

**University of São Paulo  
“Luiz de Queiroz” College of Agriculture  
Center of Nuclear Energy in Agriculture**

**Community structure of anurans along an altitudinal gradient: the  
role of topographic and climatic variables and their implications for  
conservation**

**Rodrigo Augusto Matavelli**

Thesis presented to obtain the degree of Doctor in  
Science. Area: Applied Ecology

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**Rodrigo Augusto Matavelli**  
**Bachelor in Biological Sciences**

**Community structure of anurans along an altitudinal gradient: the role of topographic and climatic variables and their implications for conservation**

versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:  
Prof. Dr. **JAIME BERTOLUCI**  
Co-Advisor:  
Prof. Dr. **MILTON CEZAR RIBEIRO**

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## RESUMO

### **Estrutura da comunidade de anuros ao longo do gradiente altitudinal: o papel das variáveis topográficas e climáticas e suas implicações para a conservação**

Os ecossistemas montanhosos cobrem aproximadamente 22 a 25% da superfície terrestre desde o nível do mar até mais de 8.000 m, abrigam mais de um terço da biodiversidade do planeta incluindo metade dos hotspots globais de biodiversidade. Entre os gradientes geográficos (latitudinais ou altitudinais), o padrão latitudinal de riqueza de espécies é o mais reconhecido e estudado. Embora não tão intensamente estudados como os gradientes latitudinais, os gradientes altitudinais também fornecem ótimos padrões de distribuição de riqueza de espécies. Apesar dos processos que impulsionam os padrões de riqueza de espécies ainda serem pouco compreendidos, três principais padrões tem sido relatados ao longo dos gradientes altitudinais: 1) decréscimo da riqueza de espécies com o aumento da altitude; 2) aumento da riqueza de espécies com o aumento da altitude and 3) aumento da riqueza de espécies em altitudes intermediárias (unimodal padrão), seguido por uma diminuição da riqueza de espécies com o aumento da altitude. O unimodal padrão é considerado o mais comum. Uma hipótese macroecológica que explica os padrões de riqueza de espécies ao longo de gradientes geográficos com foco no tamanho das faixas altitudinais das espécies é Rapoport regra. Esta prediz uma correlação positiva entre a altitude e a distribuição do tamanho das faixas altitudinais das espécies com base nos efeitos da sazonalidade climática. Rapoport regra prediz que as espécies que podem suportar um ampla variabilidade climática podem se tornar mais amplamente distribuídas ao longo dos gradientes geográficos. No entanto, essa hipótese ainda apresenta resultados controversos o que aumentaram nosso interesse em testar a Rapoport altitudinal regra em ecossistemas montanhosos na Mata Atlântica. Entretanto, os padrões e os processos que conduzem a montagem da comunidade ao longo dos gradientes altitudinais receberam pouca atenção e ainda permanecem controversos. Com base na variação da estrutura da comunidade de anuros (riqueza, composição e abundância) ao longo dos gradientes altitudinais, o presente estudo teve como objetivo compreender e desemaranhar os efeitos topográficos e climáticos nos padrões de distribuição espacial e tamanho das faixas altitudinais das espécies em ecossistemas montanhosos na Mata Atlântica, o qual realçará como as condições topográficas e climáticas atuam na montagem de comunidades ao longo de gradientes de altitude subsidiando importantes regras para a conservação da biodiversidade.

Palavras-chave: Anfíbios; Ecossistemas montanos; Filtros ambientais; Mata Atlântica; Regra de Rapoport; Planalto de Poços de Caldas;



## ABSTRACT

### **Community structure of anurans along an altitudinal gradient: the role of topographic and climatic variables and their implications for conservation**

Montane ecosystems cover approximately 22 to 25% of land surface from sea level to more than 8,000 m a.s.l., harboring more than a third of the planet's biodiversity and include half of global biodiversity hotspots. Among geographical gradients (latitudinal or altitudinal), the latitudinal species richness pattern is the most recognized and studied. Although not so intensively studied as latitudinal gradients, altitudinal gradients also provide great patterns of species richness distributions. Despite of the processes that driving the species richness patterns are still poorly understood, three main patterns have been reported along altitudinal gradients: 1) decreasing of species richness with increasing altitude, 2) increase in species richness with increased altitude, and 3) increasing species richness at intermediate altitudes (*hump-shaped pattern*), followed by a decreasing of species richness with increasing altitude. The hump-shaped pattern is considered the most common. A macroecological hypothesis that to explain species richness patterns along geographical gradients focusing in species range size is Rapoport's rule. This rule is a positive correlation between altitude and species range size distribution based on climate seasonality effects. Rapoport's rule predicts that species that can withstand broad climatic variability can become more widely distributed along geographical gradients. However, this hypothesis still has presented controversial results and this controversial results increased our interest in testing Rapoport's altitudinal rule in Atlantic Forest mountain ecosystems biome. However, patterns and process that drives community assembly along altitudinal gradients have received little attention and remain controversial. Based on the anuran community structure variation (richness, composition and abundance) along altitudinal gradients, the present work aims to understand and disentangle the topographic and climatic effects on spatial patterns distribution and species altitudinal range size in the Atlantic Forest highlands, which will highlight how topographic and climate conditions acting in communities' assembly along altitudinal gradients subside important rules to biodiversity conservation.

**Keywords:** Amphibians; Environmental filters; Atlantic Forest; Montane ecosystem; Rapoport rule; Plateau Poços de Caldas;

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## 1. GENERAL INTRODUCTION

Montane ecosystems cover approximately 22 to 25% of land surface and are worldwide distributed (Martinelli 2007, Romeo et al. 2015, Egan and Price 2017), occurring from sea level to more than 8,000 m a.s.l. (Hoorn et al. 2018). Furthermore, these ecosystem harboring more than a third of the planet's biodiversity (Chape et al. 2008) including half of global biodiversity hotspots (Körner et al. 2004, Mittermeier et al. 2011, Spehn et al. 2011, Antonelli 2015, Hoorn et al. 2018).

Mountainous regions also provide some ecosystem service as cultural (education, recreation, scenic beauty, tourism, cultural heritage, aesthetic values, spiritual and religious values), regulating/supporting (climate and air quality, potable water flow, and reduce erosion) and provisioning (food and fiber/fodder and timber, genetic resources and mainly potable freshwater), being considered important resources for human life maintenance around the world (Egan and Price 2016, Körner et al. 2017).

A most critical ecosystem service provided by mountainous regions is freshwater production, so mountains are the 'water towers' of the world (Viviroli et al. 2007, Vanham and Rauch 2009). On the other hand, nowadays million of people live in mountain regions (bases, slopes or highlands) worldwide (Romeo et al. 2015, Körner et al. 2017), where at least half of the planet's population depends on water originating from mountain tops or use soil to food production. Thus, mountain ecosystems have experienced high rates of landscape transformation around the world (Martinelli 2007), affecting its biodiversity and ecosystem services production.

In South America, the main mountain ranges are located in the Andes, Bolivian Altiplano, Guiana Highlands, and Brazilian Highlands (Hoorn et al. 2018). In Brazil, the largest mountain ranges are located in the Amazon, Caatinga, Cerrado and Atlantic Forest biomes (Benites et al. 2007, Martinelli 2007, Ribeiro et al. 2007, Gontijo-Pascutti et al. 2012). However, the most studied mountain ecosystems are the "rocky fields" in the Cerrado biome (Medina and Fernandes 2007, Velten and Garcia 2007, Borges et al. 2011, Nunes et al. 2016, Perillo et al. 2017, Mota et al. 2018) and the "altitude fields" in the Atlantic Forest biome (Giaretta et al. 1999, Safford and Martinelli 2000, Caiafa and Silva 2007, Ribeiro et al. 2007, Joly et al. 2012, Eisenlohr et al. 2013). On the other hand, despite the high number of rocky outcrops in highlands within the Amazonia and Caatinga biomes (Martinelli 2007), to our knowledge no studies about altitudinal gradients has been developed in these environments.

Among geographical gradients (latitudinal or altitudinal), the latitudinal species richness pattern is the most recognized and studied (Wallace 1878, Stevens et al. 1989, Rosenzweig 1995, Gaston 2000, Hillebrand et al. 2004) and predicts a species richness decline (for most taxa) with latitudinal increasing distance from the Equator to the poles (Stevens et al. 1989). Although not so intensively studied as latitudinal gradients, altitudinal gradients also provide great spatial species richness patterns (Stevens et al. 1992, Rahbek 1995, McCain and Grytnes 2010, Sanders and Rahbek 2012) and predicts a species richness decline (for most taxa) with increasing of altitude (Stevens et al. 1992).

Nowadays, altitudinal gradients are considered powerful natural experiments (Körner 2007) and a mirror of the latitudinal pattern, but on a smaller scale, perhaps because they offer some peculiar characteristics as rapid changes in climate, soil and vegetation over relatively short geographic distances (Körner 2007, Hoorn et al. 2018). These attributes make them ideal environments to testing ecological and evolutionary processes (Körner 2007) that shaping species distributions and community's assembly (Rahbek 1995, McCain and Grytnes 2010, Sanders and Rahbek 2012). Thus, in the last decades the interest of researchers about the altitude effects on different taxa and communities has increased as proxy to understand the community assembly and biodiversity diversification, since a great diversity of biological parameters and processes, such as interspecific interactions, productivity and habitat heterogeneity (Rosenzweig 1995, Mittelbach et al. 2001, Rowe 2009), or some evolutionary and historical processes, such as conservatism niche, isolation, phylogeny and speciation, endemism and evolutionary diversification (Brown 2001, Lomolino 2001, Hawkins et al. 2007, Li et al. 2009, Machac et al. 2011) can vary along geographical gradients and affect different taxonomic groups (Rahbek 1995, Lomolino 2001, McCain and Grytnes 2010, Sanders and Rahbek 2012, Yu et al., 2013).

Several studies conducted with different taxa and geographic regions support that biodiversity changes along altitudinal gradients around the world (Stevens 1992, Rahbek 1995, McCain and Grytnes 2010, Sanders and Rahbek 2012, Szewczyk and McCain 2016) and some mechanisms, as climatic, biological, evolutionary and historical process, have been proposed to explain spatial species richness patterns (Rahbek et al. 1995, McCain and Grytnes 2010, Sanders and Rahbek 2012). However, it is clear that species distributions are not random (Rahbek 1997) and that the processes underlying spatial species distributions along geographical gradients are still poorly understood (Grau et al. 2007, McCain and Knight 2013, Tomašových et al. 2015, Gallou et al. 2017). On the other hand, although processes driving patterns of species richness are still poorly understood, three main patterns have been

reported along altitudinal gradients: 1) decreasing of species richness with increasing altitude, 2) increase in species richness with increased altitude, and 3) increasing species richness at intermediate altitudes (*hump-shaped pattern*), followed by a decreasing of species richness with increasing altitude. The hump-shaped pattern is considered the most common (Rahbek 1995, McCain and Grytnes 2010, Sanders and Rahbek 2012, Szewczyk and McCain 2016).

A macroecological hypothesis that tries to explain species richness patterns along geographical gradients focusing in species range size is known as the Rapoport's rule (Stevens 1989, 1992). This rule was initially proposed to explain species range size along latitudinal gradients (Steven 1989), being later extended to altitudinal gradients (Stevens 1992). Rapoport's altitudinal rule is a positive correlation between altitude and species ranges size distribution (Stevens 1992) based on climate seasonality effects.

