clades in amphibians (Maciel et al. 2010).

In this sense, clades of frogs with direct development (e.g. species of genus *Ischnocnema* and *Brachycephalus*) tended to be especially sensitive to habitat conversion (Nowakowski et al. 2018). Furthermore, most of the direct developers species suffer desiccation and thermal stress in open-canopy habitats due to their frequently small bodies which have high surface-to-volume ratios and consequently low

tolerances to warm places (Catenazzi et al. 2014; Nowakowski et al. 2015; Nowakowski et al. 2017b). In fact, those traits, which are phylogenetically conserved tends to contribute to changes in amphibian assemblages following habitat conversion (Nowakowski et al. 2018). Therefore, the magnitude of the decrease due to habitat conversion is similar for both functional and phylogenetic diversity of amphibians, which reveal that while there are commonly fewer species in converted habitats, these species represent clades diffused throughout the phylogeny (Nowakowski et al. 2018).

Atlantic Forest (AF) is the most endangered ecosystems in the Neotropical region due to deforestation and habitat modification, which remaining approximately 12.4% of the original forest and most of them are small patches (<100 ha) (Ribeiro et al. 2009; S.O.S Mata Atlântica 2018). Ecosystems distributed within (AF) encompasses the most biodiverse phytogeographic units including for mammals, birds, fishes, reptiles and amphibians (Haddad et al. 2013).

Following the reasoning of the loss of habitat due to deforestation, there is an important environmental variable that increases its incidence directly on the terrestrial surface and has negative effects to the amphibians assemblages, which is RAD (Collins et al. 2009). In our fitted model and PERMANOVA, the strongest predictor with negative correlation to the variation of FD and PD across the phytogeographic units in the state of Paraná was RAD (see Table 6; Figure 8). Besides the deforestation, the anthropogenic ozone depletion is one of the main factors that has contributed to the variation of incidence intensity of RAD at Earth's surface and consequently increased ultraviolet-B radiation (UV-B) (Collins et al. 2009). Moreover, the incidence of solar radiation is higher in the phytogeographic units of lower altitude, which are those that are to the west of the state of Paraná as the APAF and part of the CE and AMF (Appendix III; Figure 8).

There is a vast literature available which evidence the negative effects of ultraviolet radiation (UVR) specifically UV-B (280 – 315nm) on amphibians species such as changes in behaviour, metabolic rate, cause developmental malformations and abnormalities, reduce growth, survival, and locomotor performance (e.g. Blaustein et al. 1998; Blaustein et al. 2003; Blaustein & Belden 2003; Stuart et al. 2004; Croteau et al. 2008; Collins et al. 2009; Alton & Franklin 2017). However, the effects of RAD depends on a variety of factors such as the species level (susceptible or not), life stage (embryos, larval, juvenile or adult), and ecological parameters (e.g. biotic and abiotic factors) (Blaustein et al. 2001).

The increase in temperature acts synergistically with solar radiation (Williamson et al. 2014), and its increase in a global warming scenario is concerning, given how sensitive to climate change amphibians, due to their metabolism (Duellman & Trueb 1994). In this scenario, dispersal across disturbed areas may not be possible for most species (Lourenço-de Moraes et al. 2018). Thus, particularly in Atlantic Forest, many species will be lost, whereas in the Cerrado, for example, will gain, mostly wide-range species (Vasconcelos et al. 2018). Accordingly, species composition is prone to change in these ecosystems (Vasconcelos et al. 2018).

Beyond sensibility to climate change, high UV-B would impair the immune system (Bancroft et al. 2008), and increasing increase pathogen loads at higher temperatures (Karvonen et al. 2010). It includes the fungus *Batrachochytrium dendrobatidis* which has been linked to population decline and extinction in these ecosystems (Lips et al. 2006). Moreover, the possible consequences of a synergistic interaction between UVB and temperature increases on disease resistance is yet another concerning matter (Cramp et al. 2014). Therefore, in all ecosystems and particularly in the state of Paraná, where is located a transitional climate zone, the effects of an

increase in solar radiation and temperature could have unprecedented negative effects on amphibians diversity.

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## APPENDIX I

**Table 1.** Listo of species of the state of Paraná compiled with three different data sets: (1) spatial data provided by the IUCN Red List of Threatened Species™ (IUCN); LC = Least concern; DD = Deficient data; NT = Near threatened. (2) LT = Literature: published studies in the state of Paraná (see Appendix II). (3) FW = Fieldwork: species registered in waterbodies in northern Paraná.

ORDER/Family/Species	Phytogeographic units					Source	
	IUCN	CE	APAF	GRSL	AMF		SMCF
<b>ANURA</b>							
<b>Alsodidae</b>							
<i>Limnomedusa macroglossa</i> (Duméril & Bibron, 1841)	LC			•	•	IUCN; LT	
<b>Brachycephalidae</b>							
<i>Brachycephalus brunneus</i> Ribeiro, Alves, Haddad & Reis, 2005	DD				•	•	IUCN; LT
<i>Brachycephalus coloratus</i> Ribeiro, Blackburn, Stanley, Pie & Bornschein, 2017				•			IUCN; LT
<i>Brachycephalus curupira</i> Ribeiro, Blackburn, Stanley, Pie & Bornschein, 2017					•		IUCN; LT
<i>Brachycephalus ferruginus</i> Ribeiro, Haddad & Reis, 2006	DD					•	IUCN; LT
<i>Brachycephalus hermogenesi</i> (Giaretta & Sawaya, 1998)	LC				•	•	IUCN; LT
<i>Brachycephalus izecksohni</i> Alves, Haddad & Reis, 2005	DD				•		IUCN; LT
<i>Brachycephalus leopardus</i> Firkowski & Pie, 2015					•	•	IUCN; LT
<i>Brachycephalus pernix</i> Wistuba & Bornschein, 1998	DD				•		IUCN; LT
<i>Brachycephalus pombali</i> Ribeiro, Haddad & Reis, 2006	DD					•	IUCN; LT
<i>Brachycephalus sulfuratus</i> Condez, Monteiro, Comitti, Garcia, Amaral & Haddad, 2016					•	•	IUCN; LT
<i>Brachycephalus tridactylus</i> Garey, Lima, Hartmann & Haddad, 2012						•	IUCN; LT
<i>Ischnocnema guentheri</i> * (Steindachner, 1864)	LC						IUCN
<i>Ischnocnema henselii</i> (Peters, 1870)	LC		•	•	•		IUCN; LT; FW
<i>Ischnocnema manezinho</i> * (Garcia, 1996)	NT						IUCN
<i>Ischnocnema paranaensis</i> (Langone and Segalla, 1996)	DD					•	IUCN; LT
<i>Ischnocnema sambaqui</i> (Castanho and Haddad, 2000)	DD					•	IUCN; LT
<i>Ischnocnema</i> cf. <i>spanios</i>						•	IUCN; LT
<b>Bufonidae</b>							
<i>Dendrophryniscus berthaltutzae</i> Izecksohn, 1994	LC					•	IUCN; LT
<i>Dendrophryniscus leucomystax</i> Izecksohn, 1968	LC					•	IUCN; LT
<i>Dendrophryniscus stawiarskyi</i> Izecksohn, 1994	DD				•		IUCN; LT

