Caldasia 44(2):408-420 | Mayo-agosto 2022
http://www.revistas.unal.edu.co/index.php/cal

CALDASIA
Fundada en 1940

ISSN 0366-5232 (impreso)
ISSN 2357-3759 (en línea)

ECOLOGY

Human density and sampling time explain richness of anurans in the brazilian biomes

La densidad humana y el tiempo de muestreo explican la riqueza de anuros en los biomas brasileños

Arielson dos Santos Protázio [1*], Lennise Costa Conceição [1], Airan dos Santos Protázio [2]

- **Received:** 17/Apr/2020
- **Accepted:** 31/May/2021
- **Online Publishing:** 02/Jun/2021

Citation: Protázio AS, Conceição LC, Protázio AS. 2022. Human density and sampling time explain richness of anurans in the brazilian biomes. Caldasia 44(2):408-420. doi: https://doi.org/10.15446/caldasia.v44n2.86114

ABSTRACT

Anuran richness patterns are strongly influenced by environmental factors. However, investigations on this issue have focused on the influence of abiotic factors without considering the joint effect of many existing variables, including the data sampling methodology and human demography. In this study we investigated the relationship between 21 environmental variables and anuran richness in brazilian biomes. Environmental variables represent a combination of human demographics, topographic, climatic and vegetation characteristics, and data sampling methodologies. We used principal component factorization and regressive and autoregressive models to select the most relevant variables for explaining anuran richness. Richness was correlated with demographic density, vegetation, accumulated rainfall, accumulated rainfall in the third and fourth quarter of the year, and accumulated rainfall in the first and second half of the year. However, the regressive and autoregressive models showed that human demographic density, sampling time, and sampling methodology were the best predictors of anuran richness. Our results highlight the importance of considering the effects of the human footprint and the methodology used for data collection on anuran species richness.

Keywords: Amphibian, climate, demography, diversity, human footprint.

[1] Centro de Ciências Agrárias, Ambientais e Biológicas, Universidade Federal do Recôncavo da Bahia, Rua Rui Barbosa, Centro, 44380-000, Cruz das Almas, Bahia, Brazil. neu_ptz@hotmail.com*, esinnelfenix@gmail.com

[2] Departamento de Ensino, Instituto Federal de Educação, Ciência e Tecnologia da Bahia, Rodovia BA 148, Km 04, Vila Esperança, 44900-000, Irecê, Bahia, Brazil. airanprotazio@yahoo.com.br

* Author for correspondence.

RESUMEN

Los patrones de riqueza de anuros están fuertemente influenciados por factores ambientales. Sin embargo, las investigaciones en este sentido se han concentrado en la influencia de factores abióticos, sin considerar, el efecto colectivo de otras variables como la metodología de muestreo y la demografía humana. En este estudio analizamos la relación entre 21 variables ambientales y la riqueza de anuros en los biomas brasileños. Las variables ambientales representan una combinación de datos demográficos de tipo humano, topográfico, vegetal, climático y de la metodología de muestreo. Se utilizan, en el presente estudio, la factorización en los componentes principales y modelos de regresión y de autorregresión para seleccionar las variables más relevantes en la explicación de la riqueza de anuros. La riqueza estuvo correlacionada con la densidad demográfica, la vegetación, la lluvia acumulada, la lluvia acumulada en los terceros y cuartos trimestres del año y la lluvia acumulada en el primero y segundo semestres del año. Sin embargo, los modelos de regresión y de autorregresión nos enseñan que la densidad demográfica, el tiempo de muestreo y la metodología de muestreo son las mejores predictoras de riqueza de anuros. Nuestros resultados evidencian la importancia de tener en cuenta los efectos de la huella humana y de la metodología empleada en la recolección de datos sobre la riqueza de especies de anuros.

Palabras clave: Anfibios, clima, demografía, diversidad, huella humana.

INTRODUCTION

Species richness is the most basic way to measure biodiversity and has persistently been used to define the levels of diversity within ecological communities, habitats and even microhabitats (Brown *et al.* 2016). In addition, richness measurement is used to define areas of great interest for conservation, guiding actions and policies that aim to mitigate the effects of landscape modification that result from human activity (Myers *et al.* 2000, Jenkins *et al.* 2013). Although some environmental factors are considered to be good predictors of variation in species richness, this seems to be determined by the joint action of several factors, which may exert different effects on different taxa (Currie 1991, Moreno-Rueda and Pizarro 2009, Wiens 2015). Thus, the patterns or general processes that influence species richness can vary regionally, with a strong effect associated with a specific geographical area (Buckley and Jetz 2007, Qian *et al.* 2007).

In general, climate, vegetation structure, geomorphology and biotic interactions are factors that are able to explain a large part of the variation in species richness because they may promote the selection of phenotypic traits that increase the chances of survival and the conquest of new niches (Willig *et al.* 2003, Wiens 2015). However, climate, topography and vegetation are the primary determinants of diversity on a large spatial scale and are responsible for the dispersion and diversification of different clades (Moura *et al.* 2016). On the other hand, the competition for resources is the final mechanism that determines the composition of species living in a given habitat (Ricklefs 2005, Brown *et al.* 2016).

Amphibia is the fourth largest group of living vertebrates, and the order Anuran accounts for 88.2 % of the species of the clade (Frost c2020). Contemporary problems, such as global warming, habitat loss, and the emergence of diseases (Collins and Storfer 2003), place the anurans as one of the planet's most threatened taxa, which justifies the special attention that is paid to the mechanisms associated with the distribution and maintenance of the species richness of the group. Despite the current understanding of the distribution of anurans is well accepted among herpetologists, with higher species richness in the Neotropical region (Jenkins *et al.* 2013), the factors and events that led to the conquest and permanence of the group in a habitat are still not well understood. The amphibians began approximately 368 million years ago, however, the explosion of their diversification and radiation, including the south American Nobleobatrachia lineage (poison frogs, toads, glass frogs, and tree frogs), occurred subsequent to the Cretaceous-Paleogene extinction event, approximately 65 million years ago (Bossuyt and Roelants 2009). This scenario implies a tropical ancestry where the diversity of species is associated with a high rate of diversification, a low rate of extinction and a greater dispersion limit guided by variables associated with climate (Pyron and Wiens 2013).

