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Release Call during Interspecific Amplexus in *Boana raniceps* (Cope, 1862): Evidence of Acoustic Variation from the Atlantic Forest of Northeast, Brazil

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Abstract

The genus *Boana* (Hylidae) comprises over 100 species distributed primarily across tropical regions of the Americas. While the advertisement calls of *Boana raniceps* are relatively well documented, information on its secondary vocalizations, such as the release call, remains scarce, particularly for populations in the Atlantic Forest. Addressing this gap, the present study provides the first description of the release call of *B. raniceps* recorded in an Atlantic Forest remnant in northeastern Brazil, during an interspecific amplexus attempt between males of *Boana albomarginata* and *B. raniceps*. The vocalization was recorded in the field using a digital recorder and directional microphone, and subsequently analyzed with Raven Pro 1.6 software. The observed release call exhibited a mean duration of 0.389 ± 0.08 s, with minimum, maximum, and dominant frequencies of 937.5 ± 35.84 Hz, 3656.25 ± 683.55 Hz, and 1593.75 ± 280.77 Hz, respectively, values notably higher than those previously reported for the species in the Cerrado biome, specifically in the state of Goiás. These differences suggest acoustic variation potentially driven by microevolutionary processes due to geographic isolation, resulting in population-specific adaptations to local environmental conditions. The vocalization was emitted as a reflexive response to the amplexus attempt by *B. albomarginata*, representing a novel behavioral record between males of these species. This description advances the understanding of secondary acoustic repertoires in *B. raniceps* and highlights their relevance for studies on acoustic communication, reproductive behavior, and taxonomic delimitation in Neotropical anurans.

Subject Areas

Biology

Keywords

Amphibia, Bioacoustics, Behavior, Vocal Repertoire

1. Introduction

Hylidae is one of the most diverse families within the Anura order, comprising over 1070 species distributed in three subfamilies and over 50 genera [1]. Species of this family are mainly encountered in tropical and subtropical regions, where they can be found in different habitats [1]. Within this family, *Boana* is a diverse genus, composed of 101 species widely represented in the Neotropical region, with species that occur in various areas, from dense forests to open habitats and temporary aquatic environments [1]. *Boana raniceps* [2] is a widely distributed species along South America, occurring from the Amazonian regions of Colombia, extending through Venezuela and to French Guiana, covering Brazil, from the Central Amazon to the states of Bahia and Amapá, as well as Paraguay, northern Argentina and eastern Bolivia, with a presumed occurrence in the Peruvian Amazon [1]. These anurans are mostly arboreal, but present morphological and behavioral adaptations that allow them to explore different reproductive habitats [3].

Vocalization is the main form of communication among anurans and is crucial for intraspecific recognition and reproductive success [4]. Advertisement calls, emitted by males to attract females and defend territory during breeding season, are the most studied type of vocalization in anurans; however, secondary vocalizations, such as stress, territory, release, and encounter calls, are equally important but less understood [4] [5]. Release calls, for example, occur when an individual is handled by another anuran or by a predator and, although more common in males, can also be emitted by females as a reflex response to unwanted interactions [4]. In some species, these responses can be accompanied by body vibrations or inflation [6].

Acoustic signals of anurans, such as advertisement and release calls, often exhibit marked geographic variations, which can be influenced by genetic divergence between populations, local selective pressures, environmental factors, and/or interspecific interactions [7] [8]. The importance of bioacoustic data, especially for secondary vocalizations such as release calls, has been increasingly recognized in understanding communication and species delimitation in anurans. Despite the wide distribution of *B. raniceps* in different ecoregions across South America, information on the geographical variation of its vocal repertoire remains scarce in the literature.