<i>Melanophryniscus alipioi</i> Langone, Segalla, Bornschein & de Sá, 2008	DD				•		IUCN; LT
<i>Melanophryniscus vilavelhensis</i> Steinbach-Padilha, 2008					•		IUCN; LT
<i>Melanophryniscus tumifrons</i> (Boulenger, 1905)	LC	•	•	•			IUCN; LT
<i>Rhinella abei</i> (Baldissera, Caramaschi & Haddad, 2004)	LC				•	•	IUCN; LT
<i>Rhinella granulosa</i> * (Spix, 1824)	LC						IUCN
<i>Rhinella henseli</i> (Lutz, 1934)	LC			•	•	•	IUCN; LT
<i>Rhinella hoogmoedi</i> Caramaschi and Pombal, 2006	LC				•	•	IUCN; LT
<i>Rhinella icterica</i> (Spix, 1824)	LC	•	•	•	•	•	IUCN; LT
<i>Rhinella ornata</i> (Spix, 1824)	LC		•	•	•	•	IUCN; LT; FW
<i>Rhinella schneideri</i> (Werner, 1894)	LC	•	•	•	•	•	IUCN; LT; FW
<b>Centrolenidae</b>							
<i>Vitreorana eurygnatha</i> * (Lutz, 1925)	LC						IUCN
<i>Vitreorana uranoscopa</i> (Müller, 1924)	LC	•	•	•	•	•	IUCN; LT; FW
<b>Ceratophryidae</b>							
<i>Ceratophrys aurita</i> * (Raddi, 1823)	LC						IUCN
<b>Craugastoridae</b>							
<i>Haddadus binotatus</i> (Spix, 1824)	LC	•	•	•	•	•	IUCN; LT; FW
<b>Cycloramphidae</b>							
<i>Cycloramphus</i> cf. <i>asper</i>						•	IUCN; LT
<i>Cycloramphus bolitoglossus</i> (Werner, 1897)	DD			•	•	•	IUCN; LT
<i>Cycloramphus diringshofeni</i> * Bokermann, 1957	DD						IUCN
<i>Cycloramphus duseni</i> (Andersson, 1914)	DD			•	•	•	IUCN; LT
<i>Cycloramphus eleutherodactylus</i> (Miranda-Ribeiro, 1920)	DD					•	IUCN; LT
<i>Cycloramphus izecksohni</i> * Heyer, 1983	DD						IUCN
<i>Cycloramphus lutzorum</i> Heyer, 1983	DD				•	•	IUCN; LT
<i>Cycloramphus mirandaribeiroi</i> Heyer, 1983	DD				•	•	IUCN; LT
<i>Cycloramphus rhyakonastes</i> Heyer, 1983	DD				•	•	IUCN; LT
<b>Hemiphractidae</b>							
<i>Gastrotheca microdiscus</i> (Andersson, 1910)	LC				•	•	IUCN; LT
<i>Fritziana goeldii</i> * (Boulenger, 1895)	LC						IUCN
<i>Fritziana</i> gr. <i>fissilis</i>						•	IUCN; LT
<b>Hylidae</b>							
<i>Aplastodiscus albosignatus</i> (A. Lutz and B. Lutz, 1938)	LC				•	•	IUCN; LT