This last issue raises an important question: what is the role of the environment in the species richness of anurans? Anurans have reproductive and behavioural aspects that are strongly influenced by the environment, which might help explain their richness patterns. Rainfall, the presence of ideal bodies of water, hydroperiod, temperature and humidity are some of the abiotic factors associated with the diversity of species (Aichinger 1987, Bertoluci and Rodrigues 2002, Oseen and Wassersug 2002). Furthermore, the presence of forests and their physical characteristics that increase habitat complexity (heterogeneity) seem to have offered the ideal scenario for the diversification of anurans because they are important mechanisms that generate greater genetic diversity (Wiens 2007).

Despite the well-known influence of these variables on anuran richness, the majority of studies that investigate richness patterns have performed isolated inferences without considering the combined effects of various environmental variables on the group's diversity (Oseen and Wassersug 2002). Moreover, the effect of the human footprint on species richness at a wide spatial scale has received little attention (Sanderson *et al.* 2002) and there are few studies that consider the effect of the methodology employed in data collection on richness (Williams *et al.* 2002). The negligence of these factors makes it difficult to identify the weight of each environmental variable independently and increases the chances that noise associated with collinearity among the analyzed variables is present (Graham 2003) or that there is a loss of predictive power due to unanalyzed variables (Montoya *et al.* 2007).

In this study, factor and regression analysis were used to assess the relationship between 21 environmental variables and anuran richness in Brazilian biomes. The environmental variables that were used represent a combination of climatic, topographic, vegetational and demographic variables, as well as variables associated with the methodology of the study to enable a more reliable prediction of richness. Brazil is a country of continental proportions, occupying most of South America and harboring the greatest diversity of anurans on Earth. There are approximately 1144 species described in Brazil (Segalla *et al.* 2021), which has been attributed to the vegetational, topographic and climatic heterogeneity observed in the country that makes some Brazilian biomes biodiversity hotspots (e.g. Atlantic Forest and Cerrado) (Myers *et al.* 2000). This scenario makes Brazil an ideal place for studies aimed at describing richness patterns at local and regional levels, motivating the search for the answer to the following question: what variables best explain the richness of anuran species in Brazil?

MATERIALS AND METHODS

Richness

The richness of anuran species was obtained from a compilation of scientific articles that characterize the anurofauna of various localities of Brazil. We used the scientific articles from the bibliographic database built by the first author, who compiled them between the years 2010 and 2017. In addition, we searched the online database of the main scientific journals that publish studies on the characterization of the herpetofauna of Brazil: Biota Neotropica, Check List, Herpetology Notes, Neotropical Biology and Conservation, and Zookeys. The search in the database of the journals was carried out directly, observing compatible studies in all issues available online. A total of 99 studies were identified, comprising a temporal frame of 18 years (1998–2016). The studies in which it was not possible to extract the data necessary for our analysis (e.g. inaccuracy in the study site, inaccuracy in the methodology used in data collection, compilation of data from several years of study, with lack of a methodological standard in data collection) were excluded. In addition, we verified the presence of outliers in the data set by means of the extreme values test, based on the mean. For localities for which more than one study was found, the one that presented the highest number of species was chosen because it indicated that the study had a more representative sample. Because our analysis considered sampling time and the methodology used in the data collection, we did not mix data from different lists as this could mask the real effect of a variable and compromise the results. A total of 83 studies distributed in 89 municipalities, six biomes and two ecotone zones were selected for our study (Fig. 1; Table S1). In each selected article the following information was extracted: location, anuran richness, biome, latitude, longitude, sampling time, and sampling methodologies.

Environmental data

Environmental data were obtained from scientific articles and different databases that provide climate and demographic information from Brazil. Twenty-one environmental variables were selected that are widely used in studies that investigate the presence of patterns in species richness on a large spatial scale. The variables were divided into six major categories:

(1) Topography: Elevation (ELE) represents the variation in relieve and was used here as a variable that defines the topographic heterogeneity. Elevation data were obtained directly from the scientific articles. For studies that lacked this information, Google Earth (c2019) was used to access the elevation of the study area from the geographical coordinates provided by the authors.

(2) Vegetation: It (VEG) was categorized according to the biome or area of the ecotone where the study was carried out. The biomes were: 1 - Caatinga; 2 - Caatinga-Atlantic Forest; 3 - Cerrado; 4 - Cerrado-Atlantic Forest; 5 - Amazonia; 6 - Atlantic Forest; 7 - Pampa; 8 - Pantanal, and 9 - Restinga. Even though the biome concept is comprehensive, it may be defined as a region in which the elements share similarities regarding the vegetation type, composition of fauna, climate and geomorphology (Coutinho 2016), but here we use this variable in a narrower sense and consider it as the indicator of the most striking vegetation type (Joly *et al.* 1999).

(3) Rainfall: We used the following variables: accumulated total rainfall (AR), accumulated rainfall divided by each year quarter (AR1T, AR2T, AR3T and AR4T); we also used

