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Article

Selection and use of calling site by *Boana leptolineata* and *Phyllomedusa distincta* during the reproductive season

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ABSTRACT. Climatic conditions and microhabitat characteristics, such as the substrate and type of vegetation, influence the choice of male anurans for calling sites that optimize their reproductive success. We evaluated the structure and selection of vocalization microhabitat of 17 individuals of *Phyllomedusa distincta* Lutz, 1950 and 25 of *Boana leptolineata* (Braun & Braun, 1977), in a subtropical forest in southern Brazil. We measure the height of the perch, the distance between the perch and the edge of the body of water and the structure of the microhabitat of quadrants used by individuals. The same attributes were measured in the quadrants available (but not used) by the individuals. We classified the microhabitats by visual estimate, assigning percentages of coverage for each quadrant by herbaceous, shrub, tree and wetland vegetation. We observed that both species selected their microhabitat, since the characteristics of the quadrants occupied by the individuals were different from those available. *Phyllomedusa distincta* was more associated with heterogeneous microhabitats, while *B. leptolineata* occurred in environments with greater coverage of tree strata. Additionally, we observed that both species used shrubs more frequently as a perch site. The selection of these microhabitat characteristics must be associated with strategies to optimize the use of the reproductive habitat, based on the morphological and behavioral characteristics of the species. Finally, it is possible to infer that the differences observed in the microhabitat structure selected by the species can facilitate the coexistence of both in the context of the heterogeneity of the environment.

KEYWORDS. Amphibian, breeding, microhabitat, resource partitioning, syntopic.

RESUMO. Seleção e uso de sítios de vocalização por *Boana leptolineata* e *Phyllomedusa distincta* durante o período reprodutivo. As condições climáticas e as características de micro-habitat, como o substrato e o tipo de vegetação, influenciam a escolha de anuros machos por sítios de vocalização que otimizem seu sucesso reprodutivo. Avaliamos a estrutura e a seleção de micro-habitat de vocalização de 17 indivíduos de *Phyllomedusa distincta* Lutz, 1950 e 25 de *Boana leptolineata* (Braun & Braun, 1977), em uma floresta subtropical no sul do Brasil. Medimos a altura do poleiro, a distância do poleiro até a margem do corpo d'água e a estrutura do micro-habitat de quadrantes utilizados pelos indivíduos. Os mesmos atributos foram mensurados nos quadrantes disponíveis (mas não utilizados) pelos indivíduos. Classificamos os micro-habitats por estimativa visual, atribuindo-se porcentagens de cobertura de cada quadrante por vegetação herbácea, arbustiva, arbórea e de área alagada. Observamos que ambas as espécies selecionaram seu micro-habitat, uma vez que as características dos quadrantes ocupados pelos indivíduos foram distintas daquelas disponíveis. *Phyllomedusa distincta* esteve mais associada a micro-habitats heterogêneos, enquanto *B. leptolineata* ocorreu em ambientes com maior cobertura de estrato arbóreo. Adicionalmente, observamos que ambas as espécies utilizaram mais frequentemente arbustos como local do poleiro. A seleção destas características de micro-habitat deve estar associada a estratégias para otimização do uso do habitat reprodutivo, baseadas em características morfológicas e comportamentais das espécies. Por fim, é possível inferir que as diferenças observadas na estrutura de micro-habitat selecionadas pelas espécies podem facilitar a coexistência de ambas no contexto da heterogeneidade do ambiente.

PALAVRAS-CHAVE. Anfíbios, reprodução, micro-habitat, partição de recursos, sintópico.

The theory of ecological niches defines fundamental niche as the set of environmental conditions that determine the limits of survival of a given species based on its physiological and behavioral characteristics (Holt, 2009). The relationship of this species with the biological community and the physical structure of the available habitat is another important dimension of this concept when determining the so-called realized niche (Holt, 2009). The habitat's physical structure deals with the different scales and forms of element organization in the environment (*e.g.*, complexity or heterogeneity of plant strata), also understood as microhabitat, when observed in the organism's scale (McCoy & Bell, 1991; Purrenhage & Boone, 2009). Thus, the effects of these microhabitat characteristics determine their suitability for the establishment and success of many taxa, including amphibians (Holt, 2009; Purrenhage & Boone, 2009).

Studies on the spatial and temporal distributions of species have been fundamental to understand patterns in anuran communities (Forti, 2009). The preference for certain habitat characteristics is an adaptive trait where individuals tend to prefer and use habitats that provide a greater probability of survival and reproductive success (Fierro-Calderón & Martin, 2019). Seasonal events, such as the reproductive cycle, can affect the microhabitat preferences of animals (Santos *et al.*, 2016). During the mating season, many anuran species share the same water body in an event called "reproductive congregations" (Toledo *et al.*, 2003). At a given calling site, males emit advertisement calls to attract females during this period (Wells & Schwartz, 1982). Calling site selection is based on many microhabitat resources, a subject that is still an open field for fieldwork studies, especially for neotropical anurans (Santos *et al.*, 2016). In general, animals exhibit preferences for specific environmental characteristics, such as substrate composition, climatic conditions and vegetation types (Reinert, 1993; Tozetti *et al.*, 2010). Adequate calling site selection improves reproductive performance of males (Hödl, 1977; Wells, 2007; Akmentins *et al.*, 2014; Solano *et al.*, 2015).

Due to the presence of many individuals in the same pond at the same time, reproductive congregations could be a challenging situation for males looking for places. Studies show that one of the main factors that allows species coexistence in the same reproductive congregation is the use of calling sites with different microhabitat conditions (Duellman, 1967; Oldham & Gerhardt, 1975; Cardoso & Vielliard, 1990; Rossa-Feres & Jim, 2001; Moser *et al.*, 2019). When individuals reproduce in the same location (*e.g.*, pond), they are immersed in a similar set of habitat resources (Moser *et al.*, 2019). However, how they use those resources could be quite different even at a temporal or spatial evaluation (Pianka, 1973; Schoener, 1974; Moser *et al.*, 2019). Additionally, due to the similarity in behavior, physiology and morphology, phylogenetically close species tend to share these resources more sharply (Heyer *et al.*, 1990).