<i>Aplastodiscus ehrhardti</i> (Müller, 1924)	LC		•	•	•	IUCN; LT
<i>Aplastodiscus leucopygius</i> * (Cruz & Peixoto, 1985)	LC					IUCN
<i>Aplastodiscus perviridis</i> Lutz, 1950	LC	•	•	•	•	IUCN; LT; FW
<i>Boana albomarginata</i> (Spix, 1824)	LC	•		•	•	IUCN; LT
<i>Boana albopunctata</i> (Spix, 1824)	LC	•	•	•	•	IUCN; LT; FW
<i>Boana bischoffi</i> (Boulenger, 1887)	LC	•	•	•	•	IUCN; LT
<i>Boana caingua</i> (Carrizo, 1991)	LC	•	•		•	IUCN; LT; FW
<i>Boana crepitans</i> * (Wied-Neuwied, 1824)	LC					IUCN
<i>Boana curupi</i> * Garcia, Faivovich & Haddad, 2007	LC					IUCN
<i>Boana faber</i> (Wied-Neuwied, 1821)	LC	•	•	•	•	IUCN; LT; FW
<i>Boana geographica</i> * (Spix, 1824)	LC					IUCN
<i>Boana guentheri</i> * (Boulenger, 1886)	LC					IUCN
<i>Boana jaguariaivensis</i> (Caramaschi, Cruz & Segalla, 2010)	LC				•	IUCN; LT
<i>Boana leptolineata</i> (P. Braun & C. Braun, 1977)	LC		•	•	•	IUCN; LT
<i>Boana prasina</i> (Burmeister, 1856)	LC	•	•	•	•	IUCN; LT; FW
<i>Boana pulchella</i> (Duméril and Bibron, 1841)	LC		•	•	•	IUCN; LT
<i>Boana punctata</i> (Schneider, 1799)	LC	•	•		•	IUCN; LT; FW
<i>Boana raniceps</i> (Cope, 1862)	LC	•	•	•	•	IUCN; LT; FW
<i>Boana semiguttata</i> (Lutz, 1925)	LC			•	•	IUCN; LT
<i>Boana semilineata</i> (Spix, 1824)	LC			•	•	IUCN; LT
<i>Boana cf. stellae</i>					•	IUCN; LT
<i>Bokermannohyla circumdata</i> (Cope, 1871)	LC				•	IUCN; LT
<i>Bokermannohyla hylax</i> (Heyer, 1985)	LC			•	•	IUCN; LT
<i>Bokermannohyla langei</i> (Bokermann, 1965)	DD					IUCN; LT
<i>Dendropsophus anceps</i> (Lutz, 1929)	LC		•	•		IUCN; LT
<i>Dendropsophus berthallutzae</i> (Bokermann, 1962)	LC					IUCN; LT
<i>Dendropsophus elegans</i> (Wied-Neuwied, 1824)	LC			•	•	IUCN; LT
<i>Dendropsophus elianeae</i> * (Napoli and Caramaschi, 2000)	LC					IUCN
<i>Dendropsophus microps</i> (Peters, 1872)	LC	•		•	•	IUCN; LT
<i>Dendropsophus minutus</i> (Peters, 1872)	LC	•	•	•	•	IUCN; LT; FW
<i>Dendropsophus nahdereri</i> (Lutz & Bokermann, 1963)	LC			•	•	IUCN; LT
<i>Dendropsophus nanus</i> (Boulenger, 1889)	LC	•	•	•	•	IUCN; LT; FW
<i>Dendropsophus rubicundulus</i> * (Reinhardt and Lütken, 1862)	LC					IUCN

<i>Dendropsophus sanborni</i> (Schmidt, 1944)	LC	●	●	●	●	IUCN; LT
<i>Dendropsophus seniculus</i> (Cope, 1868)	LC				●	IUCN; LT
<i>Dendropsophus weneri</i> (Cochran, 1952)	LC			●	●	IUCN; LT
<i>Itapotihyla langsdorffii</i> (Duméril and Bibron, 1841)	LC	●	●	●	●	IUCN; LT; FW
<i>Julianus uruguayus</i> (Schmidt, 1944)	LC			●	●	IUCN; LT
<i>Lysapsus limellum</i> Cope, 1862	LC	●	●			IUCN; LT
<i>Ololygon argyreornata</i> (Miranda-Ribeiro, 1926)	LC				●	IUCN; LT
<i>Ololygon aromothyella</i> Faivovich, 2005	DD			●	●	IUCN; LT
<i>Ololygon berthae</i> (Barrio, 1962)	LC	●	●	●	●	IUCN; LT; FW
<i>Ololygon catharinae</i> (Boulenger, 1888)	LC				●	IUCN; LT
<i>Ololygon flavoguttata</i> * (A. Lutz and B. Lutz, 1939)	LC					IUCN
<i>Ololygon littoralis</i> (Pombal & Gordo, 1991)	LC				●	IUCN; LT
<i>Ololygon</i> aff. <i>perpussila</i>					●	IUCN; LT
<i>Ololygon rizibilis</i> (Bokermann, 1964)	LC	●	●	●	●	IUCN; LT; FW
<i>Pseudis cardosoi</i> Kwet, 2000	LC			●		IUCN; LT
<i>Pseudis paradoxa</i> * (Linnaeus, 1758)	LC					IUCN
<i>Pseudis platensis</i> Gallardo, 1961	DD		●			IUCN; LT; FW
<i>Scinax alter</i> (Lutz, 1973)	LC			●	●	IUCN; LT
<i>Scinax fuscomarginatus</i> (Lutz, 1925)	LC	●	●	●	●	IUCN; LT; FW
<i>Scinax fuscovarius</i> (Lutz, 1925)	LC	●	●	●	●	IUCN; LT; FW
<i>Scinax granulatus</i> (Peters, 1871)	LC	●	●	●	●	IUCN; LT; FW
<i>Scinax hayii</i> (Barbour, 1909)	LC			●	●	IUCN; LT
<i>Scinax imbegue</i> Nunes, Kwet & Pombal, 2012	LC				●	IUCN; LT
<i>Scinax nasicus</i> (Cope, 1862)	LC	●	●	●	●	IUCN; LT
<i>Scinax perereca</i> Pombal, Haddad & Kasahara, 1995	LC	●	●	●	●	IUCN; LT; FW
<i>Scinax rossaferesae</i> Conte, Araujo-Vieira, Crivellari, & Berneck, 2016	LC			●	●	IUCN; LT
<i>Scinax squalirostris</i> (Lutz, 1925)	LC	●	●		●	IUCN; LT
<i>Scinax tymbamirim</i> Nunes, Kwet, & Pombal, 2012	LC				●	IUCN; LT
<i>Scinax x-signatus</i> * (Spix, 1824)	LC					IUCN
<i>Sphaenorhynchus caramaschii</i> Toledo, Garcia, Lingnau & Haddad, 2007	LC	●		●	●	IUCN; LT
<i>Sphaenorhynchus surdus</i> (Cochran, 1953)	LC			●	●	IUCN; LT
<i>Trachycephalus dibernardoi</i> Kwet and Solé, 2008	LC			●	●	IUCN; LT
<i>Trachycephalus imitatrix</i> * (Miranda-Ribeiro, 1926)	LC					IUCN

