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ECOLOGICAL NICHE MODELING OF *PSEUDOPALUDICOLA MOTORZINHO* (ANURA, LEPTODACTYLIDAE), WITH TWO NEW DISTRIBUTION RECORDS AND COMMENTS ON ITS ADVERTISMENT CALL VARIATION

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ABSTRACT—Species distribution is an essential factor in conservation efforts, and when new species are identified, a lack of information about their distribution may arise. In this study, we present an updated distribution map of *Pseudopaludicola motorzinho*, which includes two new occurrence records, and estimate the occurrence of the species using ecological niche modelling (ENM). The species was found in a total of 21 localities and occurs in four different ecoregions, namely Pantanal, Cerrado, Chiquitano Dry Forest, and Humid Chaco, located in the Midwest of Brazil and East Bolivia. The ENM predicted distribution remained adjusted to the occurrence points in Brazil and Bolivia, and projected the possible occurrence of the species in the eastern and central portions of Bolivia. The three advertisement calls that we recorded showed a slightly different pattern from those previously described for the species. Although *P. motorzinho* is present in other ecoregions, it is mainly associated with the Pantanal floodplains, with its life cycle intimately linked to the habitats provided by the floodplain. Unfortunately, this area has suffered severe drought and wildfires in the last years (2019-2022), which may have a direct impact on the species.

RESUMEN—La distribución de especies es un factor importante para la conservación, y cuando nuevas especies son descritas, la falta de conocimiento acerca de su distribución puede surgir. Aquí presentamos una actualización del mapa de la distribución de *Pseudopaludicola motorzinho*, incluyendo dos nuevos registros, y estimando la existencia de esta especie usando modelaje de nicho ecológico (MNE). Esta especie fue colectada en 21 localidades, y existe en cuatro ecoregiones, en Pantanal, Cerrado, Bosque Seco Chiquitano y Chaco Húmedo, en el medio oeste de Brasil, y al este de Bolivia. La distribución pronosticada por el MNE se mantuvo ajustada a los puntos de existencia de Brasil y Bolivia, y sugirió la posible existencia de la especie en las secciones orientales y centrales de Bolivia. Los tres cantos de anuncio registrados presentaron patrones un poco diferentes a los ya descritos para la especie. A pesar de existir en otras ecorregiones, *P. motorzinho* es asociada principalmente con las planicies inundables del Pantanal por tener ciclo de vida íntimamente relaciono a los hábitats provistos en las planicies inundables. Lamentablemente, esta área ha sufrido por las sequias extremas y los incendios forestales en los últimos años (2019-2022), lo que puede impactar directamente a esta especie.

The genus *Pseudopaludicola* currently comprises 25 species of small-sized leptodactylids frogs, occurring along South America (Amphibians Species of the World, https://amphibiansoftheworld.amnh.org/index.php.). The species within this genus with a T-shaped terminal phalanges are allocated in the *P. pusilla* species group (sensu Lynch, 1989), which is composed of five species (*P. boliviana*; *P. ceratophyes*; *P. llanera*; *P. motorzinho*; and *P. pusilla*) distributed from northern Argentina to Guiana Shield, covering Colombia, Venezuela, Paraguay, Bolivia, and Brazil (Costa-Campos et al., 2016; Pansonato et al., 2016; Barrio-Amorós et al., 2019).

Pseudopaludicola motorzinho is a small species (~17 mm snout-vent lenght) in relation to their congeners and is distributed in the south of Mato Grosso, west of Mato Grosso do Sul (Brazil) and in Northeastern Bolívia (Pansonato

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TABLE 1—Occurence records of localities where occurs *Pseudopaludicola motorzinho* used in the Ecological Niche Models. BOL = Bolivia; BRA = Brazil; MS = Mato Grosso do Sul state; MT = Mato Grosso State; * = type locality.