Microhabitat complexity also has a great influence on anuran use of the spatial resource in the reproductive sites (Sinsch, 2014). Several species present separation in calling and oviposition sites, with little or no overlap, in addition to using aquatic habitats differently (Bertoluci & Rodrigues, 2002). Environments with herbaceous and arboreal vegetation close to the body of water, for example, may favor anurans with arboreal habits, such as hylids, which also use the vertical stratum (Rossa-Feres & Jim, 2001).

In southern Brazil, the Araucaria Forest formation represents the southernmost extension of the Atlantic Forest. This is a subtropical region with high altitudes and which suffers with the influence of oceanic heated masses and presents low temperatures, registering frost in the coldest months (Hueck, 1972; Carlucci *et al.*, 2011). In this place, the anurans occupy several environments of temporary and permanent wetlands and ponds, where often several species congregate during their reproductive periods (Kwet *et al.*, 2010). This configuration makes it possible to carry out studies that seek to evaluate the selection of vocalization sites by males of different species.

We highlight two anuran species that were observed in syntopy in those ponds: the tree frogs *Phyllomedusa distincta* Lutz, 1950 from the Phyllomedusidae family and *Boana leptolineata* (Braun & Braun, 1977) from the Hylidae family (Frost, 2020). According to Kwet *et al.* (2010), these species vocalize at night on low vegetation and shrubs that are close to the margin. The *P. distincta* tree frog has an average of 46-71 mm and the males vocalize a low sound perched on branches of vegetation over the water. The reproduction of this species occurs in the hottest and rainiest months of the year, from October to January (Haddad & Prado, 2005; Kwet *et al.*, 2010). *Boana leptolineata* measures around 26-36 mm. Males vocalize over the vegetation on bodies of water, where the reproductive period extends throughout the year, except for periods with very low temperatures (Reinke & Deiques, 2010). In addition, the oviposition of these species is similar, since they lay their eggs on leaves in the vegetation above the water (Kwet & Di-Bernardo, 1999; Kwet *et al.*, 2010). Thus, these species are good models to investigate the characteristics and use of calling sites by understanding their microhabitats choice.

Considering the importance of the spatial resource use strategies in maintaining the dynamics and structure of communities, our objective in this work was to evaluate whether males of *P. distincta* and *B. leptolineata* select the vocalization site by the structural characteristics of the microhabitat.

MATERIALS AND METHODS

Study site. We conducted the study at the Private Reserve of Natural Heritage (RPPN in Portuguese) Pró-Mata, property of Pontifícia Universidade Católica do Rio Grande do Sul, municipality of São Francisco de Paula, southern Brazil (Fig. 1). The region's climate type is Cfb according to Köppen's scale, humid to super humid, and located at an average altitude of 912 m. The average annual temperature is 14.5°C and the average annual precipitation is 2,252 mm (Bertoletti & Teixeira, 1995). The month in which we carried out the study, January, is the hottest of the year, with average temperatures of 20°C, and maximum averages of 27°C, it is also among the rainiest month in the region (IMA-PUCRS, 2011). The Phytoecological Regions present in the RPPN Pró-Mata are Mixed Ombrophilous Forest (Araucaria Forest), Dense Ombrophilous Forest and Savanna (IBGE, 1986). We sampled individuals on the banks of a permanent and lentic wetland (29°28'55.43"S, 50°10'37.33"W), with 15 m long and seven meters wide, with a surface of approximately 90 m^2. Its southeast margin is bordered by a local access road with low vehicles flow (Fig. 1). The banks of the wetland are characterized by predominantly arboreal and shrubby vegetation inserted in a matrix of humid grasslands with herbaceous and shrubby vegetation, characterizing a heterogeneous transition habitat between flooded area and surrounding forests at different successional stages (Fig. 1).