Rapoport's altitudinal rule predicts that as climatic conditions vary more at higher altitudes, highland species must have broad climatic and/or physiological tolerances, and hence larger altitudinal ranges than lowland species that have narrower climatic and/or physiological tolerances and hence smaller altitudinal ranges due to more stable environmental conditions than highland (Teitinen et al. 2016). It is worth noting that unlike the null model (Colwell and Lees 2000, Colwell et al. 2004), Rapoport's rule attempts to explain species richness patterns based on environmental factors. Thus, Stevens (1992) proposed climatic variability hypothesis or simply Rapoport "rescue" hypothesis trying to explain this rule. The climatic variability hypothesis predicts that species that can withstand broad climatic variability can become more widely distributed along geographical gradients (Stevens 1989, 1992; see also Gaston and Chown 1999). However, Rapoport's rule still has presented controversial results (Hawkins and Diniz-Filho 2006, Tomašových et al. 2015), with works supporting (Fleishman et al. 1998, Almeida-Neto 2006, Rohner et al. 2015) and others not supporting (Ribas and Schoereder 2006, McCain and Knight 2013, Wang and Soininen 2017). Such controversial results increased our interest in testing Rapoport's altitudinal rule in mountain ecosystems in the Atlantic Forest highlands.

The Atlantic Forest is known as one of the most biodiverse regions of Earth, with high rarity, endemism and threatened species indexes, including the anurans (Morellato and Haddad 2000; Haddad et al. 2013; Rossa-Feres et al. 2017). On the other hand, this biome has been explored for high anthropic activities during the last 500 years. All these attributes make Atlantic Forest a conservation hotspot (Myers et al. 2000, Morellato and Haddad 2000, Mittermeier et al. 2004). Despite its high species richness and endemism, the Atlantic Forest is historically the oldest biome in terms of different exploitation types due to its long history

of human occupation (Dean 1996). This long-term human occupation modified the land-use and consequently reduced the native vegetation to 8-12% of its original extent (1.3 millions km<sup>2</sup>), and most of the forest fragments present less than 50 hectares (Ribeiro et al. 2009).

Considering the growing interest in spatial species distribution in response to topographic and climatic variations along altitudinal gradients, anurans (ectothermic animals) are excellent models for evaluating distribution patterns along altitudinal gradients. Its structural, morphological, behavioral and mainly physiological characteristics make anurans valuable and potential bioindicators of environmental quality (Heyer et al. 1994), particularly because they are sensitive to the effects of climatic, environmental and altitudinal variations (Fu et al. 2006, Bastazini et al. 2007, Vasconcelos et al. 2010, Hu et al. 2011).

Although Brazil has higher anurans diversity in the world, with approximately 1.040 species (Segalla et al. 2016; Frost et al. 2018), we can highlight only few studies involving altitudinal gradients (Giaretta et al. 1997, Giaretta et al. 1999, Goyannes-Araújo 2015) and a review on the influence of altitude and climatic variables on species richness in different biomes (Vasconcelos et al. 2010). Thus, based on anuran community structure (richness, composition and abundance) variation and added to the lack of studies along altitudinal gradients, the present work aims to understand and disentangle the topographic and climatic effects on spatial distribution richness pattern and in species range size along altitudinal gradient in Atlantic Forest highlands.

Understanding how communities are structured in space and time and trying to disentangle the mechanisms underlying spatial distribution richness pattern are essential issues also to we get insights and to help to direct future conservation and management strategies (Paknia and Pfeiffer 2011, Kraft et al. 2011, Olivier and van Aarde 2014, Socolar et al. 2016), mainly for tropical montane ecosystems biodiversity (Smith et al. 2007, Gradstein et al. 2008), which harbor high biodiversity with big part of the rare, endemic and endangered anurans and are considered hotspots of biodiversity and endemism (Meyers et al. 2000, Körner 2004, Orme et al. 2005, Spehn and Körner 2005, Kohler and Maselli 2009, Kohler et al. 2010, Hoorn et al. 2018).

Despite of this great biodiversity, rariry and high endemism rate found in montane ecosystems, this environments are among the most susceptible and threatened world due mainly by direct effects as habitat loss and fragmentation (Becker et al. 2007), but also in synergism with indirect effects as climate change effects (Chen et al. 2011, McCain and Colwell 2011, Catenazzi 2015, Tayleur et al. 2017) and chytridiomycosis, a disease caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Bd) (Rodriguez et al. 2014, Carvalho et

al. 2017, O’Hanlon et al. 2018). All these factors have been reported as possible enigmatic declines causes in different anuran populations and communities, which are more concentrated and severe in montane ecosystems (Stuart et al. 2004, Nogués-Bravo et al. 2007, Muths and Hero 2010, McCain and Colwell 2011, Davidson et al. 2013).

Finally, our world’s biodiversity is rapidly declining with the anthropogenic activity results (Barnosky et al. 2011, Dirzo et al. 2014) and this decline is denominated as the sixth mass extinction due to the high species extinction rates (Barnosky et al. 2011). Altitudinal gradients are natural laboratories and power tools for predicting biodiversity changes (McCain and Colwell 2011) and help us to understand community assemblage and hence to improve strategies of conservation to tropical biodiversity, which is a greatest challenge for conservation biology in the next decades (Sala et al. 2000, Foley et al. 2011, Cardinale et al. 2012, Vellend et al. 2013, Dornelas et al. 2014, Ewers et al. 2017).

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## **2. LITERATURE REVIEW**

### **2.1. The biodiversity and conservation status of mountain ecosystems**

In the Neotropical region, mountain ecosystems are very diversified with respect to their topography and geology, harboring a large proportion of the Earth's species richness and endemism (Körner 2004, Spehn and Körner 2005, Mutke and Barthlott 2005) being considered hotspots of biodiversity for different taxa (Chaverri-Polini 1998, Meyers et al. 2000, Sarmiento 2002, Orme et al. 2005).

Some Neotropical mountains are well-known for their high diversity and endemism, such as the Andes (Moraes and Beck 1992, Gentry 1995), the Guiana shield “tepui” (Gröger and Huber 2007) and the altitude forests in Central and South America (Chaverri-Polini 1998). According to Sarmiento (2002), this characteristic can be related to three factors that act in different time scales: the biotic evolution in response to the climatic and geological history, the adaptation of species to the environmental and biotic restrictions, and the exchanges with surrounding plains. Furthermore, some mountains are often isolated from each other and visually appear to form islands emerging from surrounding environments (Spehn and Körner 2005).

In Neotropical mountainous regions, climatic, geomorphologic and edaphic characteristics peculiar to these environments make them particularly sensitive to any type of anthropic activity (Chaverri-Polini 1998, Martinelli 2007). In the past, the mountains were biologically influenced by geological events (Pleistocene glaciations). Currently, the main factors influencing these environments are the edaphic-climatic (Chaverri-Polini 1998) and the anthropic activities (Martinelli 2007). The main and more common threats are the suppression of vegetation (deforestation and intense extraction of endemic or rare plant species) and the sensitivity of the soil to anthropic activities, which promote soil erosion and instability. In addition, other factors also act in synergism, such as the low competition capacity of native flora and fauna against invasive and exotic species, the criminal fire (burned), the advancement of mining areas, disorderly urban sprawl, agriculture at high altitudes (coffee growing), the installation of power transmission lines and, especially, the lack and the difficulties in applying ex-situ protection, monitoring, restoration and conservation laws (Martinelli 2007).

Although most mountain areas in Brazil are partially inserted in protected areas (Machado et al. 2004), most of these ecosystems still do not have management and

conservation plans to their peculiarities. Thus, different mountainous regions, especially those within the Atlantic Forest and Cerrado biomes, are largely degraded or threatened (Meyers et al. 2000, Klink and Machado 2005). Therefore, documenting and attempting to understand patterns and processes influencing species richness along altitudinal gradients is critical to prioritize future planning and conservation efforts (Hunter and Yonzon 1993, Vetaas and Grytnes 2002, Pimm and Brown 2004), especially in biodiversity hotspots, such as the Atlantic Forest and Cerrado highlands, which are also the most threatened in Brazil (Meyers et al. 2000, Morellato and Haddad 2000, Klink and Machado 2005).

## **2.2. Characteristics and biodiversity of the Atlantic Forest**

The Atlantic Forest is considered the second largest tropical rainforest in the American continent and also one of the 25 biodiversity hotspots worldwide (Myers 2000, Mittermeier et al. 2004, Morellato and Haddad 2000), which originally covering almost the entire Brazilian coast, to the east of Paraguay and northeast Argentina (Morellato and Haddad 2000, Galindo-Leal and Câmara 2005, Tabarelli et al. 2005).

The Atlantic Forest vegetation consists mainly of the Coastal Forest and/or Dense Ombrophylous Forest and of the Seasonal Tropical Forest and/or Seasonal Semi-deciduous Forest (Morellato and Haddad 2000). The Dense Ombrophylous Forest is mainly comprised of areas of low to medium elevations (~1,000 m a.s.l.). On the other hand, The Semideciduous Seasonal Forest occurs throughout the plateau (generally > 600 m a.s.l.), covering the center and the interior of the southeast region of the country (Morellato and Haddad 2000). In addition to the main forest formations, the ecosystems of oceanic islands, beaches, rocky shores, dunes, mangroves, restingas, altitude fields, and swamps are also found in this biome (Morellato and Haddad 2000, Haddad et al. 2013).

Among the Brazilian biomes, the greatest anuran diversity is found in the Atlantic Forest (Duellman 1999; Heyer et al. 1990, Bertoluci and Rodrigues 2002; Haddad et al. 2013, Rossa-Feres et al. 2017; Frost 2018), with approximately 550 species of amphibians (about 7% of the world biodiversity), distributed in 529 anurans and 14 caecilians, of which 472 species (80%) are endemic (Heyer 1990, Bertoluci and Rodrigues 2002, Bertoluci et al. 2007, Haddad et al. 2008, Haddad et al. 2013). The great anuran species richness observed in Atlantic Forest occurs in Dense Ombrophylous Forest, where 466 anuran species are recognized and 240 species or (51.5%) are endemic to this phytophysognomy. The Seasonal Forests (Semideciduous and Deciduous) harbor 255 anurans species, of which 42 species or

(16.5%) are endemic. In Mixed Ombrophylous Forest, 109 anurans species are known and 26 or (24%) are endemic. In Altitude fields phytophysiology, 87 anurans species are recognized and (18.4%) are endemic (Rossa-Feres et al. 2017).

This great Atlantic Forest biodiversity (species richness, rarity and high endemisms rate) is mainly related to the great heterogeneity (different phytophysionomies) created by latitudinal and altitudinal variations, high humidity gradients and to historical and biogeographic factors, which favors the different phytophysionomies formation and a wide wet microhabitats variety (Oliveira-Filho and Fontes 2000, Haddad and Prado 2005, Haddad et al. 2008, Haddad et al. 2013, Rossa-Feres et al. 2017), supporting high levels species richness and endemism (Myers 2000, Mittermeier et al. 2000, Mittermeier et al. 2004). Due to the this great phytophysionomies diversity, topographic differences and, ecosystem and climatic conditions, the anuran fauna of the Atlantic Forest highlands is still considered little studied with respect to the taxonomic status, biogeography, natural history and, ecology and conservation status (Silvano and Segalla 2005).