Here, we aim to present the first description of the release call of *B. raniceps* in northeastern Brazil, recorded during an interspecific amplexus with *Boana albomarginata* [9] and compare it with its advertisement call in different areas. This

record contributes to the acoustic characterization of the species and brings relevant data for future comparative and taxonomic studies by providing novel insights into the acoustic repertoire and behavioral ecology of *B. raniceps* and highlighting the role of release calls in interspecific interactions and population differentiation. Secondary calls are often overlooked, yet they can offer valuable insights into species-specific behaviors and mechanisms of reproductive isolation [10] [11].

2. Materials and Methods

Field activities were conducted within the Camocim Forest, a woodland remnant of approximately 200 hectares located in the municipality of São Lourenço da Mata, Pernambuco, northeastern Brazil (08˚01'59.75"S, 35˚12'3.79"W; 131 m a.s.l., DATUM WGS84), in an area of well-preserved seasonal vegetation (**Figure 1**). The area has an average annual rainfall of 1300 mm and local climate is classified as dry-summer (As) under the Köppen-Geiger system [12], with well-defined dry (September to February) and rainy seasons (March to August) [13] [14].

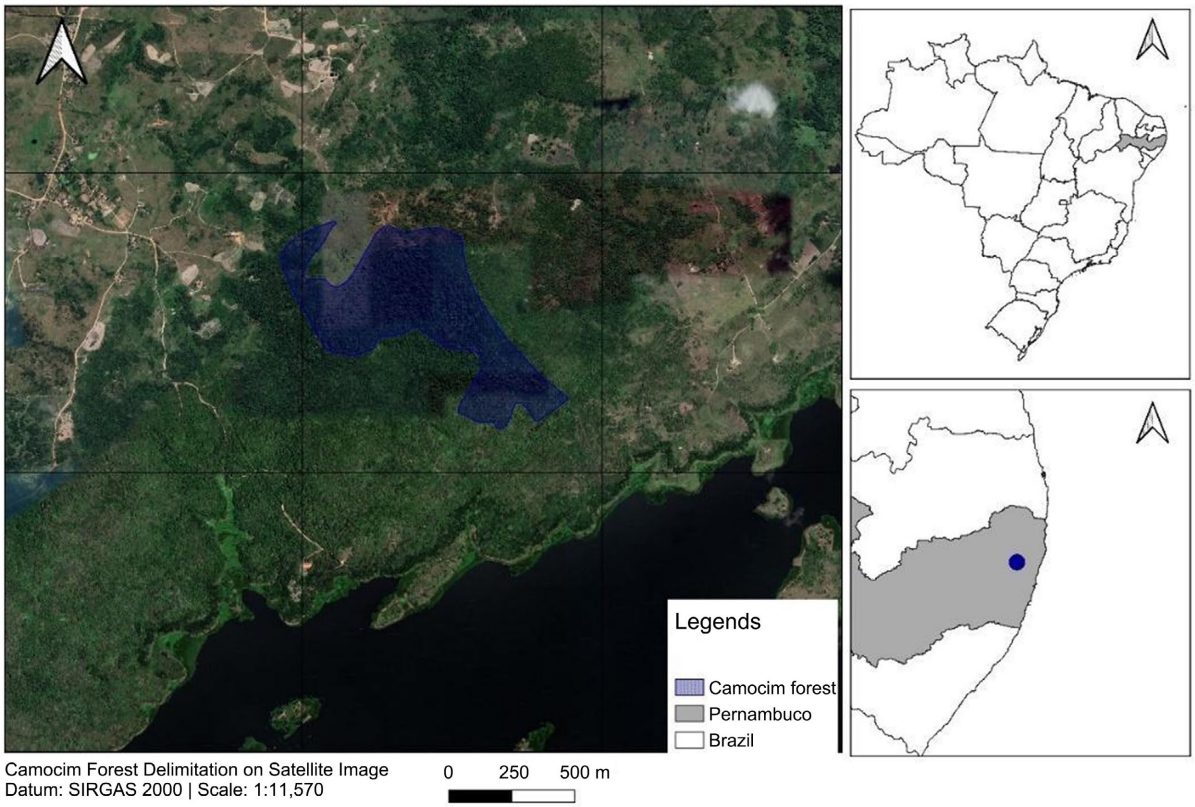

Figure 1. Location of Mata do Camocim within Pernambuco and Brazil.