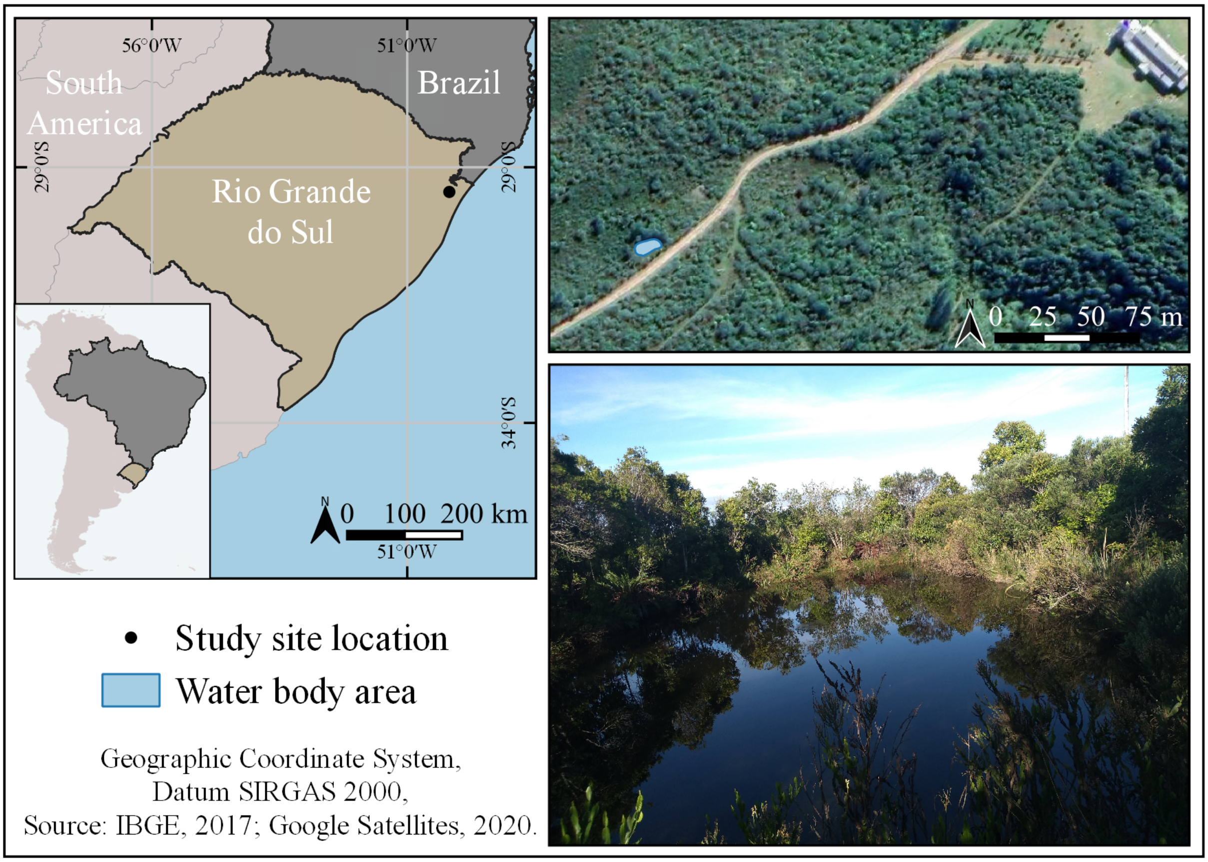

Fig. 1. Characterization of the study area. Location map of the area, São Francisco de Paula, RS, Brazil (left box). Landscape of the study site dominated by herbaceous vegetation (upper right box. in upper right corner: headquarters of the RPPN Pró-Mata; in lower right corner: forest patch). And view from the wetland's east bank showing the heterogeneity of herbaceous and tree vegetation (bottom right box).

Sampling and data collection. We manually captured males of *Boana leptolineata* and *Phyllomedusa distincta* through active search (Crump & Scott, 1994) in reproductive sites. We collected data between 08:00 p.m. and 00:00 a.m., over four consecutive nights in January 2020, totaling a sampling effort of 16 h. We packed each captured specimen in a plastic bag inflated with air and moist vegetation where they were kept for 1 to 4 h to prevent recapture in the same night of sampling. We released them at the same capture site at the end of each collection night. It is important to mention that during data collection other species were in vocalization activity (*Scinax* sp., *Dendropsophus minutus*, *Boana faber* and *Pseudis cardosoi*), but were not included in this study.

Assessment of occupied and available calling sites. For each captured vocalizing male, we evaluated the structure of the occupied microhabitat (vocalization site). For this purpose, we delimited 1 x 1 m quadrants with the individual observed location in the center. Within each quadrant, we obtained the maximum height and visually estimated (coverage percentage) the area occupied by herbaceous, shrub and tree vegetation and by flooded area (the height measurement of the occurrence of flooded area was recorded with a value of zero) (adapted from Huckembeck *et al.*, 2012; Tozetti & Martins, 2008; Santos *et al.*, 2016; Moser *et al.*, 2019). If the quadrant lacked some vegetation types, we replaced the height measurement corresponding to the absent type with the maximum height of the vegetation type present at the central point of the same quadrant. Thus, we obtained at least three height measurements for each quadrant. To characterize the microhabitat structure available to individuals, we applied the same assessment in four quadrants that were 1 m away from the occupied quadrant, oriented by each cardinal direction.

In addition, for each captured individual, we measured its height from the ground (perch height), its minimum distance to the edge of the body of water and the perch's substrate type: herbaceous, shrub, tree or water (Menin *et al.*, 2005; Prado & Pombal 2005; Wachlevski *et al.*, 2008; Santos *et al.*, 2016; Moser *et al.*, 2019).

Data analysis. Using microhabitat data, we elaborated the microhabitat structure matrices of the occupied (O) or available (A) quadrants for the anurans. We used the raw measures of strata coverage and water body per quadrant. From the height values, we estimated the strata heterogeneity of each quadrant by calculating the coefficient of variation (Cv) with the equation:

$$Cv = \frac{S}{\overline{X}} \cdot 100$$

where S is the standard deviation and $\overline{X}$ is the mean of the height values.

To observe the existence of species preference for a type of perch substrate, a chi-square test was performed, taking as the expected value the average proportion of each substrate present in all quadrants occupied by individuals of the respective species.

In order to verify the existence of microhabitat selection at the calling site by the species, we performed a Procrustes analysis (GOWER, 1975). This analysis allows us to evaluate the congruence between two data matrices through the rotation (or orthogonal transformation) of the ordering axes necessary for the overlap of the two data sets in the ordering space. From this rotation, a Procrustes correlation value (t0) is generated between the two matrices (which can be interpreted analogously to a Mantel matrix correlation), and an associated p-value based on null models from permutations (999 iterations) of the rotation and rearrangement events of the orthogonal axes (GOWER, 1975). We used the matrix of the environmental variables of the occupied quadrants in opposition to the matrix of the variables of those available to individuals of each species. Thus, we consider the existence of microhabitat selection if there was no congruence between the two matrices.

Additionally, we applied Multinomial Logistic Regression (MLR) models (KWAK & CLAYTON-MATTHEWS, 2002) for each species to observe how the microhabitat variables (percentage of the three vegetation strata, water body and stratum heterogeneity) may predict the probability of individuals to occur. MLR is an extension of binary logistic regression that allows assessing the probability of an event to occur using a response variable with more than two categories (non-hierarchical) and independent variables being metric or dichotomous (KWAK & CLAYTON-MATTHEWS, 2002).Therefore, here, the response variable was structured as five classes where the first is the presence of the individual in the quadrant and the other four are its absence in each of the alternative available quadrants. If any microhabitat variable (predictive variable) described more than 0.5 (50%) of the probability of occurrence in our model, we considered it as a predictor of microhabitat selection by any anuran species.

These analyses were performed in the R software (R CORE TEAM, 2020) with the packages "vegan" (OKSANEN *et al.*, 2019), "ade4" (DRAY & DUFOUR, 2007), "car" (FOX & WEISBERG, 2019), and functions "Nnet" (VENABLES & RIPLEY, 2002), "lmtest" (ZEILEIS & HOTHORN, 2002) and "effects" (FOX & HONG, 2009; FOX & WEISBERG, 2019). We assume the probabilistic threshold of 95% for all analyses.

RESULTS

We obtained 42 capture events (17 *Phyllomedusa distincta* and 25 *Boana leptolineata*). The microhabitats of the calling sites used by *P. distincta* males had, on average, 36.7% shrub cover (SE = ± 6.87), 28.2% herbaceous cover (± 5.03) and 25% tree cover (± 7.57). In turn, for *B. leptolineata*, the average of herbaceous coverage was 36.7% (± 3.93), tree cover was 35.6% (± 5.97) and shrub cover was 28.2% (± 3.45). The water category corresponded to less than 10% for each species (Fig. 2). As for the heterogeneity of the microhabitat strata, the Cv averages were 74.6 (± 8.20) and 61.1 (± 4.53) for *P. distincta* and *B. leptolineata* respectively (Fig. 3).