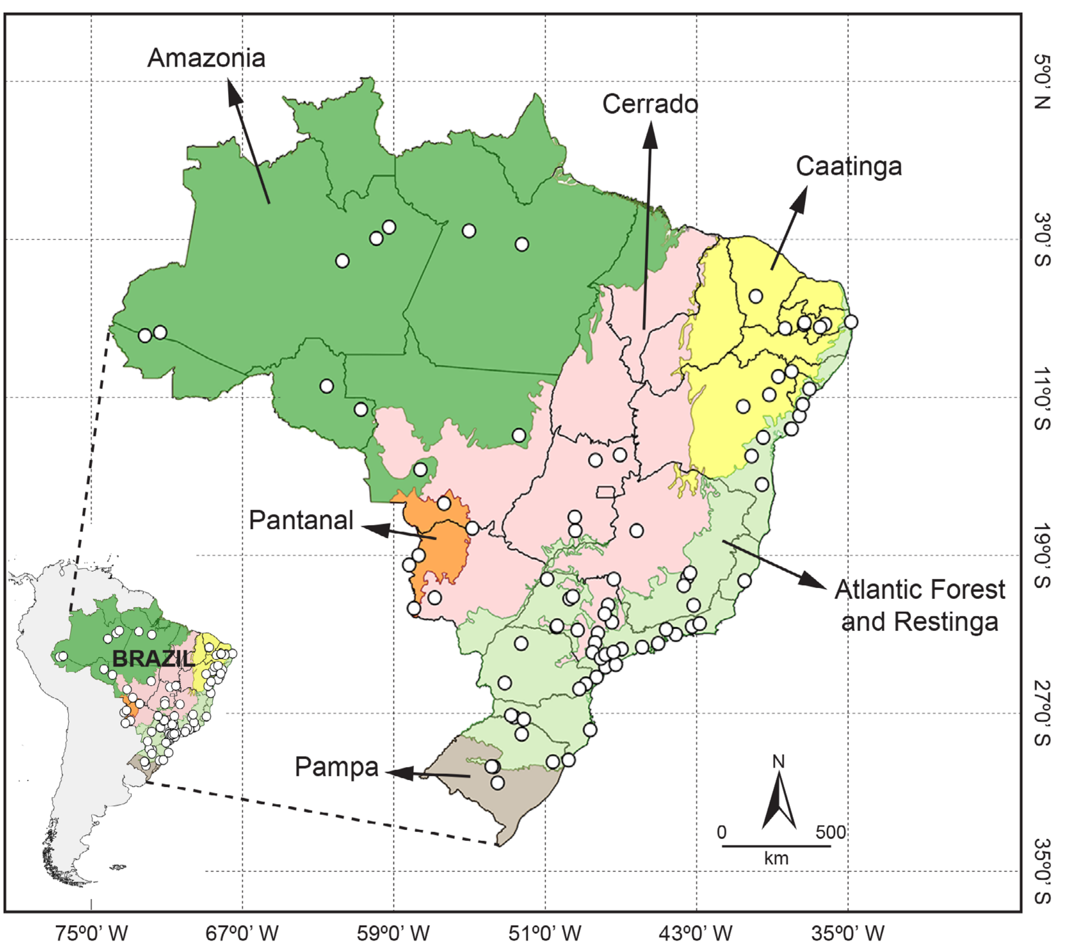

Figure 1. Map of Brazil showing biomes and localities with anuran richness data used in the study.

accumulated rainfall in the first half of the year (AR1S), accumulated rainfall in the second half of the year (AR2S), minimal rainfall in the month (MIRm), maximum rainfall for the month (MARm) and seasonality in the rainfall (SAZrain = MARm - MIRm). Rainfall data were extracted from the Climatempo Database (c2019), which provides the historical monthly average for the last 30 years by Brazilian municipality and represents the sum of the volume of rainfall per month.

(4) Temperature: We defined the following variables: the average minimum temperature (MINT), average maximum temperature (MAXT), seasonality in temperature (SAZtem = MAXT - MINT), temperature in the coldest month (Tcold) and temperature in the hottest month (Thot). Temperature data were extracted from the Climatempo Database (c2019).

(5) Demography: The population estimate (POP) (number of inhabitants) and density (DD) (hab/km^2) were used to represent the human footprint, based in Sanderson *et al.* (2002). Demographic data were obtained for each municipality where the study was developed from the database Cidades (IBGE c2017), which is a Brazilian government entity that performs the country's official statistical analyses.

(6) Sampling: It was measured in days (sampling time - ST) and methodology (sampling methodology - SM). Data for ST and SM were obtained directly from the scientific articles and were categorized according to the different methodologies employed in data collection: 1 - active search; 2 - active search + pitfall traps; 3 - active search + pitfall traps + funnel traps; 4 - active search + literature + collections; 5 - active search + pitfall traps + collections; 6 - active search + pitfall traps + literature + collections. In active searches all techniques of visual and acoustic inference were considered.

Analyses

Factor and regression analyses were used to investigate the effect of environmental variables (predictor variables) on anuran richness (response variable). Our factor analysis followed all of the suggestions of Hair *et al.* (2009) and McGarigal *et al.* (2000), and was carried out in R (R Core Team c2016). First, a Pearson correlation and a linear partial correlation were used to verify the presence of collinearity in the data set, with correlations considered significant at $P \leq 0.05$. The partial correlation coefficients were much smaller than the Pearson coefficients, which indicate that there is a combined effect of one or more variables on the dependent variable and the presence of collinearity. In this analysis the sampling time and sampling methodology variables were removed because they had a weak correlation with all of the other variables, which may contribute to the reduction of the values of sample adequacy (MSA). The suitability of the correlation matrix was verified with the Bartlett test of sphericity and the Kayser-Meyer-Olkin factor of adequacy, using the psych package (Revelle c2020). Both analyses demonstrated the suitability of the data matrix (Bartlett = 0.0; MSA = 0.6).

Principal component factorization was used to extract the most relevant variables for explaining anuran richness. For this, a Principal Component Analysis (PCA) (with correlation matrix) was performed and the criterion of latent root (Kaiser-Guttman) was used to select the most relevant components for explaining the variance in the data set, considering values above 1.0 significant. Another PCA using the varimax rotation (PCAr) was carried out with only the selected components with the latent root criterion (explanation of 90 %), using the function principal in psych package. In the PCAr the variables that had loadings higher than 0.4 in more than one component (cross-loads) or that showed commonality below 0.5 were removed from the dataset and a new adjusted PCAr was performed. Subsequently, the scores of the adjusted PCAr were used in a stepwise regression and the Akaike information criterion (AIC) was used to select the components (axes) that best explained the dependent variable. Only variables with loadings greater than 0.6 of the selected components in the stepwise regression were considered relevant for our sample size. Subsequently, a multiple regression ordinary least square (OLS) was used to verify which variables best explain anuran richness.

Analyses involving geographical samples may exhibit spatial autocorrelation, increasing the chances of type I error and biasing the data (Diniz-Filho *et al.* 2003). We verified the presence of spatial structure in anuran richness and in the residuals of the richness through of the correlogram of Moran *I*, using the function correlog in ncf package (Bjornstad c2016). The geoXY function in the SoDA package (Chambers c2013) was used to create coordinates projected on the earth's surface, from a corresponding geodesics. Later, new analyses of correlation of the dependent variable against the selected variables based on the Akaike criterion were performed using the correction of Dutilleul (1993). Finally, generalized least square (GLS) and spatial autoregressive (simultaneous autoregressive, or SAR and conditional autoregressive, or CAR) models were used to verify the association between species richness and the dependent variables. The three models are regression techniques applied to data that demonstrate the presence of spatial structure and were used to allow a better comparison and interpretation of the results. Correlogram, OLS multiple regression, GLS and spatial autoregressive models were performed in SAM software (Rangel *et al.* 2010) and all variables were log transformed before analysis, adopting a significance level of 0.05.