On March 25th, 2025, an interspecific interaction between male individuals of *B. albomarginata* and *B. raniceps* was observed (**Figure 2**). During this interaction, 37 release calls emitted by *B. raniceps* were recorded using a Tascam DR-40 digital recorder with a Yoga HT-81 directional microphone and later analyzed using Raven Pro software (v.1.6.5, K. Lisa Yang Center for Conservation Bioacoustics, 2024).

We used 27 advertisement calls of *B. raniceps* recorded from the same individual involved in the amplexus for comparison with previously published descriptions [15]. Acoustic analysis was performed with the following settings: Hann window, with a size of 512 samples, filter of 3 dB, bandwidth of 135 Hz, overlap of 50%, hop size of 256 samples, DFT with 512 samples, and frequency grid spacing of 93.8 Hz [16]. Graphs of acoustic parameters were generated using the Seewave Package within the RStudio environment [17] (**Figure 3**). To characterize the calls, the following parameters were evaluated: duration (in seconds), minimum, maximum, and dominant frequencies (in Hz), and number of pulses. The characteristics of the calls recorded in this study were compared with previously described calls with unequal variance t-tests in BioEstat 5.0 [18].

Figure 2. Interspecific amplexus between a male of *Boana albomarginata* and a male *Boana raniceps* registered in Mata do Camocim, Pernambuco, Brazil, on March 25th, 2025. Photo credit: João V. Cunegundes de Siqueira.

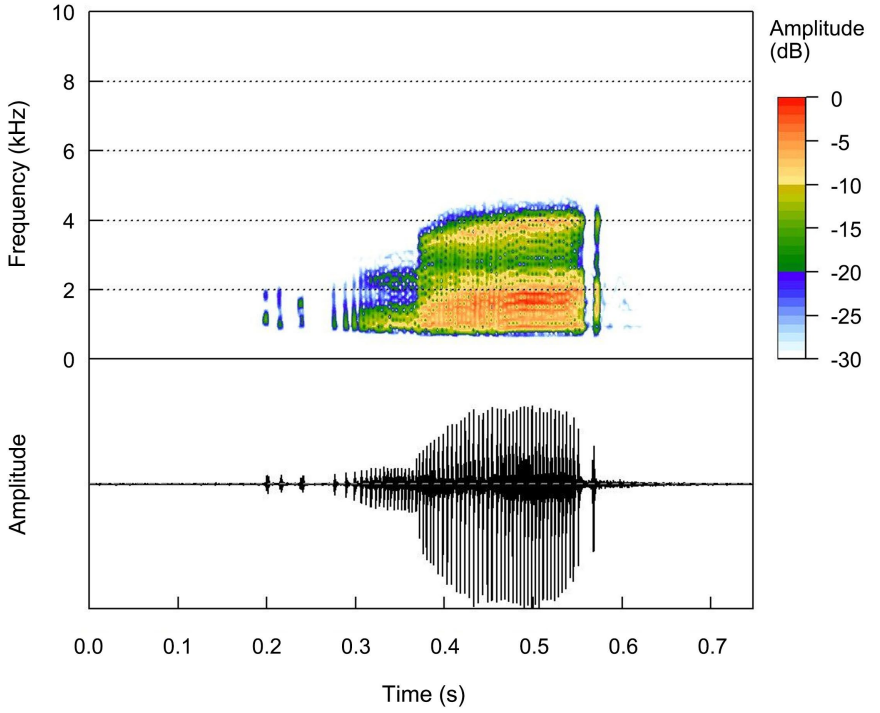

Figure 3. Release call of *Boana raniceps* recorded in Mata do Camocim, Pernambuco, Brazil, on March 25th, 2025, showing the spectrogram (top) and waveform (bottom).