<i>Trachycephalus mesophaeus</i> (Hensel, 1867)	LC			•	•	•	IUCN; LT
<i>Trachycephalus typhonius</i> (Linnaeus, 1758)	LC	•	•		•	•	IUCN; LT; FW
<b>Hylodidae</b>							
<i>Crossodactylus caramaschii</i> Bastos & Pombal, 1995	LC			•	•	•	IUCN; LT
<i>Crossodactylus schmidti</i> Gallardo, 1961	NT		•		•		IUCN; LT; FW
<i>Hylodes</i> aff. <i>asper</i> *				•	•	•	IUCN; LT
<i>Hylodes cardosoi</i> Lingnau, Canedo, & Pombal, 2008	LC				•	•	IUCN; LT
<i>Hylodes heyeri</i> Haddad, Pombal, & Bastos, 1996	DD			•	•	•	IUCN; LT
<i>Hylodes nasus</i> * (Lichtenstein, 1823)	LC						IUCN
<i>Hylodes perplicatus</i> * (Miranda-Ribeiro, 1926)	LC						IUCN
<b>Leptodactylidae</b>							
<i>Adenomera araucaria</i> Kwet & Angulo, 2002	LC				•		IUCN; LT
<i>Adenomera bokermanni</i> (Heyer, 1973)	LC				•	•	IUCN; LT
<i>Adenomera marmorata</i> Steindachner, 1867	LC			•	•	•	IUCN; LT
<i>Adenomera nana</i> (Müller, 1922)	LC				•	•	IUCN; LT
<i>Leptodactylus chaquensis</i> Cei, 1950	LC	•	•				IUCN; LT; FW
<i>Leptodactylus elenae</i> * Heyer, 1978	LC						IUCN
<i>Leptodactylus flavopictus</i> Lutz, 1926	LC				•	•	IUCN; LT
<i>Leptodactylus furnarius</i> Sazima & Bokermann, 1978	LC	•		•	•	•	IUCN; LT
<i>Leptodactylus fuscus</i> (Schneider, 1799)	LC	•	•	•	•	•	IUCN; LT; FW
<i>Leptodactylus gracilis</i> (Duméril & Bibron, 1840)	LC	•	•	•	•	•	IUCN; LT
<i>Leptodactylus labyrinthicus</i> (Spix, 1824)	LC	•	•	•	•	•	IUCN; LT; FW
<i>Leptodactylus latrans</i> (Steffen, 1815)	LC	•	•	•	•	•	IUCN; LT; FW
<i>Leptodactylus mystaceus</i> (Spix, 1824)	LC	•	•		•		IUCN; LT; FW
<i>Leptodactylus mystacinus</i> (Burmeister, 1861)	LC	•	•	•	•	•	IUCN; LT; FW
<i>Leptodactylus notoaktites</i> Heyer, 1978	LC	•	•	•	•	•	IUCN; LT; FW
<i>Leptodactylus plaumanni</i> Ahl, 1936	LC			•	•	•	IUCN; LT
<i>Leptodactylus podicipinus</i> (Cope, 1862)	LC	•			•	•	IUCN; LT; FW
<i>Physalaemus albonotatus</i> * (Steindachner, 1864)	LC						IUCN
<i>Physalaemus biligonigerus</i> * (Cope, 1861)	LC						IUCN
<i>Physalaemus centralis</i> * Bokermann, 1962	LC						IUCN
<i>Physalaemus cuvieri</i> Fitzinger, 1826	LC	•	•	•	•	•	IUCN; LT; FW
<i>Physalaemus gracilis</i> (Boulenger, 1883)	LC	•	•	•	•	•	IUCN; LT

<i>Physalaemus henselii</i> * (Peters, 1872)	LC						IUCN
<i>Physalaemus insperatus</i> Cruz, Cassini, & Caramaschi, 2008	DD			●			IUCN; LT
<i>Physalaemus lateristriga</i> (Steindachner, 1864)				●			IUCN; LT
<i>Physalaemus maculiventris</i> (Lutz, 1925)	LC			●	●	●	IUCN; LT
<i>Physalaemus marmoratus</i> (Reinhardt & Lütken, 1862)	LC	●	●				IUCN; LT
<i>Physalaemus nanus</i> (Boulenger, 1888)	LC			●	●		IUCN; LT
<i>Physalaemus nattereri</i> (Steindachner, 1863)	LC	●	●				IUCN; LT
<i>Physalaemus olfersii</i> (Lichtenstein and Martens, 1856)	LC			●	●	●	IUCN; LT
<i>Physalaemus spiniger</i> (Miranda-Ribeiro, 1926)	LC					●	IUCN; LT
<i>Pleurodema bibroni</i> Tschudi, 1838	NT			●			IUCN; LT
<i>Scythrophrys sawayae</i> (Cochran, 1953)	LC			●	●	●	IUCN; LT
<b>Microhylidae</b>							
<i>Chiasmocleis albopunctata</i> * (Boettger, 1885)	LC						IUCN
<i>Chiasmocleis leucosticta</i> (Boulenger, 1888)	LC			●	●	●	IUCN; LT
<i>Elachistocleis bicolor</i> (Guérin-Meneville, 1838)	LC	●	●	●	●	●	IUCN; LT; FW
<i>Elachistocleis cf. cesarii</i>	LC						IUCN; LT
<i>Dermatonotus muelleri</i> * (Boettger, 1885)	LC						IUCN
<b>Odontophrynidae</b>							
<i>Odontophrynus americanus</i> (Duméril & Bibron, 1841)	LC	●	●	●	●	●	IUCN; LT; FW
<i>Proceratophrys appendiculata</i> * (Günther, 1873)	LC						IUCN
<i>Proceratophrys avelinoi</i> Mercadal del Barrio and Barrio, 1993	LC	●	●	●	●	●	IUCN; LT; FW
<i>Proceratophrys boiei</i> (Wied-Neuwied, 1824)	LC			●	●	●	IUCN; LT
<i>Proceratophrys brauni</i> Kwet and Faivovich, 2001	LC			●	●		IUCN; LT
<i>Proceratophrys subguttata</i> Izecksohn, Cruz & Peixoto, 1999	LC				●	●	IUCN; LT
<b>Phyllomedusidae</b>							
<i>Phasmahyla guttata</i> (A. Lutz, 1925)	LC					●	IUCN; LT
<i>Phrynomedusa appendiculata</i> (Lutz, 1925)	NT			●	●		IUCN; LT
<i>Phyllomedusa burmeisteri</i> * Boulenger, 1882	LC						IUCN
<i>Phyllomedusa distincta</i> B. Lutz, 1950	LC			●	●	●	IUCN; LT
<i>Phyllomedusa tetraploidea</i> Pombal and Haddad, 1992	LC	●	●	●	●	●	IUCN; LT; FW
<i>Pithecopus hypochondrialis</i> * (Daudin, 1800)	LC						IUCN
<i>Pithecopus rusticus</i> Lucas, Garcia & Recco-Pimentel, 2015	LC			●			IUCN; LT