The Atlantic Forest biodiversity has been affected mainly by habitat loss and fragmentation processes (Myers et al. 2000, Tabarelli and Gascon 2005). In the past, this biome covered more than 1.5 million km<sup>2</sup>, and approximately 92% of this area was located in Brazil (Galindo-Leal and Câmara 2003). According to Ribeiro et al. (2009), the Atlantic forest has approximately 11 to 16% of its original coverage, and it is now considered one of the most threatened planet biomes (Oliveira-Filho and Fontes 2000). Furthermore, of the 12% of Atlantic Forest remaining only 40% are in protected areas (UC), and most of the remnants consist of isolated smaller than 50 ha fragments (Myers et al. 2000, Ribeiro et al. 2009). According to Pinto et al. (2006), only about 2% of the area of the original biome is inserted in an integral protection conservation unit, and 70% of the remainders are inserted in private properties, which consist mainly of secondary forests interspersed with different types of matrices. Especially for anuran fauna, Eterovick et al. (2005) reported that 31 species were declining in Brazil, mainly in the Atlantic Forest. Nowadays there are reports of amphibian population declines for 11 Brazilian localities (Verdade et al. 2011).

Considering the great diversity of species and endemism of both flora and fauna and the biological, economic and social role of organisms, it is possible to emphasize the need for preservation and management of the remnants of this biome (Almeida 2000), mainly on Atlantic Forest highlands, where there is almost no information on the ecology and distribution of anuran fauna.

### 2.3. Characteristics and biodiversity of the Cerrado Biome

The Cerrado is the second largest Brazilian biome in territorial extension, with 2 million km<sup>2</sup> and covering the North, Northeast, Central, West, and Southeast of the country, which in the past occupied 21 to 24% of the Brazilian territory (Ratter et al. 1988, Borlaug 2002). The Cerrado climate is seasonal, with a rainy period between October and March, followed by a dry period from April to September. The average annual rainfall is 1,500 mm and the average temperature varies between 22 and 27°C, being considered mild during the year. Soils are weathered, acidic, nutrient poor and have high concentrations of aluminum (Klink and Machado 2005). The Cerrado biome landscape presents plateaus, depressions and plains covered mainly by savannah, but its vegetation varies in structural terms and in floristic composition (Furley and Ratter 1998). Consequently, the Cerrado term is usually used to designate a set of ecosystems "Cerradão, Cerrado sensu stricto, Campo cerrado and Campo limpo" (Eiten 1972, Eiten 1977) and a small proportion consisting of Riparian Forests and Semi-Deciduous Forests (Oliveira-Filho and Ratter 2002, Silva and Bates 2002, Ribeiro and Walter 2008). This environmental heterogeneity is reflected in the communities of the different taxa of the Cerrado, which has high rates of endemism and currently is recognized as one of the richest regions in the world.

The Cerrado Biome is also considered biodiversity hotspots (Silva and Bates 2002, Klink and Machado 2005). One of the main factors contributing to this high diversity in different taxa, including herpetofauna, is the habitat horizontal stratification, which favors a wide variety of phytophysionomies types formation (open areas to forest areas), which can occur side by side in the landscape and harbor a species set (Colli et al. 2002, Nogueira et al. 2009). In recent decades, there has been a growing interest in the Cerrado biodiversity, and numerous studies have been developed in different phytophysionomies focusing on different species aspects (Bastos et al. 2003, Silveira 2006). However, even with the increasing number of studies, there are still gaps in relation to the Cerrado fauna (Diniz-Filho et al. 2005, Bini et al. 2006). According to Valdujo et al. (2012) the anuran fauna of the Cerrado is represented by at least 209 species, where approximately 51% are endemic. Despite the great diversity and rate of endemism, at least 137 animal species are threatened of extinction in this biome. Despite the extinction risk of a wide range of fauna and flora species, the Cerrado biome still has been deforested mainly by anthropic activities (Machado et al., 2004, Klink and Machado 2005), where approximately 55% of the 2 million km<sup>2</sup> of original vegetation cover of the Cerrado have already been destroyed (Machado et al. 2004). The main anthropogenic threats

to the biodiversity of Cerrado are also associated to the habitats loss and fragmentation processes, which are intrinsically related to the disorderly development and intensification of agriculture practices (Machado et al. 2004, Klink and Machado 2005). However, other activities are also acting in synergism, such as the dispersion of exotic species (Ratter et al. 2003), fertilizer and limestone in agricultural activities, which help to pollute aquatic environments, such as streams and rivers (Müller 2003), causing a negative impact on the diversity of different taxonomic groups, including amphibians (Pavan and Dixo 2004).

Finally, this lack of knowledge about anuran fauna in montane ecosystems in Atlantic forest and Cerrado biomes, reinforces the need for further studies focusing and analyzing on various factors that influence the species richness pattern along altitudinal gradients in these biomes, which will allow us to generate subsidies to indicate priority areas and actions for biodiversity conservation in this important mountain chain of southeastern Brazil, what has a great ecosystems diversity with different climatic, topographical and vegetation characteristics, a great number of species and a high degree of endemism of fauna and flora.

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### 3. TOPOGRAPHIC AND CLIMATIC VARIABLES DRIVE ANURAN COMMUNITY STRUCTURE ALONG AN ALTITUDINAL GRADIENT

#### ABSTRACT

Understanding the mechanisms that limit species range size variations along geographical gradients is a fundamental issue in ecology and macroecology. Studies conducted with different taxa and geographic regions supports that biodiversity changes along geographical gradients. A biogeographical hypothesis proposed to explain this change along altitudinal gradients is the Rapoport's rule. In this study, we tested the effects of topographic and climatic variables on anuran assemblages in Brazilian Atlantic Forest highland. Anurans were sampled using visual search (young and adult) and acoustic (males in calling activity) methods simultaneously. Our results revealed a species richness peak at midland "*hump-shaped pattern*" and a tendency to Rapoport altitudinal rule. In addition, species richness and species altitudinal range size variation were influenced by the altitude and temperature seasonality, highlighting the importance of both topographic and climate variables to structure anuran assembly along an altitudinal gradient in the Brazilian Atlantic Forest highlands.

Keywords: Amphibians; Atlantic Forest; Community assembly; Environmental filtering; Plateau Poços de Caldas; Rapoport's rule;

#### 3.1. Introduction

Understanding the mechanisms that limit species range size variations along geographical gradients (latitudinal or altitudinal) is an important issue in ecology and macroecology (Gaston 2003, Sexton et al. 2009, Kozak and Wiens 2010) that has fascinated biogeographers and macroecologists worldwide (Rahbek 1995, McCain and Grytnes 2010, Sanders and Rahbek 2012) and still is a current challenge (Gaston 2000).

Several studies conducted with different taxa and geographic regions supports that biodiversity changes along geographical gradients (Rahbek 1995, Rahbek 2005, Ribas and Schoereder 2006, Sanders and Rahbek 2012, Whitton et al. 2012, Sheldon et al. 2015, Szweczyk and McCain 2016) and some mechanisms as climatic, biological, evolutionary and historical process have been proposed to explain the spatial species richness patterns along geographical gradients (Sanders et al. 2003, Rahbek et al. 2005, McCain and Grytnes 2010, Sanders and Rahbek 2012). However, currently is known that species distributions are not random worldwide (Rahbek 1997) and that the processes underlying species distribution along altitudinal gradients are still poorly understood (Brown 2001, Grau et al. 2007, Gallou et al. 2017).

Three main species richness patterns along altitudinal gradients are recognized: 1) decrease of species richness with increasing altitude, 2) increase of species richness with increasing altitude (rare cases), and 3) a unimodal pattern with maximum species richness at intermediate altitudes, being the third pattern the most common (Rahbek 1995, Colwell and Lees 2000, Rahbek 2005, McCain and Grytnes 2010, Sanders and Rahbek 2012, Hutter et al. 2013, Smith et al. 2014, Szewczyk and McCain 2016). In this way, documenting and trying to understand the processes and mechanisms that drive species richness and species range size along altitudinal gradients are essential rule for biodiversity management, mainly in tropical montane ecosystems (Smith et al. 2007, Gradstein et al. 2008), which harbor high biodiversity levels with endemic and endangered species (Chaverri-Polini 1998, Meyers et al. 2000, Körner 2004, Orme et al. 2005, Kohler and Maselli 2009, Kohler et al. 2010, Hoorn et al. 2018).

A biogeographical hypothesis proposed to explain species richness patterns focusing on species ranges size along geographical gradients is the Rapoport's rule (Stevens 1989, 1992). Although Rapoport's rule has been initially proposed to explain species range size patterns along latitudinal gradients (Steven 1989), later it was also extended to explain species range size patterns along altitudinal gradients (Stevens 1992). Rapoport's altitudinal rule predicts there is a positive correlation between altitude and species altitudinal ranges size (Stevens 1992), with highland species tending to have broad ranges size due to broader climatic tolerances than lowland species and consequently covering wider altitudinal ranges. On the other hand, due to more stable environmental conditions in lowland regions, lowland species have narrower climatic tolerances and hence smaller range sizes. It is worth noting that unlike the null model (Colwell and Lees 2000, Colwell et al. 2004), Rapoport's altitudinal rule (Stevens 1992) attempts to explain the spatial species richness patterns based on the climatic variation effects on species distribution. Thus, to explain Rapoport's altitudinal rule, Stevens (1992) proposed the climatic variability hypothesis or simply Rapoport "rescue" hypothesis to trying to explain this rule, which predicts that species that have broad physiological tolerance are able to become more widely distributed (Stevens 1992, see also Gaston and Chown 1999).

Anuran are considered interesting animal to test Rapoport's rule due to their complex life cycles (Duellman and Trueb 1986, Pough et al. 2004, Becker et al. 2007), limited dispersal ability "intense phylopatry" (Duellman and Trueb 1986, Blaustein et al. 1994, Funk et al. 2005) and mainly because they are highly sensitive ectotherms organism and depending on precipitation levels mainly for reproduction and higher temperatures to the

physiological maintenance and metabolic process, which influence their development, metabolism and behaviour (Buckley and Jetz 2007, Vitt and Caldwell 2009).

All these ecological requirements added information about physiological, morphological and behavioural characteristics make anurans very sensitive to the climatic (temperature and precipitation), environmental (landscape structure and heterogeneity) and altitudinal variations (Fu et al. 2006, Bastazini et al. 2007, Blaustein and Bancroft 2007, Vasconcelos et al. 2010, Blaustein et al. 2011, Hu et al. 2011), being considered potential bioindicators quality environmental (Blaustein and Wake 1995, Heyer et al. 2001, Pounds et al. 2006) when compared to other terrestrial vertebrates (Collins and Crump 2009, Hoffmann et al. 2010).