Recorded calls were deposited in the COAXAR collection, a sound library specific for calls of anurans recorded in northeastern Brazil, which integrates the Herpetological and Paleoherpetological Studies Laboratory of the Universidade Federal Rural de Pernambuco (LEHP-UFRPE).

3. Results

During the registered amplexus, it was observed that the *B. raniceps* individual was male due to the presence of an inflated vocal sac, as a call was emitted while it was being amplexed by the *B. albomarginata* individual and after the release. The amplexus was observed for approximately 20 minutes in an area with vocalization of different species.

The release call recorded from *B. raniceps* is characterized as a single note, non-frequency modulated, pulsed call with a mean of 46 pulses (Table 1). The mean duration of the call was 0.389 ± 0.08 seconds, with a dominant frequency of 1593.75 ± 280.77 Hz (Table 1). Maximum and minimum frequencies were 3656.25 ± 683.55 Hz and 937.5 ± 35.84 Hz, respectively (Table 1). Call duration ($F_{36,9}$ = 6.0000, p = 0.0089) and maximum frequency ($F_{36,9}$ = 6.4413, p = 0.0071) were significantly different from previous recorded calls [19].

Table 1. Acoustic parameters of *Boana raniceps* release and advertisement calls recorded in the Atlantic Forest of Pernambuco, Brazil, compared with vocalizations recorded in previous studies. *p < 0.001.

Acoustic Parameters	Release Call		Advertisement Call		Distress Call	Distress Call
	Atlantic Forest (N = 37)	Cerrado (N = 10)	Atlantic Forest (N = 26)	Cerrado (N = 31)	Cerrado (N = 4)	Cerrado (N = 3)
Notes/call	-	-	3 - 5	2 - 6	-	-
Note duration (s)	-	-	0.1283 ± 0.024	0.1627 ± 0.0308	-	-
Call duration (s)	0.389 ± 0.08*	0.050 ± 0.027	0.951 ± 0.206	-	0.423 ± 0.097	0.170 ± 0.025
Bandwidth (Hz)	2718.75 ± 651.33	-	2067.188 ± 52.64	-	-	-
Dominant frequency (Hz)	1593.75 ± 280.77	758.00 ± 202.20	2140.0 ± 195.2*	761.3 ± 33.6	7265.63 ± 1761.41	1750.00 ± 108.25
Maximum frequency (Hz)	3656.25 ± 683.55*	1550.38 ± 269.33	2842.383 ± 66.14	-	8562.5 ± 1234.3	2375 ± 286.41
Minimum frequency (Hz)	937.5 ± 35.84	516.80 ± 0.00	689.062 ± 41.37	-	2625 ± 265.2	1437.5 ± 286.41
Pulses (n)	46 ± 8.97	-	4.3 ± 1.2*	5.7 ± 0.5	232.75 ± 69.21	-
Reference	This study	[19]	This study	[15]	[24]	[24]

B. raniceps advertisement call is a non-frequency modulated call with uniform notes, which can vary from three to five notes per call, with a mean duration of 0.1283 ± 0.024 s per note (Table 1). Total call presented a mean duration of 0.951 ± 0.206 s, with a dominant frequency of 2140.0 ± 195.2 (Table 1). Maximum frequency in the call was 2842.383 ± 66.14 Hz, while minimum frequency was 689,062 ± 41.37 Hz (Table 1). When compared to the first description of the advertisement call recorded for *B. raniceps* [15], the calls recorded in the present study presented

a significant difference in the dominant frequency ($F_{25,30}$ = 33.7506, p = 0.0000) and in the number of notes per call ($F_{25,30}$ = 5.7600, p = 0.0001).

4. Discussion

A record of interspecific amplexus between a male *B. albomarginata* and a female *B. raniceps* has already been registered in the literature [20], however, this is the first record involving males of both species. Abnormal amplexi between anurans can occur habitually in natural situations as males commonly confuse females with different subjects [21]. Even though such interactions may be harmless for some individuals, they can negatively impact males as they can waste time and energy trying to mate with the wrong individual [22]. This is especially hazardous during mating season, when energy requirements, time constraints, and predation risk are higher [22]. Release calls and body vibrations are specialized signals used by males and non-receptive females to avoid or discourage such interactions [4].