**Ranidae**

*Lithobates catesbeianus* (Shaw, 1802)

LC ● ● ● IUCN; LT; FW

## **GYMNOPHIONA**

### **Siphonopidae**

*Luetkenotyphlus brasiliensis* (Lütken, 1851)

DD ● ● ● IUCN; LT

*Siphonops annulatus* (Mikan, 1820)

LC ● ● ● IUCN; LT

*Siphonops paulensis* Boettger, 1892

LC ● ● ● IUCN; LT

### **Typhlonectidae**

*Chthonerpeton indistinctum* (Reinhardt & Lütken, 1862)

LC ● ● ● IUCN; LT

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## APPENDIX II

**Tabela 1.** Published studies in the state of Paraná that composed the data set of species distribution in the model and list of species.

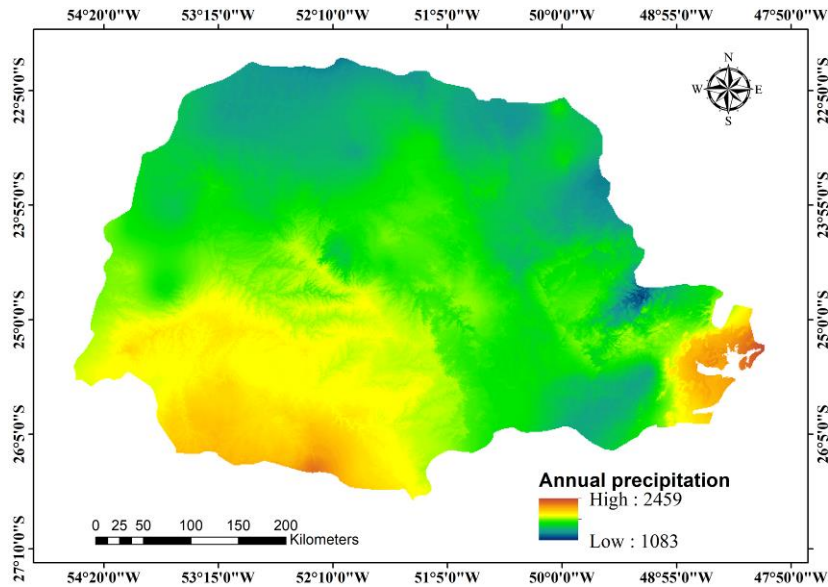
Source	Title	Journal
Affonso & Delariva (2012)	Lista comentada da anurofauna de três municípios da região Noroeste do Estado do Paraná, Brasil.	SaBios: Revista Saúde e Biologia 7(2): 102-109.
Affonso et al. (2011)	Amphibia, Anura, Leptodactylidae, <i>Leptodactylus mystaceus</i> (Spix, 1824): Distribution extension.	Check List 7(2): 198-199.
Affonso et al. (2014)	List of anurans (Amphibia: Anura) from the rural zone of the municipality of Maringá, Paraná state, southern Brazil.	Check List 10(4): 878-882.
Alves et al. (2006)	Two new species of <i>Brachycephalus</i> (Anura: Brachycephalidae) from the Atlantic Forest in Paraná State, Southern Brazil.	Herpetologica 62(2): 221-233.
Antonucci et al. (2011)	Parasitismo de <i>Amblyomma rotundatum</i> (Koch, 1844) (Acari: Ixodidae) em <i>Rhinella schneideri</i> (Werner, 1894) (Anura: Bufonidae) no estado do Paraná, Brasil.	Natureza on line 9(3): 103-105.
Armstrong & Conte (2010)	Taxocenose de anuros (Amphibia: Anura) em uma área de Floresta Ombrófila Densa no Sul do Brasil.	Biota Neotropica 10(1): 39-46.
Baldo et al. (2008)	Amphibia, Anura, Leptodactylidae, <i>Leptodactylus furnarius</i> : New country record, geographic distribution map and advertisement call.	Check List 4(2): 98-102.
Benarde & Anjos (1999)	Distribuição especial e temporal da anurofauna no Parque Estadual Mata dos Godoy, Londrina, Paraná, Brasil (Amphibia: Anura). Comunicações do Museu de Ciências e Tecnologia da PUCRS.	Série Zoologia 12: 111-140.
Bernarde & Machado (2001)	Riqueza de espécies, ambientes de reprodução e temporada de vocalização da anurofauna em Três Barras do Paraná, Brasil (Amphibia: Anura).	Cuadernos de Herpetología 14(2): 93-104.
Bokermann (1965)	<i>Hyla langei</i> , a new frog from Paraná, southern Brazil.	Journal of the Ohio Herpetological Society 5(2): 49-51.
Bornschein et al. (2015a)	Rectification of the position of the type locality of <i>Brachycephalus tridactylus</i> (Anura: Brachycephalidae), a recently described species from southern Brazil.	Zootaxa 4007(1): 149-150.
Bornschein et al. (2015b)	Rectification of the type locality of <i>Ischnocnema paranaensis</i> (Anura: Brachycephalidae), a missing species of the Atlantic Forest of Brazil.	Zootaxa 3957: 249-250.
Bornschein et al. (2016)	A new species of <i>Brachycephalus</i> (Anura: Brachycephalidae) from Santa Catarina, southern Brazil.	PeerJ 4:e2629.
Caramaschi & Cruz (2002)	Taxonomic status of <i>Atelopus pachyrhynchus</i> Miranda-Ribeiro, 1920, redescription of <i>Melanophryniscus tumifrons</i> (Boulenger, 1905), and descriptions of two new species of <i>Melanophryniscus</i> from the State of Santa Catarina, Brazil (Amphibia, Anura, Bufonidae).	Arquivos do Museu Nacional 60(4): 303-314.
Caramaschi & Rodrigues (2007)	Taxonomic status of the species of <i>Gastrotheca</i> Fitzinger, 1843 (Amphibia, Anura, Amphignathodontidae) of the Atlantic Rain Forest of eastern Brazil, with description of a new species.	Boletim do Museu Nacional 525: 1-19.
Caramaschi et al. (2010)	A new species of <i>Hypsiboas</i> of the <i>H. polytaeni</i> clade from the State of Paraná, Southern Brazil (Anura: Hylidae).	South American Journal of Herpetology 5: 169-174.
Castanho & Haddad (2000)	New species of <i>Eleutherodactylus</i> (Amphibia: Leptodactylidae) from Guaraqueçaba, Atlantic Forest of Brazil	Copeia 3: 777-781.
Clemente-Carvalho et al. (2009)	Hyperossification in miniaturized toadlets of the genus <i>Brachycephalus</i> (Amphibia: Anura: Brachycephalidae): Microscopic structure and macroscopic patterns of variation.	Journal of Morphology 270: 1285-1295.
Clemente-Carvalho et al. (2011)	Molecular phylogenetic relationships and phenotypic diversity in miniaturized toadlets, genus <i>Brachycephalus</i> (Amphibia: Anura: Brachycephalidae).	Molecular Phylogenetics and Evolution 61: 79-89.
Condez et al. (2016)	A new species of flea-toad (Anura: Brachycephalidae) from southern Atlantic	Zootaxa 4083(1): 40-56.