RESULTS

Significant correlations were identified between several predictor variables, indicating the presence of collinearity (Table S2). In the Pearson correlation anuran richness was negatively correlated with DD and positively correlated with VEG, AR, AR3T, AR4T, AR1S and AR2S. However, the highest correlation was observed between richness and AR2S. Strong correlations were observed among the accumulated rainfall in the different temporal clippings and among the different categories of temperature. The linear partial correlation revealed a decrease in the correlations in the data set, showing that controlling the influence of other variables was efficient. Richness showed no significant correlation with any of the variables, indicating that there is a joint effect of two or more variables in the richness prediction. The highest values of linear partial correlation were observed among the accumulated rainfall in the different temporal clippings and among the different categories of temperature.

The principal component factorization promoted the exclusion of the variables that presented significant contribution to more than one component (AR, AR1T, AR1S, MARm, MIRm, SAZrain, MAXT and Thot). Six components were selected in the adjusted PCAr, which together explained 95 % of the variation in the data set (Table 1). The vegetation structure and rainfall at the end of the year (VEG, AR4T and AR2S) presented a joint effect and were the most relevant variables in RC1 (rotated component), explaining 26 % of the variation. Low temperatures (MINT and Tcold) were more relevant in RC2 and explained 20 % of the variation. Demography (POP and DD) was more relevant in RC3 and explained 15 % of the variation. The rainfall in half of the year (AR2T and AR3T) was more relevant in RC4 and explained 18 % of the variation. The seasonality of temperature (SAZtem) was more relevant in RC5 and explained 11 % of the variation. Topography (ELE) was more relevant in RC6 and explained only 10 % of the variation.

Stepwise regression using the scores of the six components of the adjusted PCAr showed that RC1, RC3 and RC4 had a significant effect on species richness. Observation of the loadings indicated that the variables that express vegetation structure (VEG), rainfall (AR2T, AR3T, AR4T and AR2S) and demography (POP and DD) are the best predictors of anuran richness (Table 2). OLS multiple regression involving all of the variables selected in the stepwise regression plus ST and SM indicated significant relationship, explaining 32 % of anuran richness. In spite of this, only sampling variables had a significant relationship with richness.

The analysis of Moran *I* showed the presence of spatial structure in richness and in the residuals of the richness, with a strong effect on the largest classes of distances (Fig. 2). The correlation analysis with the Dutilleul correction showed the absence of correlation between anuran richness and the variable POP ($r = -0.10$, $P = 0.39$). The GLS, SAR and CAR models showed a significant effect of the variables DD, ST and SM over richness (Table 2), indicating that anuran richness decreases as human density increases and increases when there is an increase in the time and in the methodologies used to sample data. The results of the three models showed that the three most relevant variables explained between 37 % and 45 % of the variation in richness.

DISCUSSION

Studies that investigate anuran richness patterns often report a strong influence of climatic variables including both water availability (rainfall) and the energy that is input into the ecosystem (temperature) on species richness (Duellman 1999, Canavero *et al.* 2009, Pyron and Wiens 2013). However, our results showed a weak effect of climatic variables. Of the ten variables associated with water availability, only the accumulated rainfall at the end of the year showed some level of association with the variation in richness. Despite this, when we controlled the effect of space, none of the variables associated with the availabil-

Table 1. Loadings and communalities (h^2) of the six most relevant components in the adjusted PCAr. Values in bold represent significant loadings (> 0.60) in the axes selected through the stepwise regression. For abbreviations of variables, see Material and Methods.

Variables	RC1	RC2	RC4	RC3	RC5	RC6	h^2
POP	0.15	0.16	0.19	**0.87**	0.25	-0.12	0.91
DD	-0.08	-0.24	-0.02	**0.89**	-0.26	0.01	0.93
VEG	**0.78**	-0.18	0.31	0.11	-0.21	-0.27	0.87
ELE	-0.10	-0.19	-0.14	-0.08	0.14	0.95	0.99
AR2T	0.04	0.13	**0.96**	0.07	-0.1	-0.1	0.97
AR3T	0.37	-0.29	**0.79**	0.13	-0.29	-0.1	0.95
AR4T	**0.97**	-0.12	-0.11	-0.01	0.06	0.03	0.96
AR2S	**0.93**	-0.15	0.26	0.01	-0.09	-0.01	0.97
MINT	-0.14	0.96	0.04	-0.03	-0.09	-0.05	0.96
SAZtem	-0.12	-0.17	-0.26	-0.01	0.90	0.16	0.95
Tcold	-0.22	0.93	-0.07	-0.05	-0.06	-0.18	0.95
Eigenvalue	3.77	2.44	1.58	1.20	0.77	0.64	
% Explained	0.26	0.20	0.18	0.15	0.11	0.10	–
% Cumulative	0.26	0.46	0.64	0.79	0.90	1.00	–
AIC -311.06	-296.20	-312.38	-308.22	-307.48	-312.43	-311.22	–
Residual SS	2.79	2.32	2.44	2.46	2.32	2.36	
Stepwise P	0.001	–	0.035	0.024	–	–	–
R^2			0.24				
R^2ajusted			0.21				
F			9.00				

ity of water were relevant, indicating a strong geographic effect. The lack of an effect of climate on anuran richness was even more apparent when the temperature variables were considered. None of the five temperature variables analyzed here had a significant effect on species richness. These results contrast with those by Wright *et al.* (1993), who investigated the relationship between species richness and available energy from a compilation of 53 studies and founded that the majority of studies involving animals showed a relationship between species richness and some measure of heat (temperature, evapotranspiration or solar radiation). Canavero *et al.* (2009) found the same scenario where they identified an effect of the seasonality of temperature on the composition of species in 29 South American anuran communities.

Even though the weather is the most relevant abiotic factor for predicting anuran richness in the Neotropical region, some studies have demonstrated diverse results. For example, Azevedo-Ramos and Galatti (2002) studying similarities among thirteen anuran populations in the Brazilian Amazon, found no association between anuran richness and rainfall. The lack of an effect of climate on anuran richness has two possible explanations: first, there are other factors acting on richness such as historical influences, competition or predation (Buckley and Jetz 2007) or second, there is a joint effect among different climatic

Table 2. Significance of the most relevant variables for explaining anuran richness in four models of regression. **P* ≤ 0.05. Empty cells represent variables without correlation with richness after Dutilleul correction. In GLS models the spherical function was used.