The release call of *B. raniceps* has already been described in the Brazilian Cerrado, in the municipality of Caldas Novas, State of Goiás [19], where calls were significantly shorter and presented marked differences in the structure, with lower minimum, maximum, and dominant frequencies (**Table 1**). *Boana raniceps* is a species with a wide distribution in South America, occurring in several biomes, in addition to demonstrating high tolerance to different ecological conditions and anthropic environments [23], therefore, differences in their release calls may be a result of the heterogeneity among populations. Historical processes, such as climate fluctuations during the Pleistocene, played a central role in the genetic diversification of the species' populations, favoring isolation and reconnection events at different evolutionary moments [23].

As suggested by Köhler *et al.* (2017) [5], release calls may be useful for taxonomic purposes; in this study, considering that *B. raniceps* is a widely distributed species, the acoustic data presented may represent a relevant contribution to the systematics of the group, especially if they reflect processes of population differentiation. Notable differences were observed between the advertisement calls recorded in the present study and the original description of the species' call presented by Márquez (1993) [15]. For example, individuals recorded in Mata do Camocim present calls with higher dominant frequency and with shorter note durations compared to the first described calls (**Table 1**). Moreover, call structure also varied slightly between calls: in the present study, the number of pulses per note and the number of notes per call were smaller when compared with reference calls (**Table 1**).

When compared to distress calls previously recorded for *B. raniceps*, the release call recorded in the present study had a similar average duration to the type 1 distress call and was longer than the type 2 (**Table 1**) [24]. Despite the similarity in duration between release calls and type 1 distress calls, the other acoustic parameters clearly differentiate the types of vocalizations. The dominant frequency of the release call was substantially lower than that observed in type 1 distress calls

(**Table 1**) [24], indicating that distress calls are higher-pitched. This difference is consistent with the alarm function of distress calls, since higher frequencies tend to be more detectable at short distances and can signal emergencies more effectively [5]. The maximum and minimum frequencies of type 1 distress calls were also higher than those of the release call (**Table 1**) [24], corroborating the more intense nature of the stress signal. Moreover, the lower number of pulses in the release calls when compared to type 1 distress calls reinforces this distinction (**Table 1**) [24].

Unlike the release call, distress calls are emitted in situations of extreme threat, such as during predation attempts [4] [5]. While the release call seeks to interrupt inappropriate interactions, such as unwanted amplexi, between individuals, distress calls function as an alarm call to try to escape [4]. Release calls are short and repetitive, while distress calls are more intense and variable [4] [5] [25]. These results indicate that release calls present acoustic characteristics that functionally differentiate them from distress calls. While the release call may be related to readaptation to the environment or low-risk communication, distress calls reflect high-threat states, being higher-pitched, more intense, and prolonged.

In this context, acoustic signals emitted by anurans, such as advertisement and release calls, often exhibit marked geographic variations, influenced both by genetic divergence between populations and by local selective pressures, including environmental factors and interspecific interactions [7] [8]. Given the potential relevance of describing release calls in different populations, it is clear that these acoustic data can significantly contribute to the expansion of the systematics of widely distributed taxa, such as *Boana raniceps*. The difference found between our data and previously described release calls for the species reinforce the idea that release calls, as well as advertisement calls, can vary between geographically distinct populations, reflecting possible processes of isolation and local adaptation, offering valuable support not only for taxonomy, but also for understanding population differentiation in species with wide geographic distribution.

5. Conclusions

The present study presented the first record of an interspecific amplexus between males of the species *Boana albomarginata* and *Boana raniceps*, highlighting the complexity of reproductive interactions in anurans. The identification of *B. raniceps* as a male, confirmed by the emission of the release call during amplexus, reinforces the occurrence of behavioral confusions during mating season and their possible impacts on the individuals involved.