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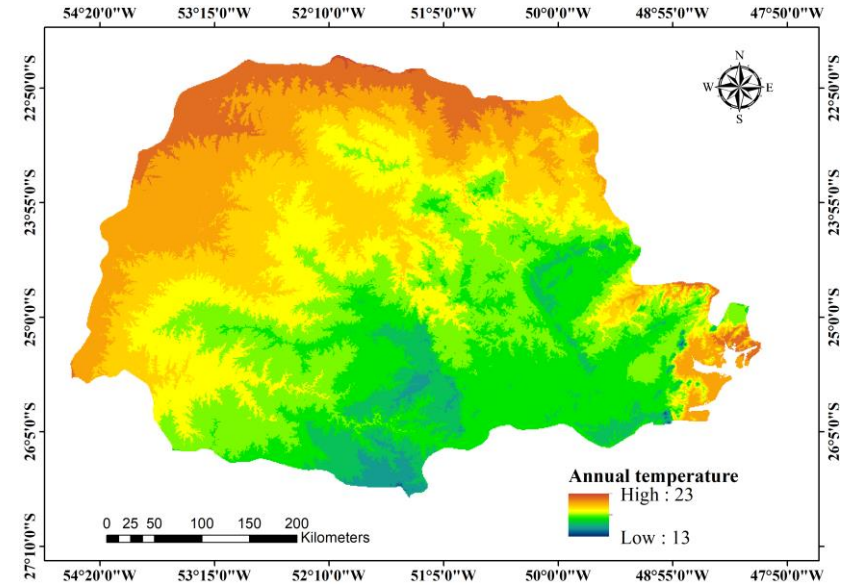
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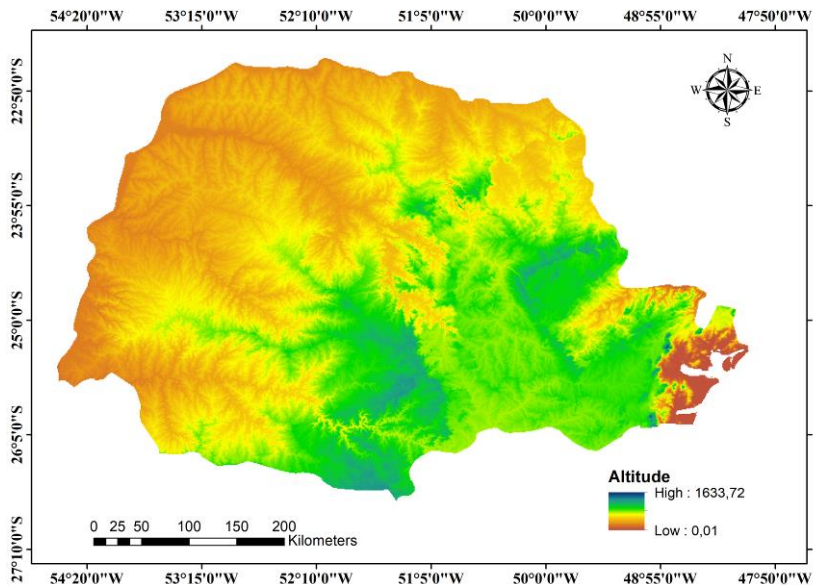
APPENDIX III



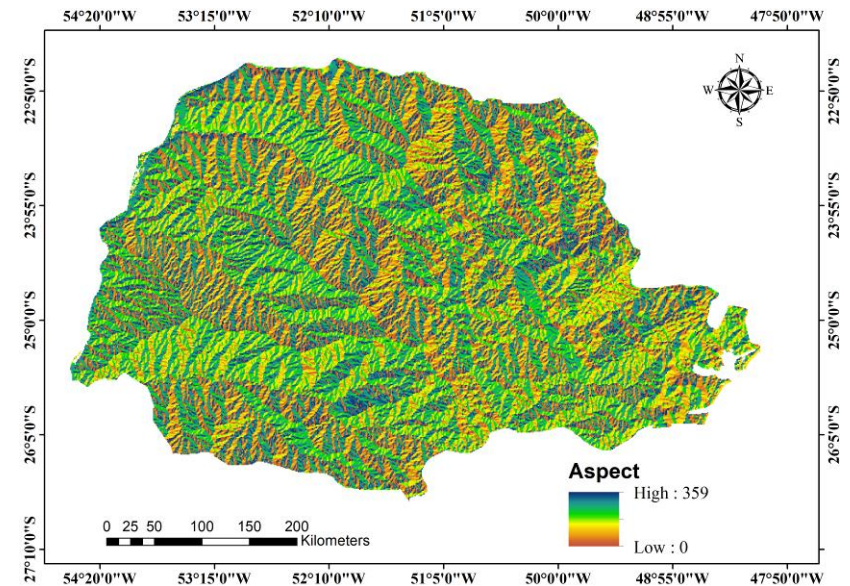
**Figure 1.** Raster of average annual precipitation in the state of Paraná, Brazil.