Studies carried out specifically with anurofauna showed that species richness peaks can be found in lowlands (Hofer et al. 1999 [Cameron], Goyannes-Araújo et al. 2015 [Brazil]), midlands (Fauth et al. 1989 [Costa Rica], Fu et al. 2006 [China], Kozak and Wiens 2010 [North America], Hu et al. 2011 [China], Hutter et al. 2013[Andes]) and highlands (Giaretta et al. 1999 [Brazil], Naniwadekar and Vasudevan 2007 [India]), depending on study region (McCain and Grytnes 2010). These controversial results also have increased our interest in testing Rapoport's altitudinal rule in the Atlantic Forest highlands.

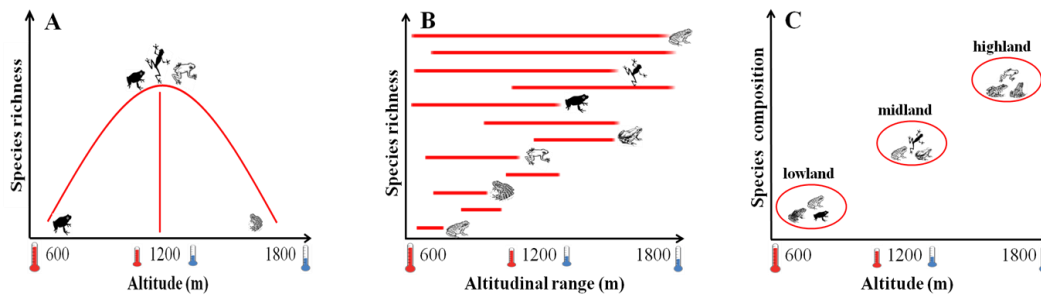
The Atlantic Forest is known as one of the most anuran biodiverse global region with rare, endemic and threatened species (Haddad et al. 2013; Rossa-Feres et al. 2017) and is listed as conservation hotspots (Myers et al. 2000, Mittermeier et al. 2004). Despite this great species richness and endemism, the Atlantic Forest is historically the oldest in terms of different exploitation types due to its long history of human occupation (Dean, 1996). The result of this long occupation historical process and of the different land uses types has been reduced the approximately 11 a 16% of its original extent (1,3 millions de km<sup>2</sup>), with majority (80%) of the fragments presenting less than 50 hectares (Ribeiro et al. 2009). However, the knowledge about altitudinal gradient effects on species richness and in the community assembly is still unknown in Atlantic Forest highlands. Thus, understanding the altitudinal effects in the communities assembly along Atlantic Forest highlands, using climate-sensitive taxa (as anurans), is essential for to try to plan better future more precise actions to conservation and management biodiversity, but also to understand the possible climate changes effects on biodiversity (Wilson et al. 2005, Chen et al. 2011, McCain and Colwell 2011, Bellard et al. 2012).

The aim of this work was to analyze the effects of topographic and climatic variables in anuran community structure along an altitudinal gradient in Atlantic Forest highlands.



Specifically, we want (1) Verify which spatial pattern the anuran species richness exhibit along altitudinal gradient; (2) Test if Rapoport's altitudinal rule explain of anuran range size distribution along altitudinal gradient and (3) Identify how anuran community structure is organized along altitudinal gradient.

Our hypotheses were: 1) Anuran species richness will exhibit an unimodal pattern (*hump-shaped pattern*) along altitudinal gradient (Figure 1A); 2) Highland species will show wider range size than lowland species (Figure 1B) and 3) Anuran community structure will be organized in three groups (lowland, midland and highland) along altitudinal gradient (Figure 1C).



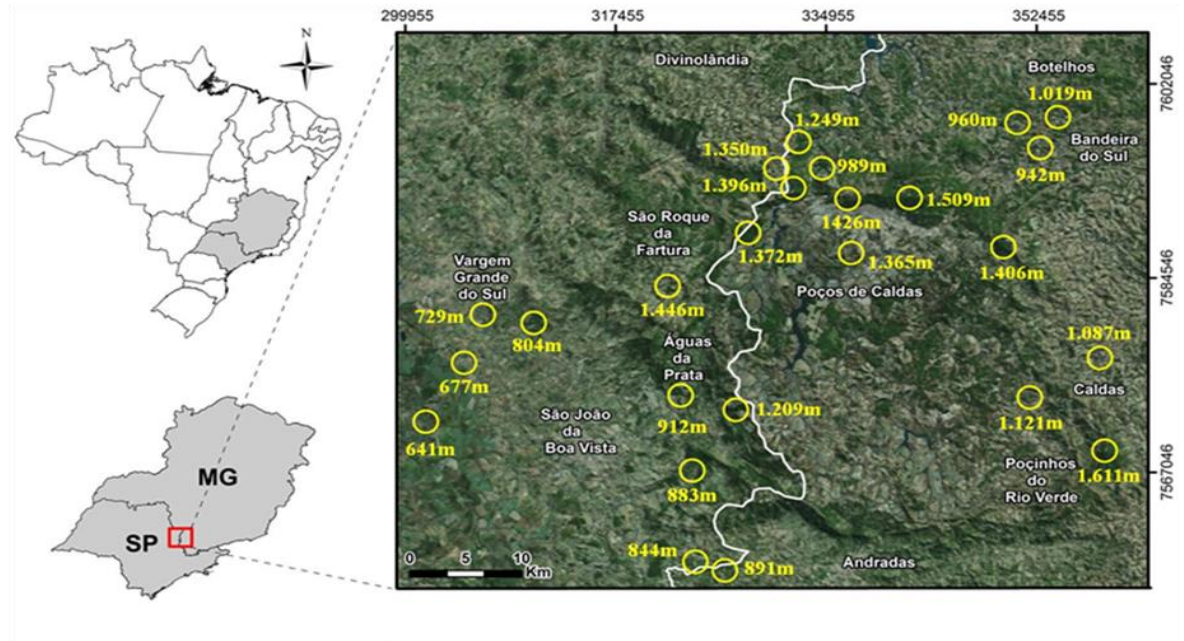
**Figure 1.** Hypothetical relationships of species richness and community composition in response to the topographic and climatic variables along an altitudinal gradient in Poços de Caldas Plateau, southeast Brazil.

## 3.2. Material and Methods

### 3.2.1. Study area

The study was carried out in Poços de Caldas Plateau (PPC) considered the largest set of alkaline rocks on Earth (Figure 2; Appendix A). The plateau is located on the extreme western edge from of the Serra da Mantiqueira, one of the largest mountain ranges in eastern South America, which occupies an extensive area in the southeastern Brazil (Christofoletti 1973, Rizzini 1997).

The Poços de Caldas Plateau (PPC) region is characterized by the presence of an almost complete main volcanic caldera forming an annular structure of 35 km in diameter (Christofoletti 1973, Ponçano et al. 1981). This volcanic caldera is located on the border of the states of Minas Gerais and São Paulo, presenting nine municipalities as boundaries: to the North (Bandeira do Sul, Botelhos, Palmeiral and Poços de Caldas), to the East (Caldas), to the South (Andradas) and to the West (Águas da Prata, São Sebastião da Grama, Caconde and Divinolândia), all of them in the state of São Paulo (Figure 2).



**Figure 2.** Study area covering 25 sampled landscapes distributed along an altitudinal gradient in Poços de Caldas Plateau, southeast Brazil. White line indicates the border between the Minas Gerais and São Paulo States. Yellow circles (buffers 1 km) represent the sample landscapes.

The climate of the region according to Köppen is Cwb "subtropical of altitude", being characterized as mesothermic with a dry winter from April to September with mild summers and rainy season in the summer from October to March, occurring approximately in the border between the states of Minas Gerais and São Paulo (Pell et al. 2007), with average temperatures between 17.6 and 18.4°C, with the average temperature of the colder month around 16.5°C and the hottest month not exceeding 22°C (Morales 2007). The mean annual precipitation of the region varies between 1300 and 1700 mm (Pell et al. 2007, Scoforo et al. 2008). The altitude of the region varies from 600 to 1800 m a.s.l. (Almeida 1964, Fraenkel et al. 1985, Moreira et al. 2002).

The region is covered by the Atlantic Forest (Ab'Saber 1989). However, the Atlantic Forest is composed of two major vegetation types: Atlantic Rain Forest and the Atlantic Semi-deciduous forest. The Atlantic Rain Forest covers mostly the low to medium elevations (1000 m a.s.l.) of the eastern slopes of the mountain chain that runs along the coastline from southern to northeastern Brazil. On the other hand, the Atlantic Semi-deciduous forest extends across the plateau (usually > 600 m a.s.l.) in the center and southeastern interior of the country (Leitão-Filho and Morellato 1997, Oliveira-Filho and Fontes 2000).

Specifically, our sampling areas are inserted in a transition area between the Atlantic Semi-deciduous forest with enclave of Cerrado and Altitude fields in the interior from the Poços de Caldas Plateau (Rizzini 1979, Velloso et al. 1991, Conforti et al. 2007).

### **3.2.2. Anuran community sampling**

We sampled the anuran communities in 25 landscapes (buffers 1 km radius) distributed along an altitudinal gradient (600 to 1800 m a.s.l) with a minimum distance of 3 km between each landscape (Figure 2; Appendix A). Landscapes with 1 km radius are considered a reasonable size area by including average dispersion and migration movements for most anuran species (Guerry and Hunter 2002, Wagner et al. 2014, Collins and Fahrig 2017) and we argue that 1km<sup>2</sup> maintain independence between tropical anuran communities along an altitudinal gradient.

The sampling was performed monthly in the rainy season and bi-monthly in the dry season during one year (December 2016 to December 2017). Visual (young and adult) and acoustic (males in calling activity) searches were used simultaneously to maximize the number of sampled species (Heyer et al. 1994). Each landscape was traversed and inspected at night between (18:00 to 24:00) for anuran species registration. All landscapes were sampled 10 times each in a randomized order (between and among landscapes) and standardized by time (1h/night) totalizing 10 hours per landscape so that the sampling effort was equivalent and to minimize false absences (Heyer et al. 1994). According Shirose et al. (1997), Crouch and Paton (2002) and Collins and Fahrig (2017) the time from 3 to 15 minutes at each sampling point are adequate to detect to most common species.

The sampled specimens that we could not identified in the field were collected (under SISBio license number #48526-1), anesthetized and euthanized with xylocaine 20%, fixed in 10% formalin, preserved in alcohol 70% and deposited in the Coleção Herpetológica do Laboratório de Zoologia de Vertebrados da ESALQ/USP (acronym VESALQ).

### **3.2.3. Topographic and bioclimatic variables**

We used altitude and slope variables from the Digital Elevation Model raster image (30-m resolution) from Geomorphometric Database of Brazil (TOPODATA) - downloaded from (<http://www.dsr.inpe.br/topodata>) and climatic (19 bioclimatic) variables from the Global Climate Data (WorldClim Version2) - downloaded from (<http://www.worldclim.org>), with spatial resolutions from 30 seconds-arc (~1 km<sup>2</sup>), to test the effects of topographic and climate variables in anuran community assembly along an altitudinal gradient. We used these variables based on the knowledge that topography and the climate are generally considered

the most important factors that determining distribution of anurans communities (Duellman 1999, Menin et al. 2007, Vasconcellos et al. 2010).