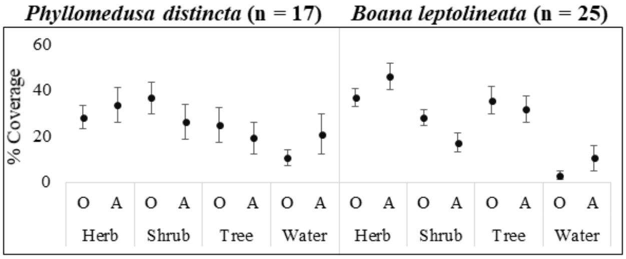

Fig. 2. Characteristics of the microhabitat of *Phyllomedusa distincta* (B. Lutz, 1950) and *Boana leptolineata* (P. Braun & C. Braun, 1977) in RPPN Pró-Mata, São Francisco de Paula, RS, Brazil. Occupied (O) and available (A) coverage microhabitat (Herb: herbaceous stratum; points: average; bars: standard error).

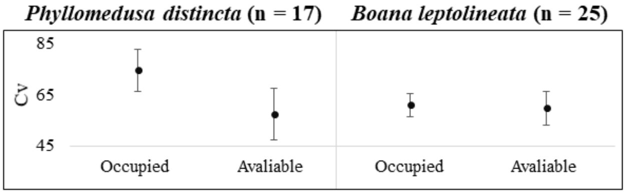

Fig. 3. Microhabitat strata heterogeneity of *Phyllomedusa distincta* (B. Lutz, 1950) and *Boana leptolineata* (P. Braun & C. Braun, 1977) in RPPN Pró-Mata, São Francisco de Paula, RS, Brazil (Cv: stratum height coefficient of variation; points: average; bars: standard error).

As characteristics of the perch location, the individuals of *P. distincta* were located on average at 68.4 (± 7.36) cm from the ground and 146 (± 50.36) cm from the water body's margin. Individuals of *B. leptolineata* were 79.8 (± 8.57) cm above the ground and 221 (± 24.22) cm away from the margin (Fig. 4). Regarding the type of substrate, both species used shrub substrates more frequently (*P. distincta* 76% and *B. leptolineata* 60% of the time, $P < 0.001$).

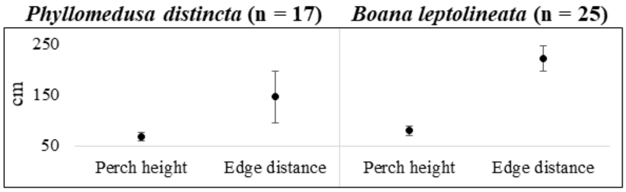

Fig. 4. Perch characteristics of *Phyllomedusa distincta* (B. Lutz, 1950) and *Boana leptolineata* (P. Braun & C. Braun, 1977) in RPPN Pró-Mata, São Francisco de Paula, RS, Brazil. (points: average; bars: standard error).

The differences between the characteristics of the occupied and available sites was confirmed by the lack of congruence between the matrices in the Procrustes analysis (*P. distincta*: $P = 0.63$, sum of squares (ss) = 0.043, t0 = 0.60; *B. leptolineata*: $P = 0.74$, ss = 0.021, t0 = 0.50 (Fig. 5).

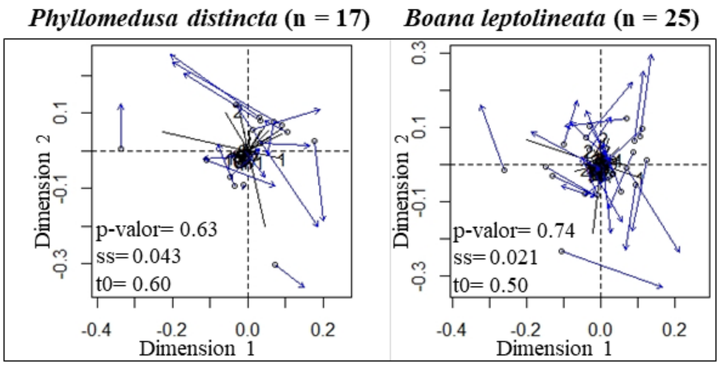

Fig. 5. Selection of microhabitat demonstrated by the dispersion diagram of the Procrustes analysis (ss, sum of squares; t0, Correlation of Procrustes. For each capture event: circles represents the matrix with environmental variables from the occupied quadrants; arrows represents the matrix of available quadrants with the same variables; line between both represents the size of congruence between matrices).

The MLR model indicated that only the strata heterogeneity of the calling site microhabitat did not show a relationship with the prediction of occurrence of *P. distincta* individuals (Fig. 6, Appendix 1). For *B. leptolineata*, the probability of occurrence (greater than 50%) was predicted only by the shrub stratum cover, where the greater the coverage of this stratum, the greater the probability of occurrence of individuals. The other variables, despite not reaching a 50% probability, indicate a tendency for individuals to be present in places with a lower proportion of herbaceous strata, water and with less strata heterogeneity (Fig. 6, Appendix 1).

DISCUSSION

Our data pointed out that males of *Phyllomedusa distincta* and *Boana leptolineata* present calling site selection based on distinct microhabitat components.

The vocalization sites of *P. distincta* were associated with microhabitats with more complex vegetation, indicating the preference for environments with proportional portions of denser coverage (shrubs and trees) and more exposed areas, with coverage of herbaceous and bodies of water. The use of this type of microhabitat as a calling site also occurs for other *Phyllomedusa* species. For instance, *P. sauvagii*, *P. tetraploidea* and *P. bursmeisteri* (Abrunhosa & Wogel, 2004; Dias *et al.*, 2013; Garcia *et al.*, 2013). However, the same does not occur for other congeners, such as *P. iheringii* e *P. azurea*, which vocalize in grasses and herbaceous above ground (Dias *et al.*, 2014, 2017).

Males of *B. leptolineata* selected calling sites with a predominance of shrub stratum in addition to a tendency to lower proportions of herbaceous strata and water body, plus less stratum heterogeneity, therefore microhabitats more covered, with less proportion of open strata. Also, the species has already been found vocalizing in emergent vegetation (Reinke & Deiques, 2010).