**Figure 2.** Raster of average annual temperature in the state of Paraná, Brazil.



**Figure 3.** Raster of altitude in the state of Paraná, Brazil.



**Figure 4.** Raster of aspect in the state of Paraná, Brazil.

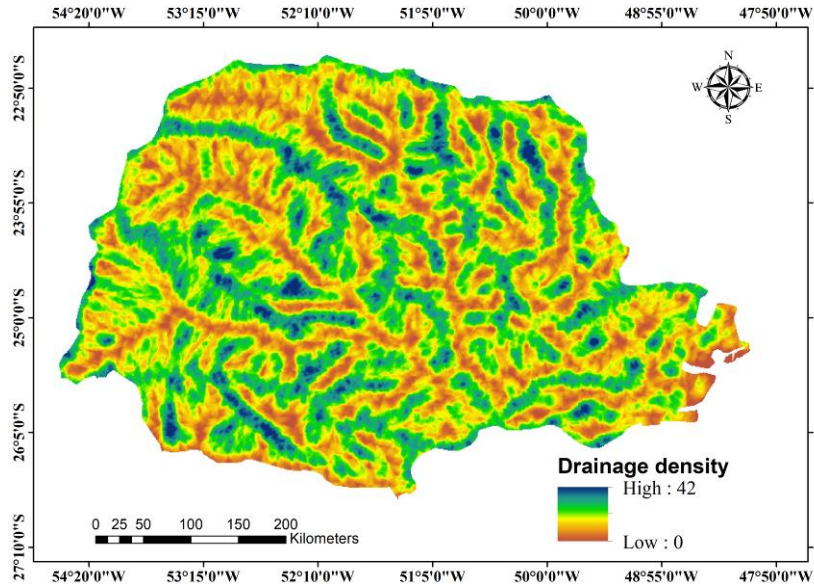


Figure 5. Raster of drainage density in the state of Paraná, Brazil.

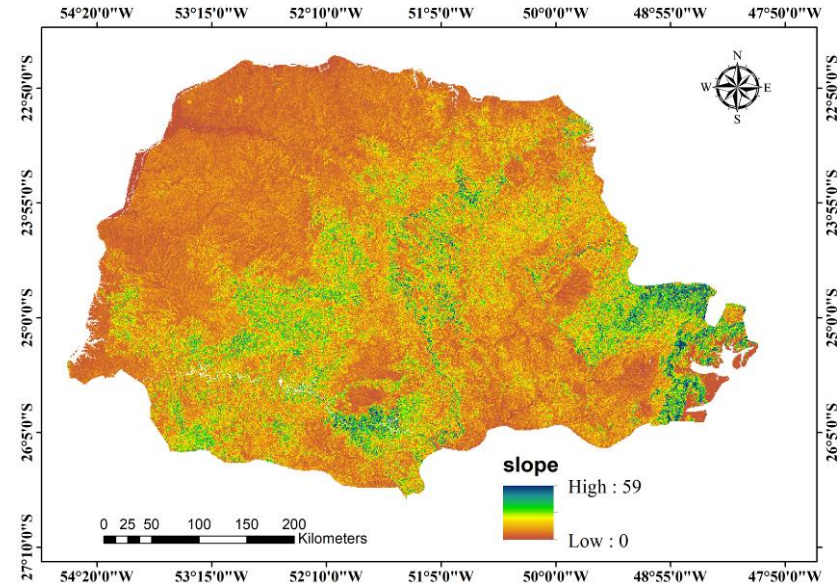


Figure 6. Raster of slope in the state of Paraná, Brazil.

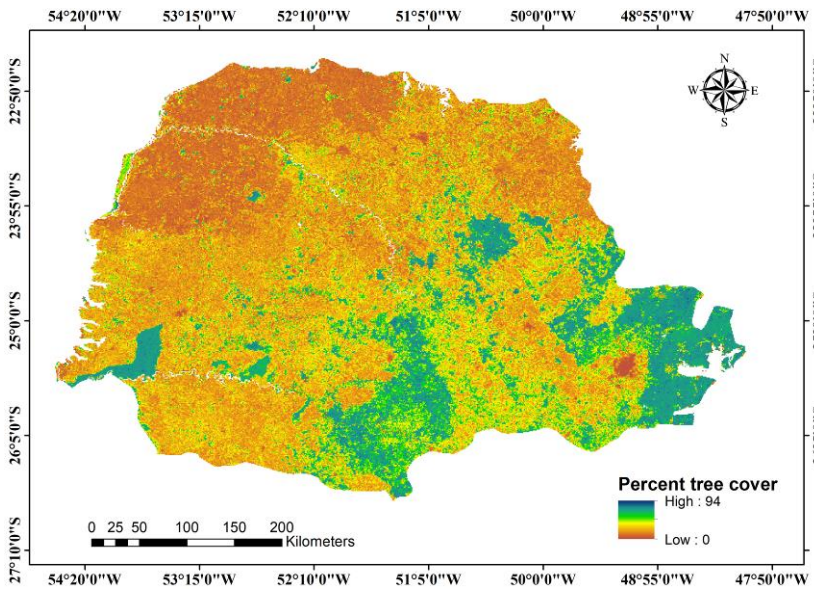


Figure 7. Raster of percent tree cover in the state of Paraná, Brazil.