### 3.3. Statistical analysis and ecological models

#### 3.3.1. Species richness and abundance patterns

To test whether anuran species richness has a unimodal (*hump-shaped pattern*) distribution along the altitudinal gradient, we first used an individual-based rarefaction curve to control for confounding effects of species abundance on richness (Gotelli and Colwell 2001). This is essentially true along altitudinal gradients because it also affects population density and size, which in turn have a positive effect on species richness. Furthermore, we combined rarefaction (interpolation) and prediction (extrapolation) to make meaningful comparison standardized by identical sampling effort (Chao et al. 2014). Whereas the interpolation was performed based on the landscape with the smallest number of individuals ( $n = 105$ ), the extrapolation was based on larger number of individuals ( $n = 1,145$ ). Chao et al. (2014) have extended the classical models of rarefaction for the most common diversity measures (species richness, Shannon, and Simpson) throughout Hill numbers. We used the nearly unbiased Shannon estimation ( $q = 1$ , Jost 2007), which represents the alpha diversity with proportional weight to rare and abundant species.

In addition, we tested whether topographic and climatic variables affect species rarefied (interpolated and extrapolated) richness. Because we expected a non-linear relationship between altitude and species richness, we used a Generalized Additive Model (GAM) that replaces a linear effects by a smooth function applied to some predictor variables. To simplify the statistical model, we started with a global model including uncorrelated predictors (see below) and compared this model with nested, simpler models. In addition, this model simplification compare linear effects with smoothed effects to obtain the best fit, as demonstrated in an example model:

```
gam.global <- gam (rarefied_richness ~ bio1 + bio4 + bio15 + alt)
summary (gam.global)
gam.mod1 <- gam (rarefied_richness ~ bio1 + bio4 + bio15 + s(alt))
summary (gam.mod1)
```

The function compares whether the smoothed (s) effects represent a best fit compares with the linear effects. Furthermore, to avoid the undesirable effects of multicollinearity on

model parameters, we first compared with a multiple correlation all predictor variables and removed those highly correlated ( $r > 0.6$ ). We have also retained only variables with a recognized biological meaning for our hypothesis.

### **3.3.2. Rapoport's altitudinal rule**

For verify anuran range size variation along altitudinal gradient, we calculated the weighted occurrence mean (lower and upper limit distribution of each species) to understand the trends (increase or decrease) of anuran ranges size and midpoint method to verify where it is more abundant.

### **3.3.3. Altitudinal variation in community composition**

To graphically visualize differences in anuran community structure along altitudinal gradient (600 to 1800 m a.s.l.), we performed a Principal Coordinates Analysis (PCoA) using the abundance-based distance method Bray-Curtis (Legendre and Legendre 2012). PCoA attempts to ordinate the samples so that the distance between points represents the dissimilarity between samples. Scores of PCoA represent a measure of species composition weighed by species abundance (when we used relative abundance values for each anuran species).

Finally, to test whether community composition varies along altitudinal gradient we performed a permutational multivariate analysis of variance (PERMANOVA - Anderson 2001, Anderson and Walsh 2013). PERMANOVA is a routine analysis for testing the simultaneous response of one or more variables to one or more factors, thus allowing the analysis of multivariate data in the context of more complex sampling structures. Probabilities were calculated with permutation method randomized 9,999 times. We also used the BETADISPER method to investigate if there is a variance heterogeneity affecting the results obtained from the PERMANOVA method (Anderson and Walsh 2013). This is an essential step because PERMANOVA confounds location (a measure of composition) and group dispersion (a measure of within-group compositional variation). These analyses were made using the R software (R Development Core Team 2017).

### 3.4. Results

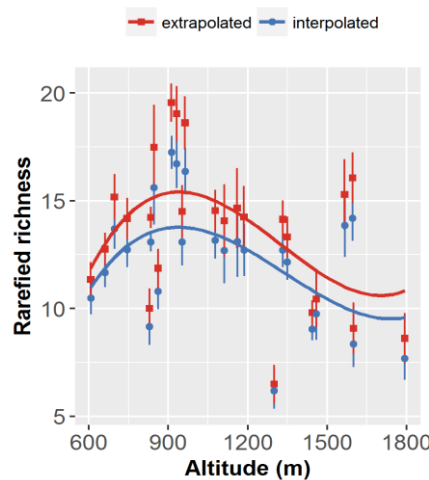
#### 3.4.1. Species richness and abundance patterns

We recorded a total of 10,935 specimens belonging to 10 families, 20 genera, and 70 species distributed along an altitudinal gradient (Appendix B), representing approximately 7% of Brazilian anuran fauna (Segalla et al. 2016, Frost 2018).

Hylidae was the richest family (37 species or 53%) followed by Leptodactylidae (12 or 17,1%), Bufonidae (5 or 7,1%), Brachycephalidae (5 or 7,1%), Hylodidae (4 or 6%), Odontophrynidae (3 or 4,2%), Craugastoridae (1 or 1,4%), Phyllomedusidae (1 or 1,4%), Microhylidae (1 or 1,4%), and Ranidae as (1 or 1,4%) the only non-native family.

The most abundant species was *Dendropsophus minutus* (N = 1,123 or 10% of sampled individuals), followed by *Physalaemus cuvieri* (N = 1,099 or 10%) and *Ischnocnema juipoca* (N = 894 or 8%), which represent approximately 28% of all sampled individuals.

In addition, our results also revealed that the higher rarefied (interpolated and extrapolated) richness showed a unimodal pattern "hump-shaped pattern" with higher species richness found in intermediary along altitudinal gradient (Figure 3).



**Figure 3.** Individual-based rarefaction curve on rarefied (interpolated and extrapolated) richness along an altitudinal gradient in Poços de Caldas Plateau, southeast Brazil.

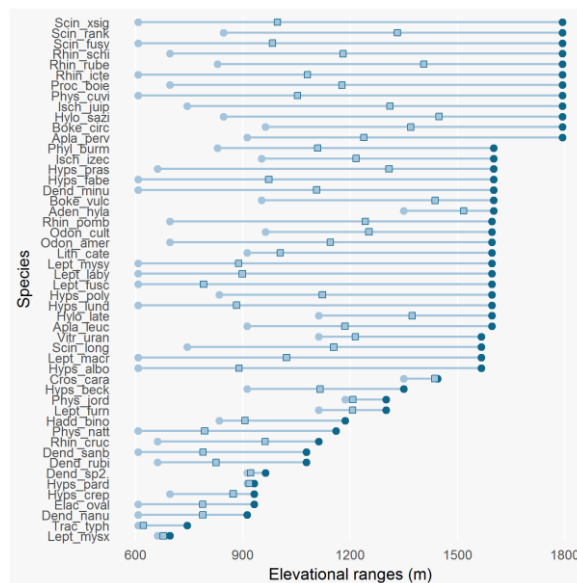
Finally, our results also revealed that among topographic (altitude and slope) and climatic (19 bioclimatic) variables tested, only altitude ( $p=0.004$ ) and temperature seasonality ( $p=0.005$ ) were that more affect anuran community along altitudinal gradient (Table 1). The other variables did not contribute significantly ( $P > 0.05$ ) to the variation of the species composition along altitudinal gradient (Table 1).

**Table 1:** Influence of the topographic and climatic variables on species rarefied (interpolated and extrapolated) richness through a Generalized Additive Model (GAM) along an altitudinal gradient in Poços de Caldas Plateau, southeast Brazil.

INTERPOLATED RICHNESS				
No linear models	Edf	Ref.df	F	P-value
Altitude (Alt)	1.00000	1.00000	10.464824	<b>0.004</b>
Temperature seasonality (Bio 04)	3.461033	4.251915	4.951471	<b>0.005</b>
EXTRAPOLATED RICHNESS				
No linear models	Edf	Ref.df	F	P-value
Temperature seasonality Bio (04)	3.450829	4.246575	4.978777	<b>0.005</b>

### 3.4.2. Rapoport's altitudinal rule

Anurans altitudinal range sizes tended to increase along altitudinal gradient with highland species showing a wider ranges size than lowland species corroborating Rapoport's altitudinal rule (Figure 4). In addition, our results also revealed that temperature seasonality influenced anuran community composition along altitudinal gradient (Table 1).

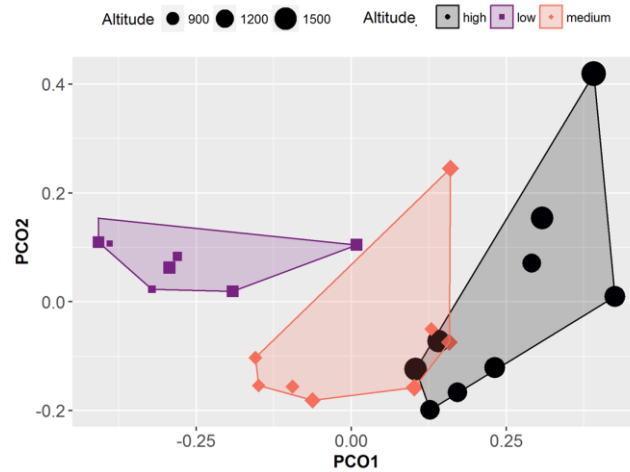


**Figure 4.** Anuran altitudinal range size distributions along an altitudinal gradient in Poços de Caldas Plateau, southeast Brazil. Bars show altitudinal range size along altitudinal gradient. Square: median abundance. The range profile was based on the lowest and highest elevation at which a species was observed.

### 3.4.3. Altitudinal variation in community composition

The Principal Coordinates Analysis (PCoA) revealed dissimilarity in anuran community structure along an altitudinal gradient defining three distinct groups (axis 1 = 41.43% and axis 2 = 14.84%): (i) one group was formed by “lowland species”, (ii) other

formed by “midland species” and (iii) “highland species” begin the last group formed (Figure 5). These results were supported by the PERMANOVA ( $F=3, 29, P=0, 001$ ).



**Figure 5.** Principal Coordinates Analysis (PCoA) ordination of anurans community structure along an altitudinal gradient in Poços de Caldas Plateau, southeast Brazil. Ordination based on the dissimilarity matrix calculated with the Bray-Curtis index (abundance) for anurans community composition.

### 3.5. Discussion

Our results revealed that higher anuran species richness was found in midlands “*hump-shaped pattern*” and that highland species tended to have wider altitudinal ranges size when compared with lowland species corroborating Rapoport’s altitudinal rule. In addition, our results also appointed that altitude and seasonality temperature were the most important variables affecting anuran community assembly along altitudinal gradient in Atlantic Forest highlands.

#### 3.5.1. Species richness and abundance patterns

Mountains are topographically and geologically highly diversified being a causal factor in their high biological diversity (Spehn and Körner 2005). Besides, this greatest anuran species richness and abundance found in our study may be related to our sample design with a largest number of landscapes sampled along altitudinal gradient. However, comparisons with other studies carried out along altitudinal gradients in Brazil and around world are limited due to used different survey methods and protocols (Haider et al. 2018).

The anuran species richness in the Plateau Poços de Caldas exhibited a unimodal pattern “*hump-shaped pattern*” along altitudinal gradient due altitude influence corroborating



several empirical studies and recent meta-analysis conducted with different taxonomic groups and regions that hump-shaped pattern is the most common (Rahbek 1995, Sanders 2002, Vetaas and Grytnes 2002, Rahbek 2005, McCain 2005, Fu et al. 2006; Chettri et al. 2010, McCain and Grytnes 2010, Sanders and Rahbek 2012, Hutter et al. 2013, Smith et al. 2014, Szewczyk and McCain 2016, Mumladze et al. 2017).