The acoustic characterization of the Release and Advertisement Calls revealed differences in relation to previously described populations, especially in duration and frequencies, indicating geographic and adaptive variations in the vocal behavior of this widely distributed species. These differences may be related to historical processes of population isolation and local selective pressures. Moreover, the comparison between Release and Distress Calls showed clear distinctions in structure

and function, indicating distinct roles in anuran communication.

Finally, the results of the present study highlight the importance of considering regional variations in acoustic analysis for taxonomic purposes and for the understanding of the ecological and evolutionary processes that shape behavioral and genetic diversity, contributing to the systematics of *Boana raniceps* and the knowledge of social and reproductive interactions in natural environments.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Frost, D. (2025) Amphibian Species of the World: An Online Reference. American Museum of Natural History. http://research.amnh.org/vz/herpetology/amphibia/

[2] Cope, E.D. (1862) Catalogues of the Reptiles Obtained during the Explorations of the Parana, Paraguay, Vermejo and Uraguay Rivers, by Capt. Thos. J. Page, U.S.N.; and of Those Procured by Lieut. N. Michler, U.S. Top. Eng., Commander of the Expedition Conducting the Survey of the Atrato River. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **14**, 346-359.

[3] Luría-Manzano, R., Pinheiro, P.D.P., Kohlsdorf, T., Haddad, C.F.B. and Martins, M. (2023) Evolution of Territoriality in Hylinae Treefrogs: Ecological and Morphological Correlates and Lineage Diversification. *Journal of Evolutionary Biology*, **36**, 1090-1101. https://doi.org/10.1111/jeb.14189

[4] Toledo, L.F., Martins, I.A., Bruschi, D.P., Passos, M.A., Alexandre, C. and Haddad, C.F.B. (2015) The Anuran Calling Repertoire in the Light of Social Context. *Acta Ethologica*, **18**, 87-99. https://doi.org/10.1007/s10211-014-0194-4

[5] Köhler, J., Jansen, M., Rodríguez, A., Kok, P.J.R., Toledo, L.F., Emmrich, M., *et al.* (2017) The Use of Bioacoustics in Anuran Taxonomy: Theory, Terminology, Methods and Recommendations for Best Practice. *Zootaxa*, **4251**, 1-124. https://doi.org/10.11646/zootaxa.4251.1.1

[6] do Nascimento Lopes, P., Varago, A., Portilho, J., Santana, D.J. and Mângia, S. (2024) Release Call of *Trachycephalus typhonius* (Anura, Hylidae) in the Cerrado, Central-Western Brazil. *Journal of Vertebrate Biology*, **73**, Article 24052-1. https://doi.org/10.25225/jvb.24052

[7] Pröhl, H., Hagemann, S., Karsch, J. and Höbel, G. (2007) Geographic Variation in Male Sexual Signals in Strawberry Poison Frogs (*Dendrobates pumilio*). *Ethology*, **113**, 825-837. https://doi.org/10.1111/j.1439-0310.2007.01396.x

[8] Erdtmann, L. and Amézquita, A. (2009) Differential Evolution of Advertisement Call Traits in Dart-Poison Frogs (Anura: Dendrobatidae). *Ethology*, **115**, 801-811.

https://doi.org/10.1111/j.1439-0310.2009.01673.x

[9] von XSpix, J.B. (1824) Animalia nova sive species novae testudinum et ranarum, quas in itinere per brasiliam annis MDCCCXVII-MDCCCXX jussu et auspiciis maximiliani Josephi I. bavariae regis suscepto collegit et descripsit. F.S. Hübschmann.

[10] Gerhardt, H.C. and Huber, F. (2002) Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions. University of Chicago Press.

[11] Nains, P.M., Feng, A.S., Fay, R.R. and Popper, A.N. (2007) Hearing and Sound Communication in Amphibians. Springer.