	OLS R^2 adjusted = 0.32 F = 5.44		GLS R^2 adjusted = 0.37 F = 6.01		SAR R^2 adjusted = 0.45 F = 5.73		CAR R^2 adjusted = 0.45 F = 5.53	
	Coefficient	*t*	Coefficient	*t*	Coefficient	*t*	Coefficient	*t*
VEG	0.048	0.28	0.013	0.08	0.001	0.01	-0.186	-1.16
POP	-0.076	-0.62	–	–	–	–	–	–
DD	-0.191	-1.46	-0.239	-2.48*	-0.23	-2.19*	-0.286	-2.59*
AR2T	-0.019	-0.11	-0.070	-0.45	-0.097	-0.53	-0.144	-0.69
AR3T	0.276	1.09	0.343	1.46	0.432	1.49	0.467	1.50
AR4T	0.373	1.20	0.384	1.29	0.433	1.24	0.299	0.76
AR2S	-0.154	-0.39	-0.175	-0.49	-0.211	-0.50	-0.019	-0.04
ST	0.260	2.85*	0.246	2.83*	0.245	2.64*	0.248	2.54*
SM	0.264	2.86*	0.257	2.90*	0.165	1.72	0.226	2.35*

variables (i.e. collinearity) (Moura *et al.* 2016). Many studies have postulated the existence of a positive correlation between species richness and precipitation and temperature, based on information from current evapotranspiration and net primary productivity. Although these four environmental variables are strongly correlated amongst themselves (Hawkins *et al.* 2003, Gouveia *et al.* 2013), it is possible that the direct analysis of rainfall and temperature does not reflect the effect of evapotranspiration or productivity, which might explain why no relationship was found. Thus, we do not disregard the influence of productivity as a relevant variable in the explanation of anuran richness in Brazilian biomes.

In all of our regression and autoregression models, the sampling variables showed considerable power to explain richness. Thus, studies that registered a greater number of species were those that spent more time collecting field data and employed a larger number of methodologies. The effect of the sample design on the measurement of species richness present in a given locality is already widely known by the ecologists (Gotelli and Chao 2013). Studies where the researchers spent less time measuring richness proportionally increased the chance of missing rare species or those that are difficult to detect (Magurran 2004), which also increases the chances of biasing the results. The relationship between the number of inventories (knowledge of biodiversity) and description of new species, for example, was revealed for anurans in the Brazilian Cerrado (Diniz-Filho *et al.* 2005). This result indicates that better sampled regions have greater richness and corroborates our findings.

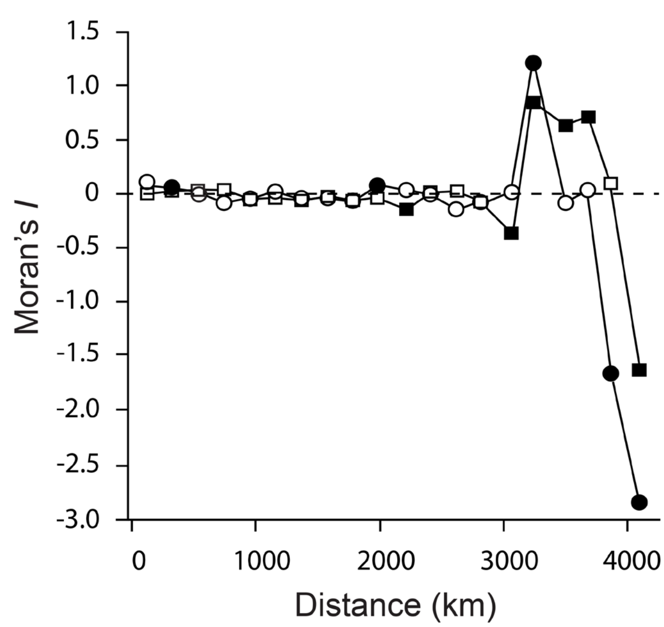

Figure 2. Correlograms (20 distance classes) of anuran richness (circles) and residuals of the multiple regression OLS (squares). Black symbols indicate significant autocorrelations (*P* ≤ 0.05).

Despite this, the effect of this variable has been neglected both in studies that seek to measure species richness at a local level and in studies that use these data to reveal patterns of species richness on a global scale (Ferrer *et al.* 2006). It is possible that the short time devoted to measuring species richness is more closely related to factors external to the fieldwork than to the lack of a methodological contribution in the sample design. The lack of logistical apparatus and financial resources that allow the maintenance of long-term studies, coupled with the urgency to deliver results and reports of biological information for the implementation of policies for environmental preservation, seem to be some of the reasons for the short execution time of the fieldwork. Heyer *et al.* (1994) presented a number of comments about the negative effects of random sampling on measuring the richness and abundance of amphibians and argued that it makes comparative analyses among different studies impossible.

Due to the existing correlation between species richness and sampling time, some questions can be raised, such as: Could researchers be spending more time studying notably more diverse areas and promoting a subsampling of less diverse areas? Or could the existence of vast areas not sampled lead to a misinterpretation about areas that have a great species richness? It is perfectly possible that both scenarios are real and hinder our interpretation of the richness pattern of anurans in Brazil. Thus, studies that take into account the formation of regional patterns of richness variation can help to clarify these issues. Our results highlight the need to consider the effect of sampling time on studies aimed at measuring species richness at a small or large spatial scale, in order to avoid bias in results and interpretations.

Another interesting result was the influence of human density on anuran richness. Demographic density is the most frequently used indicator to analyze the level of human activity on the landscape and species diversity (Sanderson *et al.* 2002, Huston 2005) and its use in the identification of the influence of human activities on biodiversity is not a new approach (Holdren and Ehrlich 1974). Since concern for the preservation and management of environmental resources has gained prominence in the world, the relationship between human activity and species richness has helped to delimit areas with important biodiversity, define threatened species and direct environmental policies toward better resource management (Cincotta and Engelman 2000, Chown *et al.* 2003, Diniz-Filho *et al.* 2006, Luck 2007). The relationship between species richness and the human density has already been demonstrated for many species and has different effects on different taxa (Luck 2007). The diversity of mammals, birds, butterflies and amphibians seems to present a strong congruence to the human population density, revealing a threat to biodiversity. On the other hand, the diversity of reptiles seems to be inversely proportional to the increase of population human (Luck *et al.* 2004). Many authors have argued that the congruence between species richness and the human footprint can be associated with the primary productivity and energy levels of ecosystems (Gaston 2000, Luck 2007). Humans tend to establish settlements in regions with high availabilities of energy, notably warmer and productive regions (Gaston 2005). Thus, there is a tendency of humans and other animal lineages to spread over areas with the same environmental characteristics, such as higher temperatures and higher humidity. For humans these conditions are conducive to agricultural development, while for other animals they provide a higher availability of food, breeding sites and shelter (Chown *et al.* 2003, Gaston 2005).