### **3.5.2. Rapoport's altitudinal rule**

Our results indicated that anuran altitudinal ranges size showed a tendency to increase along altitudinal gradient corroborating Rapoport's altitudinal rule (Stevens 1992) being consistent with other empirical studies encompassing different taxa as butterflies (Fleishman et al. 1998), ants (Sanders 2002), spiders (Chatzaki et al. 2005), opiliones (Almeida-Neto et al. 2006), dung beetles (Herzog et al. 2013), which also documented Rapoport's altitudinal rule in some regions. On the other hand, this hypotheses still has presented controversial results (Hawkins and Diniz-Filho 2006, McCain and Knight 2013) with works supporting (Fleishman et al. 1998, Sanders 2002, Navas 2003, Almeida-Neto 2006, Rohner et al. 2015) and other not supporting (Ribas and Schoereder 2006, Bhattarai and Vetaas 2006, Bernal and Lynch 2008, Hu et al. 2011, McCain and Knight 2013, Goyannes et al. 2015, Wang and Soininen 2017) the Rapoport's altitudinal rule. Nevertheless, Stevens (1992) never suggested that Rapoport's (latitudinal or altitudinal) rule should be valid for all taxa, and the lack of this consensus as a universal rule is a common basis for criticism (Gaston et al. 1998, Ruggiero and Werenkraut 2007).

One of the most common mechanism proposed by Stevens (1992) to explain Rapoport's altitudinal rule was the climatic variability hypothesis or simply Rapoport "rescue" hypothesis. The climatic variability hypothesis predicts that species that can withstand broad climatic variability are able to become more widely distributed (Stevens 1992, see also Gaston and Chown 1999). Our results revealed positive explanation of seasonality temperature on anuran altitudinal range size variations along altitudinal gradient being consistent with the climatic variability hypothesis corroborating with previous studies that also supported climatic variability hypothesis in different taxa, as dung beetle (Gaston and Chown 1999), ants (Sanders et al. 2003), vertebrates (McCain 2009) and moths (Beck et al. 2016).

Tropical biodiversity is strongly influenced by climatic stability (Barron 1995) mainly the ectothermic organisms that are highly dependent on the ambient temperature to

maintain their body temperature (Bakken and Angilletta 2014). Temperature has a profound influence on behaviour, physiology, and ecological performance of organisms and can alter everything from growth rates, metabolism, geographic ranges, and species diversity (Navas 2006, Navas et al. 2008, Angilletta 2009), which impose selective pressures on the geographical ranges of terrestrial vertebrates (McCain 2009b, Sheldon et al. 2015, Chan et al. 2016) having a direct impact on the probability of extinction. According to Whitton et al. (2012) and Pintor et al. (2015) the idea that current climate patterns are consistently the best predictor for a correct interpretation amphibian altitudinal range size variations in a regional scale.

Different taxonomic group's exhibit diversity peaks at distinct altitudes probably reflecting both physical and physiological constraints (Guo et al. 2013). The climatic variability hypothesis was proposal by Stevens (1992) to explain monotonically decreasing species richness patterns. However, our results revealed a combination between a peak in richness in midland (*hump-shaped pattern*) and a tendency the Rapoport's altitudinal rule along altitudinal gradient. It has been noted that the data provided by Stevens (1992) to support his version of Rapoport' altitudinal rule actually show a peak at midlands (Colwell and Hurtt 1994, Rahbek 1997) and similar result combinations have been found in previous works with different taxa as butterflies (Fleishman et al. 1998), ants (Sanders 2002), moths (Brehm et al. 2007) and Tibetan loaches (Feng et al. 2017) in different regions.

This mixed of results has led to the Rapoport's (latitudinal or altitudinal) rule questioning as a universal macroecological pattern (Ruggiero and Werenkraut 2007). Some reasons for this confusion are in the use of different methodologies, taxa (ectothermic and endothermic), and in the differences ecological or historical of each montane ecosystems (Lawton et al. 1987, McCoy 1990, Lomolino 2001). All these factors cited above and added the lack of such relationships in some taxa and regions have considered Rapoport's rule a phenomenon restricted to certain ecosystems and regions (Rohde et al. 1993, Colwell and Hurtt 1994, Rhode 1996, Ruggiero and Werenkraut 2007). Finally, ours results added to other similar result combinations (Fleishman et al. 1998, Sanders 2002, Brehm et al. 2007, Feng et al., 2017) highlight that Rapoport's altitudinal rule maybe preferred help understand and explain species altitudinal range size and peak in species richness at midland (*hump-shaped pattern*) rather than monotonically decreasing richness along altitudinal gradient.

### 3.5.3. Altitudinal variation in community composition

Anuran community structure exhibited a clear and striking segregation pattern along altitudinal gradient due to the altitude influence forming three (low, median and high) distinct communities. This community segregation has already been documented to birds (Rahbek 1997), dung beetle (Davis et al. 1999, da Silva et al. 2018), gastropods (Presley et al. 2011), small mammals (Andrade and Monjeau 2014) and anurans (Malonza and Veith 2012) in other montane tropical ecosystems. Our results also were consistent with Ngalason and Mkonyi (2011), which also found dissimilarity in anuran community between lowland and highland due to altitude influence along an altitudinal gradient in Uluguru south Mountains in Tanzania. Hu et al. (2011) studying spiny frogs in Asian mountains also found a distinct segregation pattern in community composition along an altitudinal gradient between higher altitudinal species (highland group) from the lower altitudinal species (lowland group) by strongly influenced of the basin stepped geomorphology. Another example is Zancolli et al. (2014) that also found a clear segregation in anuran community between lowland (colline–submontane transition) and highland (submontane–montane transition) by altitude influence along altitudinal gradient in Mount Kilimanjaro in Tanzania. Similar findings also has been documented for anuran communities in other montane tropical ecosystems as in Mount Kupe in Cameroon (Hofer et al. 1999), Kibale National Park in Uganda (Vonesh 2001), Mount Kinabalu in Borneo (Malkmus et al. 2002), Taita hills in Kenya (Malonza and Veith 2012) and in some mountains of the Eastern Arc in Tanzania as Mahenge Mountains (Loader et al. 2004), and Udzungwa Mountains (Menegon and Salvidio 2005), which all also observed communities distinct because on altitude influence along its altitudinal gradients.

Communities' segregation observed in our results corroborates the idea that altitude is an important drive to maintain and organized community assembly along altitudinal gradients. However, other factors as isolation, dispersal capacity, geological physical barriers, climatic history (past and present) and historical and evolutionary processes (Currie 1991, Brown 2001, Hawkins et al. 2003, Kreft and Jetz 2007, Field et al. 2009, Khatiwada and Hugaasen 2015, Oliveira et al. 2017, Laiolo et al. 2018) are not mutually exclusive and also can influence anuran community assembly.

### 3.6. Implications for conservation

Montane tropical ecosystems will experience the highest biodiversity losses in next decades (McCain and Colwell 2011, Davidson et al. 2013) due mainly habitat loss and fragmentation process (Becker et al. 2007) and climate changes effects (Chen et al. 2011, Catenazzi 2015; Tayleur et al. 2017). Thus, preserve biodiversity in an era denominated as the sixth mass extinction due to the high species extinction rates (Barnosky et al. 2011) will be the greatest challenge this century (Sala et al. 2000, Foley et al. 2011, Ruffell et al. 2017) mainly in the montane tropical ecosystems considered biodiversity hotspots (Orme et al. 2005, Kohler et al. 2010, Hoorn et al. 2018).

Our research revealed a clear segregation pattern with three anuran distinct (lowland, midland and highland) communities and also showed very species with small altitudinal range size along altitudinal gradient. These results highlight that common, rare and threatened species distributions are not complementary and that all further conservation and management plans need encompass entire altitudinal variation and not just total diversity hotspots (Orme et al. 2005, Grenyer et al. 2006) or simple an altitudinal range size (low, mid or highlands).

Species altitudinal ranges size is determined by a complex interplay of species characteristics and environmental factors that limit viable dispersal (Gaston 2003) and should be considered as an strong indirect conservation status indicator in a wider conservation context because species altitudinal range size variations is one of the main extinction risk predictors in different taxonomic groups (Davidson 2009, Lee and Jetz 2010, Böhm et al. 2016a), whereas species with large altitudinal range size tend to have high dispersal ability, broad environmental tolerances (Jablonski and Roy 2003) and lower extinction risk (Böhm et al. 2017) and species with small altitudinal range size (rare or endemic taxa) may be vulnerable and more threatened to climate change, higher extinction risk (Gaston and Blackburn 1996, Arita et al. 1997, Mace et al. 2001), which should be the focus main in conservation efforts along altitudinal gradients. Therefore, our findings show that studies on altitudinal Rapoport rule may not only help us in species altitudinal range size understanding, but also be helpful to conserve biodiversity mainly to species that present small altitudinal range (rare or endemic taxa) along tropical altitudinal gradient.

Finally, in an era denominated as the sixth mass extinction, understand the spatial species richness patterns and the potential degradation effects are crucial issues to we get insights, which will help us to direct future for biodiversity conservation plans and management actions the long-term (Zhang et al. 2012, Zancolli et al. 2013) in a of the most

fragile and threatened ecosystems currently worldwide (Becker et al. 2007, McCain and Colwell 2011).

### 3.7. Conclusion

Our results revealed higher anuran species richness at midland “*hump-shaped pattern*” and a tendency to Rapoport’s altitudinal rule. Moreover, altitude and seasonality temperature were the main anuran communities assembly drivers along an altitudinal gradient in Atlantic Forest highlands. In addition, our results highlight that all further conservation and management plans need encompass entire altitudinal variation and not just total diversity hotspots or simple an altitudinal range size (low, mid or highlands).

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## APPENDIXES

### APPENDIX A.

**Table 2:** Altitude and geographic coordinates of the 25 sampled landscapes along an altitudinal gradient in Poços de Caldas Plateau, southeast Brazil.

<b>Sites</b>	<b>Altitude</b>	<b>Decimal degrees (WGS 84)</b>	
<b>1</b>	641.408	-46.916366	-21.954404
<b>2</b>	677.184	-46.885657	-21.906595
<b>3</b>	729.092	-46.870502	-21.867467
<b>4</b>	804.027	-46.829673	-21.874171
<b>5</b>	844.826	-46.699812	-22.068319
<b>6</b>	883.846	-46.702342	-21.994218
<b>7</b>	891.676	-46.676119	-22.075324
<b>8</b>	912.928	-46.711655	-21.933053
<b>9</b>	942.000	-46.441055	-21.711138
<b>10</b>	960.900	-46.422819	-21.731736
<b>11</b>	989.850	-46.597963	-21.748286
<b>12</b>	1.019.96	-46.408642	-21.706596
<b>13</b>	1.087.92	-46.374818	-21.902666
<b>14</b>	1.121.09	-46.431235	-21.934823
<b>15</b>	1.209.73	-46.667329	-21.944845
<b>16</b>	1.249.91	-46.616971	-21.726901
<b>17</b>	1.350.38	-46.634817	-21.748533
<b>18</b>	1.365.47	-46.574704	-21.81722
<b>19</b>	1.372.70	-46.657421	-21.800849
<b>20</b>	1.396.83	-46.621207	-21.764399
<b>21</b>	1.406.00	-46.452422	-21.81247
<b>22</b>	1.426.00	-46.577634	-21.77314
<b>23</b>	1.446.63	-46.722102	-21.844113
<b>24</b>	1.509.57	-46.52775	-21.772414
<b>25</b>	1.611.82	-46.371095	-21.978024

**APPENDIX B.****Table 3:** Anurofauna recorded in 25 sampled landscapes distributed along an altitudinal gradient (600 to 1800m a.s.l.) in Poços de Caldas Plateau, southeast Brazil.