For anurans, a conflicting demand is recognized regarding the characteristics of the vocalization site (Duellman & Trueb, 1986; Bernarde, 2012). More exposed environments, with less dense vegetation and higher perch selection, represent advantages for the male to better propagate its vocalization, becoming more attractive to females (Bernarde, 2012); on the other hand, this behavior makes it more visible and vulnerable to predators, in addition to suffering greater dehydration (Santos *et al.*, 2007; Santos *et al.*, 2016). Thus, the intensity of the predation pressure in this environment, as well as the quality of the conditions for its reproduction, should influence which strategy will be adopted and, consequently, what type of microhabitat will be selected (Wells, 2007; Santos *et al.*, 2016; Moser *et al.*, 2019). Our data describe possible strategies that these species may be adopting in selecting microhabitat to improve the reproductive habitat use, which is possibly associated with the species' morphological and behavioral characteristics (Bertoluci & Rodrigues, 2002).

In this sense, it is also important to investigate the calling characteristics of anurans which may be influenced by environmental restrictions (Zimmerman, 1983). Some studies show that, in response to characteristics of their habitat, anuran species can adjust their acoustic emissions (Lardner & Bin Lakim, 2002; Slabbekoorn & Peet, 2003; Patricelli & Blickley, 2006). Thus, when selecting the calling site, males should consider the best location for sound propagation to increase the chances of attracting females (Ziegler *et al.*, 2011; Santos *et al.*, 2016), and prevent conspecific male competition and/or in the acoustic niche at community level.

Furthermore, the preference of these amphibians for the shrub substrate as a perch site may be related to their reproductive modes. Abrunhosa & Wogel (2004) suggest that the selection of calling sites in microhabitats composed of arboreal substrate, with less dense vegetation, may facilitate visual communication during reproduction for *P. bursmeisteri.* Furthermore, according to Kwet *et al.* (2010), females of *P. distincta* lay their eggs wrapped in leaves hanging from the vegetation above the water. In its turn, the gelatinous posture of *B. leptolineata* is trapped delicately in the vegetation under the water and the tadpoles develop among the rocks at the bottom of the puddle (Kwet & Di-Bernardo, 1999; Kwet *et al.*, 2010). Thus, the location of the males on this substrate may also be related to the proximity to the oviposition site (Forti, 2009; Kwet *et al.*, 2010; Reinke & Deiques, 2010).

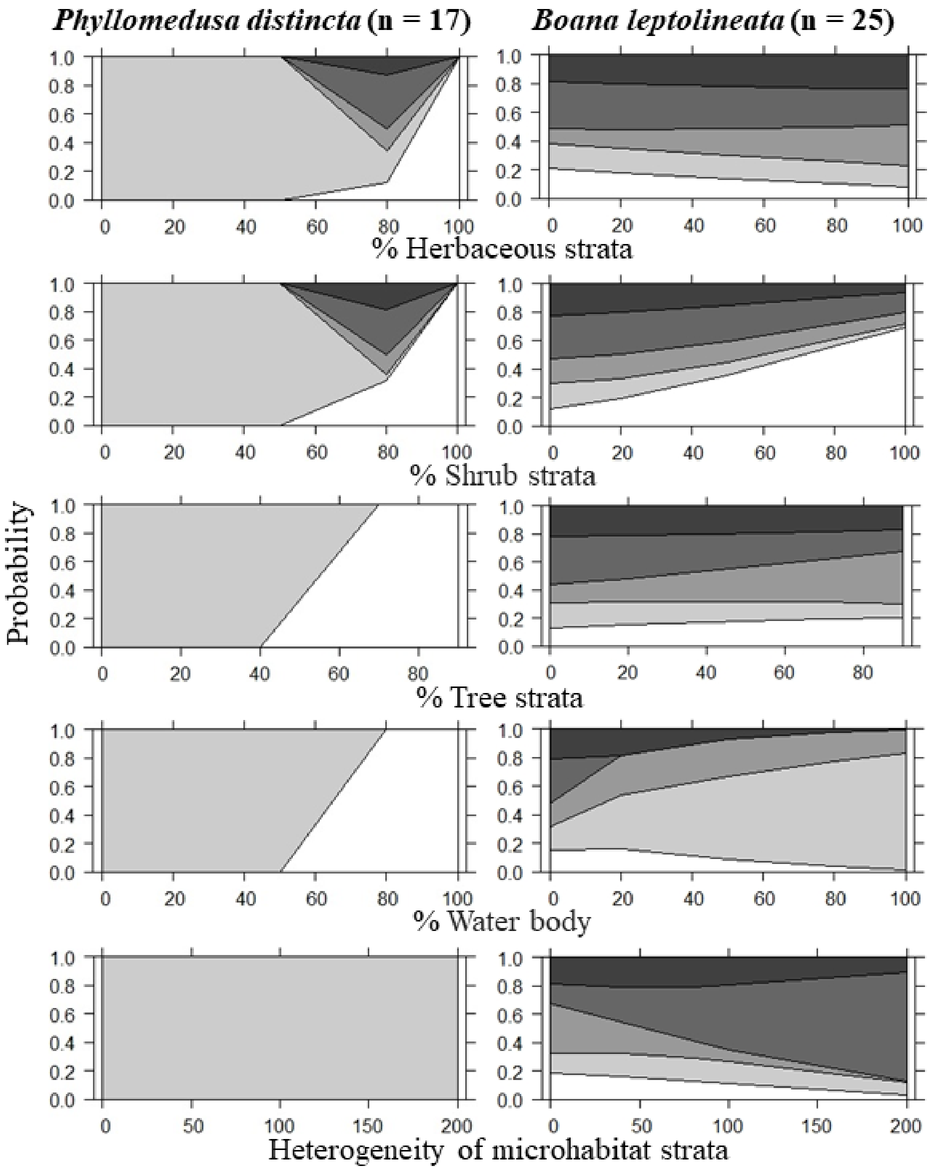

Fig. 6. Occurrence probability (y-axis) of *Phyllomedusa distincta* (B. Lutz, 1950) and *Boana leptolineata* (P. Braun & C. Braun, 1977) individuals in the quadrants based on the microhabitat environmental variables (x-axis) used in the Multinomial Logistic Regression model. Notes: white indicates the condition of individuals occurrence, while the shades of gray indicate the other four available conditions (quadrants) of individuals non-occurrence. A detailed table containing the outcomes is provided in Appendix 1.