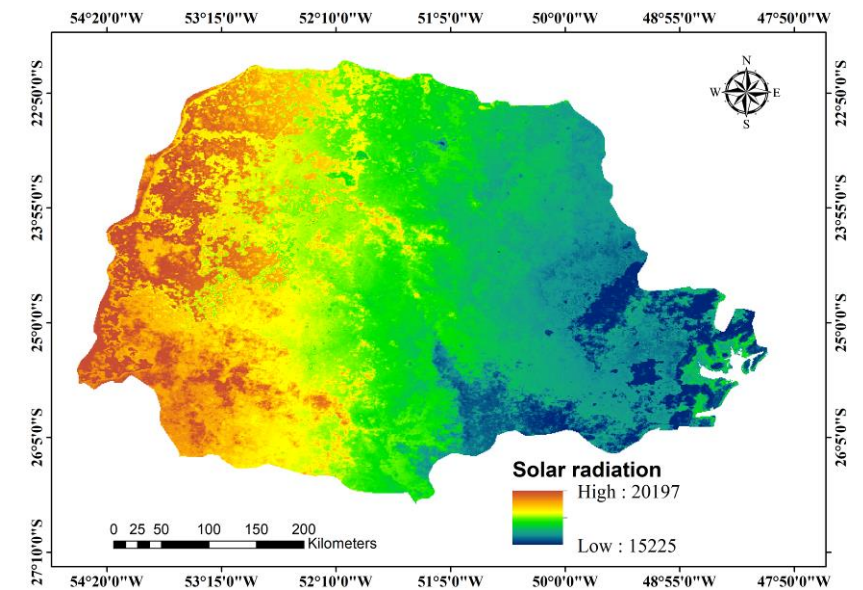
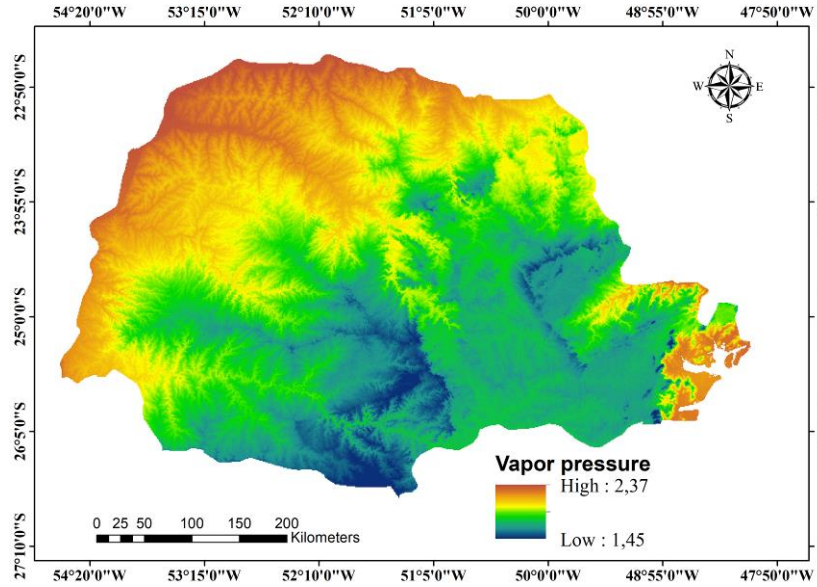
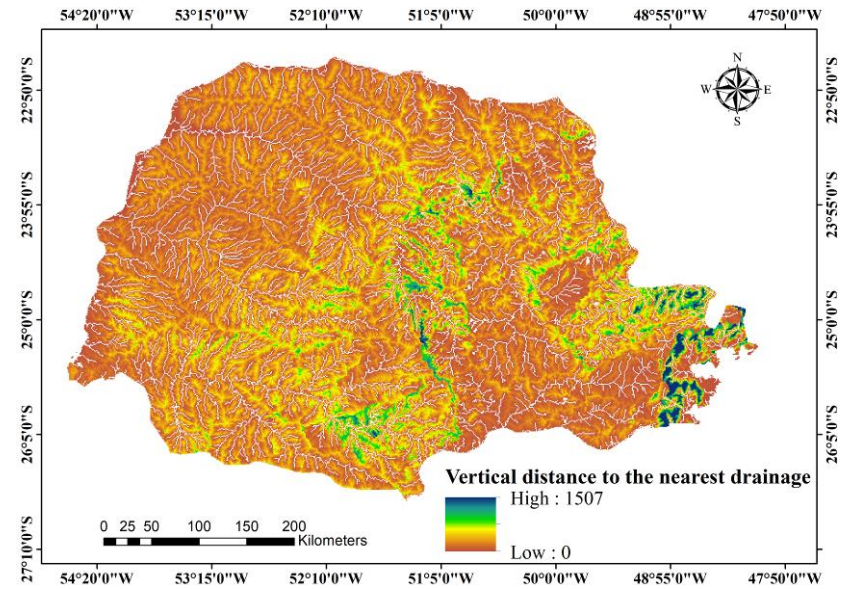


Figure 8. Raster of average solar radiation in the state of Paraná, Brazil.



**Figure 9.** Raster of average vapor pressure in the state of Paraná, Brazil.



**Figure 10.** Raster of vertical distance to the nearest drainage in the state of Paraná, Brazil.

## CONSIDERAÇÕES FINAIS

- O uso de modelos de distribuição de espécies ao longo dos ecossistemas, especialmente para aquelas espécies com distribuição restrita, podem servir como uma ferramenta para a tomada de decisões e indicar locais prioritários a serem preservados.
- Os microhabitats são importantes para anfíbios já que fornecem diversas implicações positivas desde a proteção de juvenis e adultos até a disponibilização de sítios de canto. Assim, a quantidade de microhabitats disponíveis nos ambientes aquáticos promovem o aumento da riqueza de anfíbios anuros.
- A radiação solar é um forte preditor na variação da diversidade funcional e filogenética dos anfíbios anuros em escala fitogeográfica, que por sua vez são sensíveis às mudanças climáticas e mudanças no habitat.
- Para todos os ecossistemas e particularmente para o estado do Paraná que se encontra em uma zona de transição climática os efeitos do aumento da radiação solar e temperatura pode ter efeitos negativos sem precedentes para a diversidade de anfíbios.