[12] Alvares, C.A., Stape, J.L., Sentelhas, P.C., de Moraes Gonçalves, J.L. and Sparovek, G. (2013) Köppen's Climate Classification Map for Brazil. *Meteorologische Zeitschrift*, **22**, 711-728. https://doi.org/10.1127/0941-2948/2013/0507

[13] de Lyra-Neves, R.M., Oliveira, M.A.B., Telino-Júnior, W.R. and dos Santos, E.M. (2007) Comportamentos interespecíficos entre callithrix jacchus (Linnaeus) (Primates, Callitrichidae) e algumas aves de Mata atlântica, pernambuco, brasil. *Revista Brasileira de Zoologia*, **24**, 709-716. https://doi.org/10.1590/s0101-81752007000300022

[14] Moura, G.J., Azevedo, J.S.M. and El-Deir, A.C.A. (2012) A biodiversidade da es-tação ecológica de tapacurá: Uma proposta de manejo e conservação. NU-PEEA.

[15] Marquez, R., la Riva, I.D. and Bosch, J. (1993) Advertisement Calls of Bolivian Species of Hyla (Amphibia, Anura, Hylidae). *Biotropica*, **25**, 426-443. https://doi.org/10.2307/2388866

[16] K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology (2024) Raven Pro: Interactive Sound Analysis Software (Version 2.0.0 Build 67 Beta) [Computer Software]. The Cornell Lab of Ornithology.

[17] Sueur, J., Aubin, T. and Simonis, C. (2008) Seewave, a Free Modular Tool for Sound Analysis and Synthesis. *Bioacoustics*, **18**, 213-226. https://doi.org/10.1080/09524622.2008.9753600

[18] Ayres, M., Ayres Junior, M., Ayres, D.L. and Santos, A.A.S. (2007) BioEstat 5.0: Aplicações estatísticas nas áreas das ciências biológicas e médicas. Socie-Dade Civil Mamirauá.

[19] Guerra, V., de Queiroz Costa, N. and Bastos, R.P. (2018) Interspecific Behaviour between Two Species of Boana (Anura: Hylidae) from Central Brazil. *Herpetology Notes*, **11**, 489-493.

[20] Rocha, S.M., Santana, D.O., Silva, I.R.S. and Faria, R.G. (2015) *Interspecific am-plexus* between *Hypsiboas albomarginatus* (Spix, 1824) and *Hypsiboas raniceps* Cope, 1862 (Anura: Hylidae) in Northeastern Brazil. *Herpetology Notes*, **8**, 213-215.

[21] Serrano, F.C., Díaz-Ricaurte, J.C. and Martins, M. (2022) Finding Love in a Hopeless Place: A Global Database of Misdirected Amplexus in Anurans. *Ecology*, **103**, e3737. https://doi.org/10.1002/ecy.3737

[22] Pearl, C.A., Hayes, M.P., Haycock, R., Engler, J.D. and Bowerman, J. (2005) Observations of Interspecific Amplexus between Western North American Ranid Frogs and the Introduced American Bullfrog (Rana Catesbeiana) and an Hypothesis Concerning Breeding Interference. *The American Midland Naturalist*, **154**, 126-134. https://doi.org/10.1674/0003-0031(2005)154[0126:ooiabw]2.0.co;2

[23] Turchetto-Zolet, A.C., Pinheiro, F., Salgueiro, F. and Palma-Silva, C. (2012) Phylogeographical Patterns Shed Light on Evolutionary Process in South America. *Molecular Ecology*, **22**, 1193-1213. https://doi.org/10.1111/mec.12164

[24] Guerra, V., Andrade, M.S., de Andrade, S.P., Ramalho, W.P. and Bastos, R.P. (2018)

Defensive Behaviour of *Boana raniceps* (Anura: Hylidae). *Herpetology Notes*, **11**, 433-436.

[25] Wells, K.D. (2019) The Ecology and Behavior of Amphibians. University of Chicago Press.