According to Pautasso (2007), the influence of humans on biodiversity can have a bias associated with the scale of study employed in the analysis. Thus, investigations on a large scale might find a positive correlation between humans and diversity, indicating congruence between these two variables. In another way, investigations on a fine scale, which consider patterns of biodiversity in sample units of up to 1 km^2, might find a negative correlation. Looking exclusively for amphibians, many studies have demonstrated the absence of a negative relationship between the diversity of amphibians and human occupation. Diniz-Filho *et al.* (2006) found a positive relation between anuran richness and size of the human population in the Brazilian Cerrado. In addition, regions with a higher richness of anurans coincided with less populated areas, which tend to minimize conflicts regarding the preservation of the Cerrado biome. Moreno-Rueda and Pizarro (2009) found similar results when they analyzed patterns of vertebrate richness in Spain, where amphibian richness was positively correlated with the humanized surface area (modification of the landscape for agriculture), suggesting that the irrigation and cultivable areas may promote the formation of bodies of water that favor anuran survival. Evans *et al.* (2006) investigated the influence of human population growth on anuran richness in South Africa from a temporal clipping of five years (1996–2001) and did not find any correlation, suggesting that the increase in human population does not affect anuran richness.

Contrary to these views, we found a negative correlation between anuran richness and human density. Some studies have also demonstrated an inversely proportional relationship between the two variables, with strong implications for the risk of extinction of different species of the group, diversification rate, or evolution of phenotypic traits (Jenkins *et al*. 2013, Trimble and Van Aarde 2014, Escoriza and Ruhí 2014). Furthermore, there are evidences that the changes caused by human activity can also change the ecology of many species, promoting an adaptive advantage to the species that fit their requirements in accordance with resource availability in the environment (López *et al*. 2015), which may reflect the composition of existing species in the habitat (Rocha *et al*. 2008). The identification of a negative correlation between anuran richness and human activity on a large spatial scale has been reported in independent studies from various parts of the world (Assunção-Albuquerque *et al*. 2012, Trimble and Van Aarde 2014, Cruz-Elizalde *et al*. 2016). Despite evidence for variation in the effects of the human footprint due to the scale of the study, a negative correlation among these variables seems to also be evident at a fine scale (Cruz-Elizalde *et al*. 2016), suggesting a tendency for anuran species richness to decrease in places with great human activity or presence.

Based on the evidences mentioned above, the negative relationship between the human footprint and anuran richness found here should be interpreted with caution. This negative relationship does not absolutely mean that the greatest concentration of humans, and consequently the increased landscape change, is the factor of that causes a decrease of anuran richness in Brazilian biomes. It is possible that sites that have higher anuran richness do not coincide with sites that have a higher density of humans. The highest density of humans occurs in places with high energy and productivity (Gaston 2005). In contrast, the relationship between anuran richness and productivity can be guided by the geographic variation that influences different spatial patterns of species richness (Gouveia *et al*. 2013), which might determine the non-congruence between anuran richness and human density.

The strong spatial effect on anuran richness and also on the residual richness observed in our study suggests that the environmental variables that were investigated by us do not have sufficient weight to explain the spatial autocorrelation, This finding indicates that patterns of anuran richness in Brazilian biomes can be molded by other mechanisms, and help us understand why our model explained only between 37 and 45% of the variation in species richness. Thus, the history of diversification and dispersion of different species in the different biomes and geographical areas of Brazil seems to be a plausible hypothesis to help explain the mechanisms that regulate the distribution of species across different geographical areas. Some studies have already evidenced the influence of the history of the diversification of anurans on the richness patterns on a large spatial scale (Gonzalez-Voyer *et al*. 2011, Chejanovski *et al*. 2014), which may justify our interpretation. Thus, analyses of other environmental mechanisms that may influence richness such as origin, rate of speciation, extinction, dispersion, and interactions in the community, as well as qualitative analyses considering the composition of species in each locality, can reveal new information to better define this scenario. Finally, we suggest that further studies that analyze the pattern of anuran richness at different spatial scales take into consideration the time of sampling and the influence of the human footprint, thus preventing the results from being biased by uninvestigated variables that may exert some degree of effect on species richness.

AUTHOR'S CONTRIBUTION

ArSP conceived the study; LCC collected data and built database; ArSP and AiSP designed and perfomed the statistical analyses; ArSP and AiSP wrote the manuscript with significant imput from LCC.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We are grateful to numerous authors of the articles that us utilized for built the database. We are also grateful to reviewers for the valuable suggestions and comments that have improved this manuscript. Our special thanks to Juliana Paiva Santiago and Francisco Javier Chain Villar for help with the Spanish language.

LITERATURE CITED

Aichinger M. 1987. Annual activity patterns of anurans in seasonal Neotropical environment. Oecologia 71: 583–592. doi: http://doi.org/10.1007/BF00379302

Assunção-Albuquerque MJT, Rey Benayas JM, Rodríguez MA, Albuquerque FS. 2012. Geographic patterns of vertebrate diversity and identification of relevant areas for conservation in Europe. Anim. Biodiv. Conserv. 35(1): 1–11. http://doi.org/10.32800/abc.2012.35.0001

Azevedo-Ramos C, Galatti U. 2002. Patterns of amphibian diversity in Brazilian Amazonia: conservation implications. Biol. Conserv. 103(1): 103–111. doi: http://doi.org/10.1016/S0006-3207(01)00129-X

Bertoluci J, Rodrigues MT. 2002. Seasonal patterns of breeding activity of Atlantic Rainforest anurans at Boracéia, Southeastern Brazil. Amphibia-Reptilia 23(2): 161–167. doi: http://doi.org/10.1163/156853802760061804

Bjornstad ON. c2016. ncf: spatial nonparametric covariance functions. R package version 1.1-7. [Last accessed: 02 Dez 2019]. https://cran.rproject.org/web/packages/nfc

Bossuyt F, Roelants K. 2009. Frogs and toads (Anura). In: Hedges SB, Kumar S, editors. The Timetree of Life. Oxford: Oxford University Press. p. 357–364.

Brown RL, Jacobs LA, Peet RK. 2016. Species richness: small scale. In: Encyclopedia of Life Sciences. Chichester: John Wiley & Sons. p. 1–8.