In addition, the use of vertical dimension of vegetation as a perch for vocalization sites had already been recorded in the behavior for *B. leptolineata* (Kwet *et al.*, 2010; Reinke & Deiques, 2010) and *P. distincta* (Forti, 2009; Kwet *et al.*, 2010) in other studies. This conservative characteristic is also reported for other anuran species (Rossa-Feres & Jim, 2001; Bertoluci & Rodrigues, 2002; Santos & Rossa-Feres, 2007; Moser *et al.*, 2019) which optimizes the use of space in their reproductive congregations (Bertoluci & Rodrigues, 2002).

Finally, with this work, we demonstrated the existence of selection of microhabitat by the studied species. Our data did not allow us to detect the mechanisms that lead to differences in the use of microhabitat between species, but to infer that, even though the two species present similar historical characteristics of life (such as the arboreal habit and the laying of eggs), the structural characteristics of the microhabitat they select may permit their coexistence. These results highlight the possible role of different structural characteristics of the microhabitat to promote niche diversification to higher trophic levels (McCoy & Bell, 1991; Holt, 2009; Purrenhage & Boone, 2009). Lastly, we suggest that future studies could be designed to better understand possible mechanisms for the selection of this microhabitat characteristics and its role promoting the coexistence between different anuran species at reproductive congregations.

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REFERENCES

Abrunhosa, P. A. & Wogel, H. 2004. Breeding behavior of the leaf-frog *Phyllomedusa burmeisteri* (Anura, Hylidae). **Amphibia-Reptilia 25**:125-135.

Akmentins, M. S.; Pereyra, L. C.; Sanabria, E. & Vaira, M. 2014. Patterns of daily and seasonal calling activity of a direct-developing frog of the subtropical Andean forests of Argentina. **Bioacoustics 24**:89-99.

Bernarde, P. S. 2012. **Anfíbios e répteis: introdução ao estudo da herpetofauna brasileira.** São Paulo, Anolis Books. 320p.

Bertoletti, J. J. & Teixeira, M. B. 1995. Centro de Pesquisas e Conservação da Natureza Pró-Mata: Termo de Referência. **Divulgações do Museu de Ciências e Tecnologia – UBEA/PUCRS 2**:1-47.

Bertoluci, J. & Rodrigues, M. 2002. Utilização de habitats reprodutivos e micro-habitats de vocalização em uma taxocenose de anuros (Amphibia) da Mata Atlântica do sudeste do Brasil. **Papéis Avulsos de Zoologia 11**:287-297.

Cardoso, A. J. & Vielliard, J. 1990.Vocalizações de anfíbios anuros de um ambiente aberto, em Cruzeiro do Sul, Estado do Acre. **Revista Brasileira de Biologia 1**:229-242.

Carlucci, M. B.; Jarenkow, J. A.; Duarte, L. & Pillar, V. P. 2011. Conservação da Floresta com Araucária no extremo sul do Brasil. **Natureza & Conservação 9**:111-114.

Crump, M. L. & Scott, N. J. J. R. 1994. Visual encounter surveys. *In*: Heyer W. R.; Donnelly, M. A.; McDiarmid, R. W.; Hayek, L. A. C. & Foster, M. S. eds. **Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians.** Washington, Smithsonian Institution Press, p. 84-92.

Dias, T. M.; Maragno, F. P.; Madalozzo, B.; Prado, C. B. & Cechin, S. Z. 2013. Breeding sites of the leaf frog *Phyllomedusa tetraploidea* (Hylidae, Phyllomedusinae) in a forest remnant in southern Brazil. **North-western Journal of Zoology 9**:422-424.

Dias, T. M.; Maragno, F. P.; Prado, C. P. A. & Cechin, S. Z. 2014. Reproductive site selection in the leaf-frog *Phyllomedusa azurea* Cope, 1862 (Anura: Hylidae) in altered areas of the Brazilian Cerrado. **Journal of Natural History 48**:2689-2699.

Dias, T. M.; Santos, T. G.; Maragno, F. P.; Oliveira, V. F.; Lima, C. & Cechin, S. Z. 2017. Breeding biology, territoriality, and reproductive site use by *Phyllomedusa iheringii* (Anura: Phyllomedusidae) from the South American Pampa in Brazil. **Salamandra 53**:257-266.

Dray, S. & Dufour, A. 2007.The ade4 package: implementing the duality diagram for ecologists. **Journal of Statistical Software 4**:1-20.

Duellman, W. 1967.Courtship isolating mechanisms in Costa Rican hylid frogs. **Herpetologica 3**:169-183.

Duellman, W. E. & Trueb, L. 1986. **Biology of amphibians**. Baltimore, The Johns Hopkins University Press. 696p.

Forti, L. 2009. Temporada reprodutiva, micro-habitat e turno de vocalização de anfíbios anuros em lagoa de Floresta Atlântica, no sudeste do Brasil. **Revista Brasileira de Zoociências 1**:89-98.

Fierro-Calderón, K. & Martin, T. E. 2019. Does vegetation change over 28 years affect habitat use and reproductive success? **The Auk 137**:1-9.

Fox, J. & Hong, J. 2009.Effect Displays in R for Multinomial and Proportional-Odds Logit Models: Extensions to the effects Package. **Journal of Statistical Software 1**:1-24.

Fox, J. & Weisberg, S. 2019. **An R companion to applied regression**. Thousand Oaks, Sage. 474p.

Frost, D. R. 2020. **Amphibians Species of the World 6.1: An Online Reference**. American Museum of Natural History. Available at <https://amphibiansoftheworld.amnh.org/>. Accessed on 21 March 2021.

Garcia, C. G.; Lescano, J. N. & Leynaud, G. C. 2013. Oviposition-site selection by *Phyllomedusa sauvagii* (Anura:Hylidae): an arboreal nester inhabiting arid environments. **Acta Oecologica 51**:62-65.

Gower, J. C. 1975. Generalized Procrustes Analysis. **Psychometrika 1**:33-51.

Haddad, C. F. B. & Prado, C. P. A. 2005. Reproductive modes in frogs and their unexpected diversity in the Atlantic forest of Brazil. **BioScience 3**:207-217.