<b>FAMILIES</b>	<b>SPECIES</b>
<b>Bufonidae</b>	<i>Rhinella crucifer</i> (Wied-Neuwied, 1821) <i>Rhinella icterica</i> (Spix, 1824) <i>Rhinella pombali</i> (Baldisserra, Caramaschi and Haddad, 2004) <i>Rhinella rubescens</i> (Lutz, 1925) <i>Rhinella schneideri</i> (Werner, 1894)
<b>Brachycephalidae</b>	<i>Ischnocnema izecksohni</i> (Caramaschi and Kisteumacher, 1989 "1988") <i>Ischnocnema juipoca</i> (Sazima and Cardoso, 1978) <i>Ischnocnema</i> gr. <i>lactea</i> <i>Ischnocnema</i> sp.1 <i>Ischnocnema</i> sp.2
<b>Craugastoridae</b>	<i>Haddadus binotatus</i> (Spix, 1824)
<b>Phyllomedusidae</b>	<i>Phyllomedusa burmeisteri</i> Boulenger, 1882
<b>Hylidae</b>	<i>Aplastodiscus perviridis</i> Lutz, 1950 <i>Aplastodiscus leucopygius</i> (Cruz and Peixoto, 1985) <i>Boana albopunctata</i> (Spix, 1824) <i>Boana</i> aff. <i>beckeri</i> (Caramaschi and Cruz, 2004) <i>Boana crepitans</i> (Wied-Neuwied, 1824) <i>Boana faber</i> (Wied-Neuwied, 1821) <i>Boana lundii</i> (Burmeister, 1856) <i>Boana pardalis</i> (Spix, 1824) <i>Boana</i> aff. <i>polytaenia</i> (Cope, 1870) <i>Boana prasina</i> (Burmeister, 1856) <i>Boana stenocephala</i> (Caramaschi and Cruz, 1999) <i>Boana</i> sp.1 <i>Bokermannohyla vulcaniae</i> De Vasconcelos and Giaretta, 2005 <i>Bokermannohyla</i> aff. <i>circumdata</i> (Cope, 1871) <i>Bokermannohyla</i> sp.1 <i>Dendropsophus microps</i> (Peters, 1872) <i>Dendropsophus minutus</i> (Peters, 1872) <i>Dendropsophus nanus</i> (Boulenger, 1889) <i>Dendropsophus rubicundulus</i> (Reinhardt and Lütken, 1862) <i>Dendropsophus sanborni</i> (Schmidt, 1944) <i>Dendropsophus</i> sp.1 <i>Dendropsophus</i> sp.2 <i>Dendropsophus</i> sp.3

FAMILIES	SPECIES
	<i>Oloolygon fuscovarius</i> (A. Lutz, 1925)
	<i>Oloolygon longilinius</i> (B. Lutz, 1968)
	<i>Oloolygon ranki</i> (Andrade and Cardoso, 1987)
	<i>Oloolygon</i> sp.1
	<i>Oloolygon</i> sp.2
	<i>Scinax caldarum</i> Lutz, 1968
	<i>Scinax fuscomarginatus</i> (A. Lutz, 1925)
	<i>Scinax x-signatus</i> (Spix, 1824)
	<i>Scinax hayii</i> (Barbour, 1909)
	<i>Scinax</i> sp.1
	<i>Scinax</i> sp.2
	<i>Trachycephalus imitatrix</i> (Miranda-Ribeiro, 1926)
	<i>Trachycephalus typhonius</i> (Linnaeus, 1758)
	<i>Vitreorana uranoscopa</i> (Müller, 1924)
<b>Hylodidae</b>	
	<i>Crossodactylus</i> aff. <i>caramaschi</i> Bastos & Pombal, 1995
	<i>Crossodactylus</i> sp.1
	<i>Hylodes</i> aff. <i>lateristrigatus</i> (Baumann, 1912)
	<i>Hylodes</i> gr. <i>sazimae</i>
<b>Leptodactylidae</b>	
	<i>Adenomera</i> aff. <i>hylaedactyla</i> (Cope, 1868)
	<i>Leptodactylus cunicularius</i> Sazima and Bokermann, 1978
	<i>Leptodactylus furnarius</i> Sazima and Bokermann, 1978
	<i>Leptodactylus fuscus</i> (Schneider, 1799)
	<i>Leptodactylus labyrinthicus</i> (Spix, 1824)
	<i>Leptodactylus mystaceus</i> (Spix, 1824)
	<i>Leptodactylusmystacinus</i> (Burmeister, 1861)
	<i>Leptodactylus macrosternum</i> Miranda-Ribeiro, 1926
	<i>Leptodactylus</i> sp.1
	<i>Physalaemus cuvieri</i> Fitzinger, 1826
	<i>Physalaemus nattereri</i> Steindachner, 1863
	<i>Physalaemus jordanensis</i> Bokermann, 1967
<b>Microhylidae</b>	
	<i>Elachistocleis ovalis</i> (Schneider, 1799)
<b>Odontophrynidae</b>	
	<i>Odontophrynus americanus</i> (Duméril and Bibron, 1841)
	<i>Odontophrynus cultripes</i> Reinhardt and Lütken, 1862
	<i>Proceratophrys boiei</i> (Wied-Neuwied, 1825)
<b>Ranidae</b>	
	<i>Lithobates catesbeianus</i> (Shaw, 1802) – <b>Exotic species</b>



## First record of *Oloolygon longilinea* (Anura: Hylidae) for the state of São Paulo, southeastern Brazil

Rodrigo Matavelli<sup>1,2,\*</sup>, Juliano Oliveira<sup>2</sup>, Ederson Godoy<sup>2</sup>, Milton Cezar Ribeiro<sup>2</sup> and Jaime Bertoluci<sup>1</sup>

The anuran family Hylidae Rafinesque, 1815 is composed of 693 species arranged in seven subfamilies plus “*Hyla*” *imitator* (Barbour and Dunn, 1921) *incertae sedis* (Frost, 2017). The subfamily Scinaxinae Duellman, Marion, and Hedges, 2016 contains four genera and 133 species, which range mostly across parts of South America (from Uruguay and northern Argentina to northwestern Peru), northward through Central America to southern and eastern Mexico, and in the islands of Tobago, Trinidad, and Saint Lucia (Frost, 2017).

The Neotropical genus *Oloolygon* Fitzinger, 1843 includes 44 species distributed in the Atlantic Forest of eastern Brazil, extending southward to northeastern Argentina and westward into the Brazilian Cerrado, where the species can be found in gallery forests (Frost, 2017). This genus was recently removed from the synonymy of *Scinax* by Duellman et al. (2016) and corresponds to the former *Scinax catharinae* group (Faivovich, 2002; Faivovich et al., 2005; Duellman et al., 2016).

The highest diversity of species of *Oloolygon* occurs primarily in the Atlantic Forest domain of Brazil (Lourenço et al., 2014; Frost, 2017), except for *O. aromothyella* (Faivovich, 2005) and *O. berthae* (Barrio, 1962), which also occur in open areas of Argentina, Uruguay and Paraguay (Brusquetti and Lavilla, 2006; Laufer et al., 2009; Busin et al., 2010; Pereyra et al., 2012). However, some species (*O. canastrensis*, *O. centralis*, *O. longilinea*, *O. luizotavioi*, *O. machadoi*,

and *O. skaios*) are also found in the Cerrado biome and in transitional areas between Cerrado and Atlantic Forest (Faivovich, 2002; Canelas and Bertoluci, 2007; Bertoluci et al., 2007; Bertoluci et al., 2009; Horta et al., 2010; Pombal et al., 2010; Lourenço et al., 2013; Lourenço et al., 2016).

*Oloolygon longilinea* (Lutz, 1968) was originally described as *Hyla longilinea* based on a single specimen collected at the water reservoir of Morro de São Domingos, Poços de Caldas, Minas Gerais State (21.7854° S, 46.5619° W), between 1,200 and 1,300 meters elevation (holotype MNRJ 4060, by original designation) (Lutz, 1968). This small tree frog has nocturnal and arboreal habits and is usually observed in riparian forests and forest edges, on vegetation near streams and pools where it reproduces. It is found in mountains of southeastern Brazil, between 700 and 1,200 m a.s.l. (Frost, 2017; IUCN, 2017).

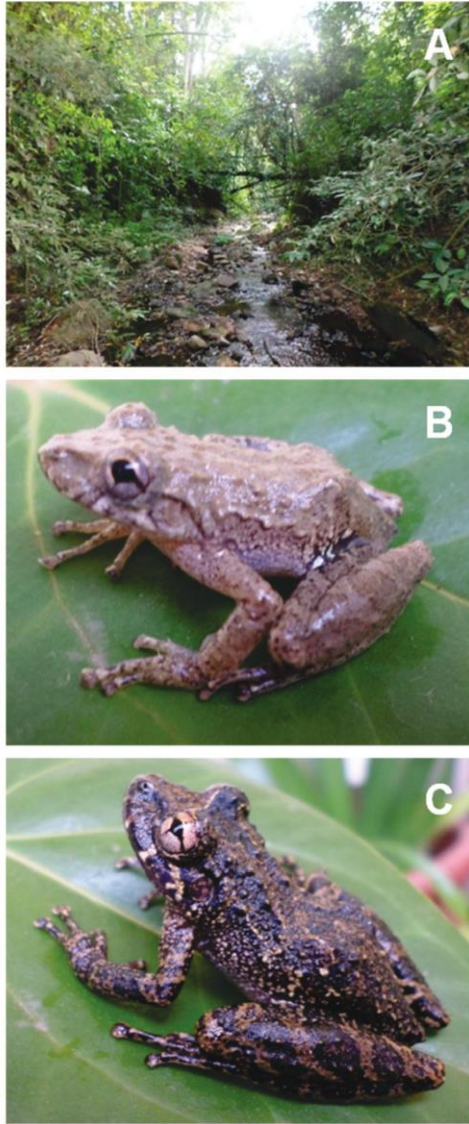


**Figure 2.** Geographic distribution of *Oloolygon longilinea* (Lutz, 1968). Type locality (star), new record for São Paulo state (circle), and literature records (triangles).

<sup>1</sup> Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo (ESALQ/USP). Av. Pádua Dias 11, 13418-900, Piracicaba, SP, Brazil.

<sup>2</sup> Laboratório de Ecologia Espacial e Conservação (LEEC), Departamento de Ecologia, Universidade Estadual Paulista (UNESP). Av. 24A, 1515, 13506-900, Rio Claro, SP, Brazil.