Buckley LB, Jetz W. 2007. Environmental and historical constraints on global patterns of amphibian richness. Proc. R. Soc. B. 274(1614): 1167–1173. doi: http://doi.org/10.1098/rspb.2006.0436

Canavero A, Arim M, Brazeiro A. 2009. Geographic variations of seasonality and coexistence in communities: the role of diversity and climate. Austral Ecol. 34(7): 741–750. doi: http://doi.org/10.1111/j.1442-9993.2009.01980.x

Chambers JM. c2013. Package 'SoDA': Functions and examples for "software for data analysis". R package version 1.0-6. [Last accessed: 02 Dez 2019]. http://cran.r-project.org/web/packages/SoDA

Chejanovski ZA, Wiens JJ. 2014. Climatic niche breadth and species richness in temperate treefrogs. J. Biogeogr. 41(10): 1936–1946. doi: http://doi.org/10.1111/jbi.12345

Chown SL, Van Rensburg BJ, Gaston KJ, Rodrigues ASL, Van Jaarsveld AS. 2003. Energy, species richness, and human population size: conservation implications at a national scale. Ecol. Appl. 13(5): 1233–1241. doi: http://doi.org/10.1890/02-5105

Cincotta RP, Engelman R. 2000. Nature's Place: Human Population and the Future of Biological Diversity. Washington: Smithsonian Institute.

Climatempo Database. c2019. Climatologia. [Last accessed: 03 Mar 2021]. http://climatempo.com.br

Collins JP, Storfer A. 2003. Global amphibian declines: sorting the hypotheses. Divers. Distrib. 9(2): 89–98. doi: http://doi.org/10.1046/j.1472-4642.2003.00012.x

Coutinho LM. 2016. Biomas Brasileiros. São Paulo: Oficina de Textos.

Cruz-Elizalde C, Berriozabal-Islas C, Hernández-Salinas U, Martínez-Morales MA, Ramírez-Bautista A. 2016. Amphibian species richness and diversity in a modified tropical environment of central Mexico. Trop. Ecol. 57(3): 407–417.

Currie DJ. 1991. Energy and large-scale patterns of animal-and plant-species richness. Am. Nat. 137(1): 27–49. doi: http://doi.org/10.1086/285144

Diniz-Filho JAF, Bastos RP, Rangel TFLVB, Bini LM, Carvalho P, Silva RJ. 2005. Macroecological correlates and spatial patterns of anuran description dates in the Brazilian Cerrado. Global Ecol. Biogeogr. 14(5): 469–477. http://doi.org/10.1111/j.1466-822X.2005.00165.x

Diniz-Filho JAF, Bini LM, Hawkins BA. 2003. Spatial autocorrelation and red herrings in geographical ecology. Global Ecol. Biogeogr. 12(1): 53–64. doi: http://doi.org/10.1046/j.1466-822X.2003.00322.x

Diniz-Filho JAF, Bini LM, Pinto MP, Rangel TFLVB, Carvalho P, Bastos RP. 2006. Anuran species richness, complementarity and conservation conflicts in Brazilian Cerrado. Acta Oecol. 29(1): 9–15. doi: http://doi.org/10.1007/s10531-006-9010-4

Duellman WE. 1999. Patterns of Distribution of Amphibians: A Global Perspective. Baltimore: John Hopkins University Press.

Dutilleul P, Clifford P, Richardson S, Hemon D. 1993. Modifying the t test for assessing the correlation between two spatial processes. Biometrics 49(1): 305–314. doi: https://doi.org/10.2307/2532625

Escoriza D, Ruhí A. 2014. Macroecological patterns of amphibian assemblages in the western Palearctic: implications for conservation. Biol. Conserv. 176: 252–261. doi: http://doi.org/10.1016/j.biocon.2014.05.030

Evans KL, Van Rensburg BJ, Gaston KJ, Chown EL. 2006. People, species richness and human population growth. Global Ecol. Biogeogr. 15(6): 625–636. doi: http://doi.org/10.1111/j.1466-8238.2006.00253.x

Ferrer X, Carrascal LM, Gordo O, Pino J. 2006. Bias in avian sampling effort due to human preferences: an analysis with catalonian birds (1900 - 2002). Ardeola 53(2): 213–227.

Frost DR. c2020. Amphibian Species of the World: an online reference. Version 6.0. American Museum of Natural History, New York, USA. [Last accessed: 3 Feb 2020]. http://amphibiansoftheworld.amnh.org

Gaston KJ. 2000. Global patterns in biodiversity. Nature 405: 220–227. doi: http://doi.org/10.1038/35012228

Gaston KJ. 2005. Biodiversity and extinction: species and people. Prog. Phys. Geogr. 29(2): 239–247. doi: http://doi.org/10.1191/0309133305pp445pr

Gonzalez-Voyer A, Padial JM, Castroviejo-FIsher S, De La Riva I, Vila C. 2011. Correlates of species richness in the largest Neotropical amphibian radiation. J. Evol. Biol. 24(5): 931–942. doi: http://doi.org/10.1111/j.1420-9101.2011.02243.x

Google Earth. c2019. Google Earth Pro. Version 7.3. Google LLC. [Last access: 03 Mar 2021]. http://google.com.br/earth

Gotelli NJ, Chao A. 2013. Measuring and estimating species richness, species diversity, and biotic similarity from sampling data. In: Levin SA, editor. Encyclopedia of Biodiversity, 2 edition, 5 volume. Waltham: Academic Press .p. 195-211.

Gouveia SF, Hortal J, Cassemiro FAS, Rangel TF, Diniz-Filho JAF. 2013. Nonstationary effects of productivity, seasonality, and historical climate changes on global amphibian diversity. Ecography 36(1): 104–113. doi: http://doi.org/10.1111/j.1600-0587.2012.07553.x

Graham MH. 2003. Confronting multicollinearity in ecological multiple regression. Ecology 84(11): 2809–2815. doi: http://doi.org/10.1890/02-3114

Hair JF, Black WC, Babin BJ, Anderson RE, Tatham RL. 2009. Análise Multivariada de Dados. Porto Alegre: Bookman.

Hawkins BA, Porter EE, Diniz-FIlho JAF. 2003. Productivity and history as predictors of the latitudinal diversity gradient of terrestrial birds. Ecology 84(6): 1608–1623. doi: http://doi.org/10.1890/0012-9658(2003)084[1608:PAHAPO]2.0.CO;2

Heyer WR, Donnely MA, McDiarmid RW, Hayek LAC, Foster MS. 1994. Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Washington: Smithsonian Institution Press.

Holdren JP, Ehrlich PR. 1974. Human population and the global environment: population growth, rising per capita material consumption, and disruptive technologies have made civilization a global ecological force. Am. Sci. 62(3): 282–292.