Heyer, R.; Rand, S.; Cruz C. A. G.; Peixoto, O. & Nelson, C. 1990. Frogs of Boracéia. **Arquivos de Zoologia 4**:231-410.

Hödl, W. 1977. Call differences and calling site segregation in anuran species from central Amazonian floating meadows. **Oecologia 28**:351-363.

Holt, R. D. 2009. Bringing the Hutchinsonian niche into the 21st century: ecological and evolutionary perspectives. **Proceedings of the National Academy of Sciences of the United States of America 106**:19659-19665.

Huckembeck, S.; Claudino, M.; Correa, F.; Bastos, R. F.; Loebmann, D.; Tozetti, A. M. & Garcia, A. M. 2012. Pattern activity and microhabitat use of *Pseudis minuta* Günther, 1858 (Anura, Hylidae) in a subtropical Biosphere Reserve in Brazil. **Brazilian Journal of Biology 72**:331-336.

Hueck, K. 1972. **As florestas da América do Sul: ecologia, composição e importância econômica**. São Paulo, Polígono. 466p.

IBGE.1986. **Projeto RADAM** Brasil**: levantamento de recursos naturais, folha SH.22, Porto Alegre e parte das folhas SH.21, Uruguaiana e SI.22, lagoa Mirim**. Rio de Janeiro, Instituto Brasileiro de Geografia e Estatística. v.33.

IMA-PUCRS - Instituto de meio ambiente da Pontifícia Universidade Católica do Rio Grande do Sul. 2011. **Plano de manejo centro de pesquisa e conservação da natureza PRÓ-MATA**. Porto Alegre, Instituto de Meio Ambiente, Pontifícia Universidade Católica do Rio Grande do Sul. 258p.

Kwak, C. & Clayton-Matthews, A. 2002. Multinomial Logistic Regression. **Nursing Research 6**:404-410.

Kwet, A. & Di-Bernardo, M. 1999. **Pró-Mata: Anfíbios. Amphibien, Amphibians.** Porto Alegre, Edipucrs. 108p.

Kwet, A.; Lignau, R. & Di-Bernardo, M. 2010. **Pró-Mata: Anfíbios da Serra Gaúcha, sul do Brasil–Amphibien der Serra Gaúcha, Südbrasilien–Amphibians of the Serra Gaúcha, South of Brazil.** Porto Alegre, Edipucrs. 148p.

Lardner, B. & Bin Lakim, M. 2002. Tree-hole frogs exploit resonance effects. **Nature 420**:475.

McCoy, E. D. & Bell, S. S. 1991. Habitat structure: the evolution and diversification of a complex topic. *In*: Bell, S. S.; McCoy, E. D. & Mushinsky, H. R. eds. **Habitat structure: the physical arrangement of objects in space.** Dordrecht, Springer, p. 3-27.

Menin, M.; Rossa-Feres, D. & Giaretta, A. 2005. Resource use and coexistence of two syntopic hylid frogs (Anura, Hylidae). **Revista Brasileira de Zoologia 22**:61-72.

Moser, C. F.; Olmedo, G.; Oliveira, M. & Tozetti, A. M. 2019. Movement ecology and habitat use in males of two species of *Boana* (Anura: Hylidae) during the breeding season. **Herpetology Notes 12**:885-893.

Oksanen, F. J.; Blanchet, G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P. R.; O'Hara, R. B.; Simpson, G. L.; Solymos, P.; Stevens, M. H. H.; Szoecs, E. & Wagner, H. 2019. **Vegan: Community Ecology Package, version 2.5-7**. R package. Available at: <https://cran.r-project.org/web/packages/vegan/index.html>. Accessed on: 23 Mar 2021.

Oldham, R. & Gerhardt, C. 1975. Behavioral isolating mechanisms of the tree frogs *Hyla cinerea* and *Hyla gratiosa*. **Copeia 2**:223-231.

Patricelli, G. L. & Blickley, J. L. 2006.Avian communication in urban noise: causes and consequences of vocal adjustment. **The Auk 123**:639-649.

Pianka, E. 1973.The structure of lizard communities. **Annual Review of Ecology and Systematics 4**:53-74.

Prado, G. M. & Pombal, J. P. 2005. Distribuição espacial e temporal dos anuros em um brejo da Reserva Biológica de Duas Bocas, sudeste do Brasil. **Arquivos do Museu Nacional 4**:685-705.

Purrenhage, J. L. & Boone, M. D. 2009. Amphibian community response to variation in habitat structure and competitor density. **Herpetologica 65**:14-30.

R Core Team. 2020. R: **A language and environment for statistical computing**. R Foundation for Statistical Computing, Vienna, Austria. Available at: <http://www.R-project.org/.>. Accessed on: 17 Jan 2020.

Reinert, H. K. 1993. Habitat selection in snakes. *In*: Seigel R. A.; Collins, J. T. & Novak, S. S. eds. **Snakes: Ecology and Evolutionary Biology**. New York, MacMillan Publishing Company, p. 201-240

Reinke, M. & Deiques, C. H. 2010. Natural history of *Hypsiboas leptolineatus* (Anura: Hylidae) in Aparados da Serra National Park, Rio Grande do Sul, Brazil. **Neotropical Biology and Conservation 3**:189-196.

Rossa-Feres, D. & Jim, J. 2001.Similaridade do sítio de vocalização em uma comunidade de anfíbios anuros na região noroeste do Estado de São Paulo, Brasil. **Revista Brasileira de Zoologia 2**:439-454.

Santos, N. P.; Colombo, P.; Avila, F. P.; Oliveira, M. & Tozetti, A. M. 2016. Calling site selection by the south American tree frog *Hypsiboas pulchellus* (Anura, Hylidae) in subtropical wetlands. **South American Journal of Herpetology 3**:149-156.

Santos, T. G. & Rossa-Feres, D. 2007. Similarities in calling site and advertisement call among anuran amphibians in southeastern Brazil. **South American Journal of Herpetology 2**:17-30.

Santos, T. G.; Rossa-Feres, D. & Casatti, L. 2007. Diversidade e distribuição espaço-temporal de anuros em região com pronunciada estação seca no sudeste do Brasil. **Iheringia**, Série Zoologia **97**(1):37-49.