\* Corresponding author. E-mail: ram\_eco@yahoo.com.br



**Figure 1.** (A) Habitat where *Oloolygon longilinea* (Lutz, 1968) specimens were collected in São João da Boa Vista municipality, state of São Paulo, southeastern Brazil. Adult male (B) and adult female (C) of *O. longilinea* in life.

Until now, *O. longilinea* has been found in the municipalities of Alfenas (Ferrante *et al.*, 2017); Poços de Caldas (Lutz, 1968; Andrade and Cardoso, 1991); Nova Lima (Nascimento *et al.*, 1994; Bertoluci *et al.*,

2007); Rio Acima (Grandinetti and Jacobi, 2005); Brumadinho and Congonhas (Leite *et al.*, 2008); Morada Nova de Minas (Torres and Eterovick, 2010); Belo Horizonte (Leite *et al.*, 2008; Del Lama *et al.*, 2011; Fatorelli *et al.*, 2015); Ouro Branco (Leite *et al.*, 2008; São-Pedro and Feio, 2011); and Ouro Preto (Pedralli *et al.*, 2001; Pirani *et al.*, 2013; Hepp *et al.*, 2017). Furthermore, studies have been performed on tissues of *O. longilinea* specimens from different municipalities (Caeté, Ibertioga, Ibituruna, Itabira, Lagoa Santa, Moeda, Pains, Sabará and Sete Lagoas) (Lourenço *et al.*, 2016). All the municipalities cited above are in the state of Minas Gerais, southeastern Brazil. We provide here the first record of *Oloolygon longilinea* for the state of São Paulo, southeastern Brazil.

During field surveys carried out on 15 February 2017, two *O. longilinea* individuals were collected perched on a shrub near a gallery forest in a transitional area between Cerrado and Atlantic Forest, in the municipality of São João da Boa Vista, central-east region of São Paulo state (21.8844° S, 46.8376° W, 720 m a.s.l.) (Figure 1A). An adult male (26.1 mm SVL) and an adult female (40.1 mm SVL) were captured between 21:30 and 22:00 h (Figure 1B, C), euthanized with 5% xylocaine, fixed in 10% formalin and preserved in 70% alcohol. Voucher specimens were deposited in the herpetological collection of Escola Superior de Agricultura Luiz de Queiroz (VESALQ 1031-2), under the SISBio collection license number 48526-1.

This new record extends the distribution of *Oloolygon longilinea* to São Paulo state and to approximately 30 km directly west-southwest from the type locality (Figure 2). Although this new locality is relatively near the other points of the species distribution, our record is important because it adds a species to the list of anurans of São Paulo state, which has its own state list of threatened fauna. According to Pimenta and Andrade (2004), populations of *O. longilinea* are decreasing, and the major threats are probably related to habitat loss due to wood plantations, logging, mining and human settlement.

**Author Contributions:** RM, JO and EG carried out the fieldwork; RM, MCR and JB wrote the manuscript.

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2), and Instituto Chico Mendes de Conservação da Biodiversidade (ICMbio/SISBio) for the collection license (Authorization #48526-1). JB and MCR are researchers of CNPq (processes 309017/2016-5 and 312045/2013-1, respectively).

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APPENDIX D. Short note published in Herpetology Notes in May 2018.

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## Expanding the known range of *Trachycephalus imitatrix* (Anura: Hylidae) in the state of Minas Gerais, southeastern Brazil

Rodrigo Matavelli<sup>1,2,\*</sup>, Juliano Oliveira<sup>2</sup>, Ederson Godoy<sup>2</sup> and Milton Cezar Ribeiro<sup>2</sup> and Jaime Bertoluci<sup>1</sup>

The genus *Trachycephalus* Tschudi, 1838 actually is composed of 17 species widely distributed from lowlands of Mexico, Central and South America east of the Andes, south to north Argentina and eastern Brazil (Frost, 2017; IUCN, 2017). It is noteworthy that five species [*Trachycephalus hadroceps* (Duellman and Hoogmoed, 1992), *Trachycephalus jordani* (Stejneger and Test, 1891), *Trachycephalus macrotis* (Andersson, 1945), *Trachycephalus "vermiculatus"* (Cope, 1877), and *Trachycephalus quadrangulum* (Boulenger, 1882)] have not yet been registered in Brazil up to date (Frost, 2017; IUCN, 2017).

Actually 12 species of the genus *Trachycephalus* are known for Brazil (Frost, 2017), six of which [*Trachycephalus atlas* Bokermann, 1966, *Trachycephalus mambaiensis* Cintra, Silva-Jr., Garcia and Zaher, 2009, *Trachycephalus mesophaeus* (Hensel, 1867), *Trachycephalus nigromaculatus* Tschudi, 1838, *Trachycephalus typhonius* (Laurenti, 1768), and *Trachycephalus imitatrix* (Miranda-Ribeiro, 1926)] have already been record in the state of Minas Gerais (Cruz et al., 2009; IUCN, 2017).

*Trachycephalus imitatrix* (Miranda-Ribeiro, 1926) was originally described as *Hyla imitatrix* (holotype MNRJ 154, by original designation) based on two specimens collected at Parque Nacional da Serra dos Orgãos (22.4956 S, 43.0736 W) municipality of Teresópolis, Rio de Janeiro, Brazil (Frost, 2017). It is a poorly known

arboreal treefrog that has a large and robust body (female SVL 5.3 cm and male SVL 7.1 cm, on average), which exhibits an explosive breeding spawning in permanent water bodies of the forest, being considered a rare species (Cruz et al., 2009; Haddad et al., 2013; Frost, 2017; IUCN, 2017). This species is found in the canopy of the primary forest in mountainous parts of southern and southeastern Brazil (from southern Minas Gerais to northern Rio Grande do Sul) and Argentina (west to east-central Misiones Province) in altitudes from 400 to 1,700 meters a.s.l. (Bertoluci, 2001; Bertoluci and Rodrigues, 2002; Frost, 2017; IUCN, 2017). However, it is worth mentioning that *Trachycephalus imitatrix* was very confused with *Trachycephalus dibernardoi* in the southern region of Brazil prior to its description (Kwet, 1997a; Kwet, 1997b; Kwet, 1998; Kwet and Di-Bernardo, 1999; Kwet, 2001; Frost, 2017).

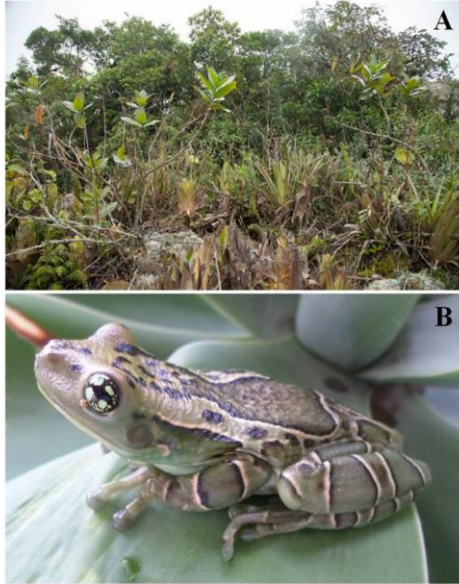


**Figure 2.** Geographic distribution of *Trachycephalus imitatrix* (Miranda-Ribeiro, 1926) in the state Minas Gerais, southeastern Brazil. Type locality (triangle), new record (star), and the only record known to the state (circle).

<sup>1</sup> Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo. Avenida Pádua Dias 11, CEP 13.418-900, Piracicaba, SP, Brazil.

<sup>2</sup> Laboratório de Ecologia Espacial e Conservação (LEEC), Departamento de Ecologia, UNESP-Universidade Estadual Paulista, Avenida 24A, 1515, CEP 13.506-900, Rio Claro, SP, Brazil.

\* Corresponding author. E-mail: ram\_eco@yahoo.com.br



**Figure 1.** (A) Habitat where *Trachycephalus imitatrix* (Miranda-Ribeiro, 1926) was collected in the municipality of Caldas, southwestern Minas Gerais, Brazil. (B) Adult male of *T. imitatrix* (SVL 70.0 mm) in life.

Nevertheless, *T. imitatrix* has been recorded until now in the state of Minas Gerais only at Parque Estadual do Ibitipoca (21.7166 S, 43.9029 W), which is inserted in the Atlantic Forest domain (Cruz *et al.*, 2009). Here we report the second record of *T. imitatrix* (Miranda-Ribeiro, 1926) in the state Minas Gerais, southeastern Brazil.

During field surveys carried out on 21 February 2017, an adult male of *T. imitatrix* (Figure 1A) was found and captured between 22:00 and 22:30 h inside a bromeliad on a rocky outcrop (21.9796 S, 46.3678 W, 1,730 m a.s.l.) in a transition area (Figure 1B). This spot is a vegetational transition zone (see Rosa and Monteiro, 2012) that belongs to the Reserva Biológica da Pedra Branca, which is inserted at Área de Proteção Ambiental “Santuário Ecológico da Pedra Branca”, municipality of Caldas, southwestern of Minas Gerais state (Figure 2). Voucher specimen was euthanised with 5% xylocaine, fixed in 10% formalin, preserved in 70% alcohol and deposited in the herpetological collection of Escola Superior de Agricultura Luiz de Queiroz (VESALQ 1141), under the SISBio collection license number #48526-1.

This second record of *T. imitatrix* to Minas Gerais expands its distribution in approximately 345 km from the type locality (Parque Nacional da Serra dos Orgãos; Frost, 2017), and approximately 260 km from Parque Estadual do Ibitipoca, the only record in the state of Minas Gerais up to date (Cruz *et al.*, 2009).

*T. imitatrix* does not tolerate highly disturbed habitats, and the major threats are habitat loss due to agriculture, livestock grazing, wood plantations, logging, and human settlement and for these reasons the population of this species is probably decreasing (IUCN, 2017). In the study region the major threats to the diversity of the flora and fauna are agriculture, pasture areas, and mining, but currently disorderly tourism is also another problem (Rosa and Monteiro, 2012; Rezende *et al.*, 2013; Williams *et al.*, 2014).

Another important fact is that *Trachycephalus imitatrix* is not well-known in any protected areas in Brazil (IUCN, 2017), but it has already been recorded in different protection areas besides the type locality at Parque Nacional da Serra dos Orgãos (Frost, 2017), but also at Parque Nacional da Bocaina (Garey *et al.*, 2014), Parque Estadual do Intervales (Bertoluci, 2001; Bertoluci and Rodrigues, 2002), Parque Estadual da Serra do Mar (Silva *et al.*, 2017), Parque Estadual do Ibitipoca (Cruz *et al.*, 2009), and now at Reserva Biológica da Pedra Branca, which reinforces the need for preservation of the different types of reserves in Brazil, which despite the poor management, still harbor a great diversity of endemic and rare species.

This new record is important because of the scarcity of data about this species, considered rare (Bertoluci, 2001; Bertoluci and Rodrigues, 2002; Haddad *et al.*, 2013; IUCN, 2017), corroborating with some authors that additional information on the geographical distribution of species is one of the most important criteria used to assess species conservation status and also to plan for future conservation strategies (Drummond *et al.*, 2005; Bressan *et al.*, 2009).

**Author Contributions:** RM, JO and EG collected the data, RM, MCR and JB wrote the text.

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