Huston MA. 2005. The three phases of land-use change: implications for biodiversity. Ecol. Appl. 15(6): 1864–1878. doi: http://doi.org/10.1890/03-5281

IBGE] Insituto Brasileiro de Geografia e Estatística. c2017. Cidades. IBGE. [Last access: 03 Mar 2021]. http://cidades.ibge.gov.brc

Jenkins CN, Pimm SL, Joppa LN. 2013. Global patterns of terrestrial vertebrate diversity. PNAS 110(28): E2602–E2610. doi: http://doi.org/10.1073/pnas.1302251110

Joly CA, Aidar MPM, Klink CA, McGrath DG, Moreira AG, Moutinho P, Nepstad DC, Oliveira AA, Pott A, Rodal MJN, Sampaio EVSB. 1999. Evolution of the Brazilian phytogeography classification systems: implications for biodiversity conservation. Ciência e Cultura, 51(5/6): 331–348.

López JA, Scarabotti PA, Ghirardi R. 2015. Amphibian trophic ecology in increasingly human-altered wetlands. Herpetol. Conserv. Biol. 10(3): 819–832.

Luck GW, Ricketts TH, Daily GC, Imhoff M. 2004. Alleviating spatial conflict between people and biodiversity. Proc. Natl. Acad. Sci. USA. 101(1): 182–186. doi: http://doi.org/10.1073/pnas.2237148100

Luck GW. 2007. A review of the relationships between human population density and biodiversity. Biol. Rev. 82(4): 607–645. doi: http://doi.org/10.1111/j.1469-185X.2007.00028.x

Magurran AE. 2004. Measuring biological diversity. Oxford: Blackwell.

McGarigal K, Cushman S, Stafford S. 2000. Multivariate Statistics for Wildlife and Ecology Research. New York: Springer.

Montoya D, Rodríguez MA, Zavala MA, Hawkins BA. 2007. Contemporary richness of holarctic trees and the historical pattern of glacial retreat. Ecography 30(2): 173–182. doi: http://doi.org/10.1111/j.0906-7590.2007.04873.x

Moreno-Rueda G, Pizarro M. 2009. Relative influence of habitat heterogeneity, climate, human disturbance, and spatial structure on vertebrate species richness in Spain. Ecol. Res. 24: 335–344. doi: http://doi.org/10.1007/s11284-008-0509-x

Moura MR, Villalobos F, Costa GC, Garcia PCA. 2016. Disentangling the role of climate, topography and vegetation in species richness gradients. Plos One 11(3): 1–16. doi: http://doi.org/10.1371/journal.pone.0152468

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature 403: 853–858. doi: http://doi.org/10.1038/35002501

Oseen KL, Wassersug RJ. 2002. Environmental factors influencing calling in sympatric anurans. Oecologia 133(4): 616–625. doi: http://doi.org/10.1007/s00442-002-1067-5

Pautasso M. 2007. Scale dependence of the correlation between human population presence and vertebrate and plant species richness. Ecol. Lett. 10(1): 16–24. doi: http://doi.org/10.1111/j.1461-0248.2006.00993.x

Pyron RA, Wiens JJ. 2013. Large-scale phylogenetic analyses reveal the causes of high tropical amphibian diversity. Proc. Biol. Sci. 280(1779): 1–10. doi: http://doi.org/10.1098/rspb.2013.1622

Qian H, Wang X, Wang S, LI Y. 2007. Environmental determinants of amphibian and reptile species richness in China. Ecography 30: 471–482. doi: http://doi.org/10.1111/j.0906-7590.2007.05025.x

R Core Team. c2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [Last accessed: 25 Sep 2017]. http://www.r-project.org

Rangel TF, Diniz-Filho JAF, Bini LM. 2010. SAM: a comprehensive application for spatial analysis in macroecology. Ecography 33: 1–5. doi: http://doi.org/10.1111/j.1600-0587.2009.06299.x

Revelle W. c2020. Psych: procedures for personality and psychological research. Northwestern University, Evanston, Illinois. R package version 2.0.12. [Last access: 03 Mar 2021]. http://CRAN.R-project.org/package=psych

Ricklefs RE. 2005. Historical and ecological dimensions of global patterns in plant diversity. Biol. Skr. 55: 583–603.

Rocha CFD, Hatano FH, Vrcibradic D, Van Sluys M. 2008. Frog species richness, composition and β-diversity in coastal Brazilian restinga habitats. Braz. J. Biol. 68(1): 101–107. doi: http://doi.org/10.1590/S1519-69842008000100014

Sanderson EW, Jaiteh M, Levy MA, Redford KH, Wannebo AV, Woolmer G. 2002. The human footprint and the last of the wild. BioScience 52(10): 891–904. doi: https://doi.org/10.1641/0006-3568(2002)052[0891:THFATL]2.0.CO;2

Segalla MG, Berneck B, Canedo C, Caramaschi U, Cruz CAG, Garcia PCA, Grant T, Haddad CFB, Lourenço ACC, Mângia S, Mott T, Nascimento LB, Toledo LF, Werneck FP, Langone JA. 2021. List of Brazilian amphibians. Herpetol. Bras. 10(1): 121–216.

Trimble MJ, Van Aarde RJ. 2014. Amphibian and reptile communities and functional groups over a land-use gradient in a coastal tropical forest landscape of high richness and endemicity. Anim. Conserv. 17(5): 441–453. doi: http://doi.org/10.1111/acv.12111

Wiens JJ. 2007. Global Patterns of diversification and species richness in amphibians. Am. Nat. 170(S2): S86–S106. doi: http://doi.org/10.1086/519396

Wiens JJ. 2015. Explaining large-scale patterns of vertebrate diversity. Biol. Lett. 11(7): 1–4. doi: http://doi.org/10.1098/rsbl.2015.0506

Williams PH, Margules CR, Hilbert DW. 2002. Data requirements and data sources for biodiversity priority area selection. J. Biosci. 27: 327–338. doi: http://doi.org/10.1007/BF02704963

Willig MR, Kaufman DM, Stevens RD. 2003. Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. Annu. Rev. Ecol. Evol. Syst. 34: 273–309. doi: http://doi.org/10.1146/annurev.ecolsys.34.012103.144032

Wright DH, Currie DJ, Maurer BA. 1993. Energy supply and patterns of species richness on local and regional scale. In Ricklefs RE, Schluter D, editors. Species diversity in ecological communities: historical and geographical perspectives. Chicago: The University of Chicago Press. p 66–74.