Schoener, T. 1974. Resource partitioning in ecological communities. **Science 4145**:27-39.

Sinsch, U. 2014. Movement ecology of amphibians: from individual migratory behaviour to spatially structured populations in heterogeneous landscapes. **Canadian Journal of Zoology 6**:491-502.

Slabbekoorn, H. & Peet, M. 2003. Birds sing at a higher pitch in urban noise. **Nature 424**:267.

Solano, L.; Lizcano, D. J. & Mercado-Gómez, J. 2015. Selection of calling sites by the Neotropical tree frog *Hypsiboas crepitans*. **Revista Biodiversidad Neotropical 5**(1):71-75.

Toledo, L. F.; Zina, J. & Haddad, C. B. 2003. Distribuição espacial e temporal de uma comunidade de anfíbios anuros do município de Rio Claro, São Paulo, Brasil. **Holos Environment 2**:136-149.

Tozetti, A. M. & Martins, M. 2008. Habitat use by the South American rattlesnake (*Crotalus durissus*) in southeastern Brazil. **Journal of Natural History 42**:1435-1444.

Tozetti, A. M.; Pontes, G. M. F.; Borges-Martins, M. & Oliveira, R. B. 2010. Temperature preferences of *Xenodon dorbignyi*: field and experimental observations. **Herpetological Journal 20**:277-280.

Venables, W. N. & Ripley, B. D. 2002. **Modern Applied Statistics with S.** New York, Springer. 501p.

Wachlevski, M.; De Souza, P. H.; Kopp, K. & Eterovick, P. C. 2008. Microhabitat use and feeding habits of *Crossodactylus bokermanni* Caramaschi and Sazima, 1985 (Anura, Hylodidae) at a site in south-eastern Brazil. **Journal of Natural History 42**:19-20.

Wells, K. D. 2007. **The ecology and behavior of amphibians.** Chicago, The University of Chicago Press.1148p.

Wells, K. D. & Schwartz, J. J. 1982. The effect of vegetation on the propagation of calls in the Neotropical Frog *Centrolenella fleischmanni*. **Herpetologica 38**:449-455.

Zeileis, A. & Hothorn, T. 2002. Diagnostic checking in regression relationships. **R News 3**:7-10.

Ziegler, L.; Arim, M. & Narins, P. M. 2011. Linking amphibian call structure to the environment: the interplay between phenotypic flexibility and individual attributes. **Behavioral Ecology 22**:520-526.

Zimmerman, B. L. 1983. A comparison of structural features of calls of open and forest habitat frog species in central Amazon forest. **Herpetologica 39**:235-246.

Appendix 1. Outcomes of the Multinomial Logistic Regression showing the predicted probability of two anuran species to occur independently in five different niches (one occupied vs. four available quadrants) according to environmental variables sampled within their corresponding microhabitat. Notes: The niche cells are shown in gray color scale to facilitate the interpretation of Figure 6 in the main manuscript.

	Phyllomedusa distincta					*Boana leptolineata*				
	Herbaceous strata									
Quadrant	0%	20%	50%	80%	100%	0%	20%	50%	80%	100%
Available IV	0.00	0.00	0.00	0.13	0.00	0.18	0.19	0.21	0.23	0.23
Available III	0.00	0.00	0.00	0.37	0.00	0.32	0.31	0.29	0.27	0.25
Available II	0.00	0.00	0.00	0.15	0.00	0.11	0.13	0.18	0.23	0.27
Available I	1.00	1.00	1.00	0.21	0.00	0.16	0.16	0.16	0.15	0.14
Occupied	0.00	0.00	0.00	0.12	0.99	0.21	0.18	0.13	0.10	0.08
	Shrub strata									
Quadrant	0%	20%	50%	80%	100%	0%	20%	50%	80%	100%
Available IV	0.00	0.00	0.00	0.18	0.00	0.22	0.19	0.15	0.09	0.06
Available III	0.00	0.00	0.00	0.31	0.00	0.30	0.29	0.25	0.18	0.13
Available II	0.00	0.00	0.00	0.13	0.00	0.16	0.16	0.14	0.10	0.08
Available I	1.00	1.00	1.00	0.03	0.00	0.18	0.14	0.08	0.04	0.02
Occupied	0.00	0.00	0.00	0.31	0.99	0.11	0.19	0.35	0.56	0.68
	Tree strata									
Quadrant	0%	20%	40%	70%	90%	0%	20%	40%	70%	90%
Available IV	0.00	0.00	0.00	0.00	0.00	0.21	0.21	0.20	0.18	0.16
Available III	0.00	0.00	0.00	0.00	0.00	0.34	0.30	0.26	0.19	0.15
Available II	0.00	0.00	0.00	0.00	0.00	0.12	0.16	0.21	0.30	0.37
Available I	1.00	1.00	1.00	0.00	0.00	0.17	0.16	0.14	0.12	0.10
Occupied	0.00	0.00	0.00	0.99	1.00	0.13	0.15	0.16	0.19	0.20
	Water body									
Quadrant	0%	20%	50%	80%	100%	0%	20%	50%	80%	100%
Available IV	0.00	0.00	0.00	0.00	0.00	0.21	0.18	0.07	0.02	0.009
Available III	0.00	0.00	0.00	0.00	0.00	0.30	0.00	0.00	0.00	0.00
Available II	0.00	0.00	0.00	0.00	0.00	0.16	0.27	0.26	0.20	0.16
Available I	1.00	1.00	1.00	0.00	0.00	0.16	0.37	0.58	0.73	0.81
Occupied	0.00	0.00	0.00	1.00	1.00	0.15	0.16	0.08	0.03	0.01
	Heterogeneity of microhabitat (coeficiente of variation)									
Quadrant	0	50	100	150	200	0	50	100	150	200
Available IV	0.00	0.00	0.00	0.00	0.00	0.18	0.21	0.20	0.19	0.10
Available III	0.00	0.00	0.00	0.00	0.00	0.13	0.24	0.38	0.45	0.76
Available II	0.00	0.00	0.00	0.00	0.00	0.34	0.21	0.11	0.08	0.01
Available I	1.00	1.00	1.00	1.00	1.00	0.14	0.16	0.16	0.15	0.08
Occupied	0.00	0.00	0.00	0.00	0.00	0.18	0.16	0.13	0.11	0.03