Country	State/Department	Municipality	Altitude (m)	Lat	Long	Reference	Call
BOL	Santa Cruz	Provincia Angel Sandoval	102	−17.0386	−58.5286	Pansonato et al. (2016)	—
BOL	Santa Cruz	Província Velasco	199	−14.9105	−61.0725	Pansonato et al. (2016)	X
BRA	MS	Campo Grande	506	−20.5374	−54.7547	**This study**	X
BRA	MS	Aquidauana	117	−19.7561	−55.8630	Neves et al. (2020)	—
BRA	MS	Corumbá	105	−18.9880	−56.6191	Neves et al. (2020)	—
BRA	MS	Corumbá	142	−17.8775	−57.5577	Pansonato et al. (2016)	X
BRA	MS	Miranda	90	−19.5525	−57.0388	**This study**	X
BRA	MS	Miranda	115	−20.0866	−56.6155	Neves et al. (2020)	—
BRA	MS	Porto Murtinho	78	−21.7000	−57.8750	Pansonato et al. (2016)	—
BRA	MS	Porto Murtinho	96	−21.7019	−57.7208	Neves et al. (2020)	—
BRA	MS	Porto Murtinho	123	−21.4922	−57.5869	Neves et al. (2020)	—
BRA	MT	Barão de Melgaço*	126	−16.5886	−56.2805	Pansonato et al. (2016)	X
BRA	MT	Cáceres	207	−15.9744	−57.5297	Pansonato et al. (2016)	X
BRA	MT	Cáceres	237	−16.2997	−58.1525	Pansonato et al. (2016)	X
BRA	MT	Cuiabá	194	−15.6591	−55.9416	Pansonato et al. (2016)	X
BRA	MT	Jaciara	379	−15.9558	−54.9752	Pansonato et al. (2016)	X
BRA	MT	Nossa Senhora do Livramento	129	−16.3661	−56.3091	Pansonato et al. (2016)	X
BRA	MT	Poconé	107	−16.6522	−57.1561	Pansonato et al. (2016)	—
BRA	MT	Poconé	117	−16.5161	−56.7480	Pansonato et al. (2016)	—
BRA	MT	Pontes e Lacerda	252	−14.9277	−59.5702	Pansonato et al. (2016)	—
BRA	MT	Vila Bela da Santíssima Trindade	198	−15.0166	−59.9500	Lobo (1995)	—

et al., 2016; Neves et al., 2020). It differs from all other species of the group *P. pusilla* by the absence of palpebral tubercles on the upper eyelids, a presence of a conspicuous conical tubercle on upper edge of the heel and its advertisement call (Pansonato et al., 2016). This species is commonly found in open areas, on the flooded natural grasslands on forest border and is abundant where occurs (Valério-Brun et al., 2010; Pansonato et al., 2011, 2016).

Once new species of amphibians are described (Moura and Jetz, 2021), a lack of data about distribution of this identities emerge (e.g. Motta et al., 2020). The use of methods, such as the ecological niche modelling (ENM), to predict distribution of species is crucial for conservation programs (Blank and Blaustein, 2012; Guisan et al., 2013), to indicate geographical distribution in the past (Bandeira et al., 2021) or even anthropogenic effects (Wiens et al., 2009; Enriquez-Urzelai et al., 2019). Herein, we provide an updated distribution map of *P. motorzinho* with two new occurrence records. Additionally, we estimate the occurrence of *P. motorzinho* using the ENM framework to access potential areas for the occurrence of species.

MATERIAL AND METHODS—*Species data collection*—We constructed a database of occurrence records of *P. motorzinho* by compiling information from literature (Lobo, 1995; Pansonato et al., 2016; Neves et al., 2020), and data collected by us during fieldwork in the state of Mato Grosso do Sul, Brazil. On September 21, 2016, at Base de Estudos do Pantanal, in Corumbá municipality, we recorded a *P. motorzinho* male (ZUFMS-AMP7589) calling during the day in a flooded area (around 1600 h). On December 01, 2017, at RPPN Brejo Bonito, Campo Grande municipality, around 18.45 h during a field work, we recorded two *P. motorzinho* males calling (ZUFMS-AMP11532 and ZUFMS-AMP11533) in the border of a pond. The vouchers were euthanized using a liquid solution of 2% lidocaine chlorhydrate, fixed in 10% formalin, and transferred to permanent storage in 70% ethanol. The samplings were authorized by the System of Authorization and Information in Biodiversity (SISBIO/MMA, number 55579).

We analyzed three calls with Raven Pro 1.6.1 for Windows (Center for Conservation Bioacoustics, 2019) and audiospectrograms were constructed in "R" software using the package "seewave" (Sueur et al. 2008; R Development Core Team, 2021) with the following parameters: FFT window width = 256, Frame = 100, Overlap = 75, and flat-top filter. Terminology used follows Köhler et al. (2017) and morphological and acoustic comparisons were made using the original publication (Pansonato et al., 2016).

Environmental niche modeling—We performed an Ecological Niche Modeling (ENM) to estimate the geographic distribution of *P. motorzinho* based on the climatically suitable regions for the species. The ENM uses the association of environmental variables with occurrence data of a species to map its potential distribution (Guisan and Thuiller, 2005). We used 21 localities (Table 1) to generate an ENM.

Three groups of climate predictors were used: bioclimate, evapotranspiration, and UV-B variables. We downloaded 19 bioclimatic variables from the WorldClim database (see http://www.worldclim.org/ for variable descriptions) interpolated to 10 minutes resolution (Fick and Hijmans, 2017), averaged over the 1970–2000 period; Slope (defined as a measure of the concavity and convexity of

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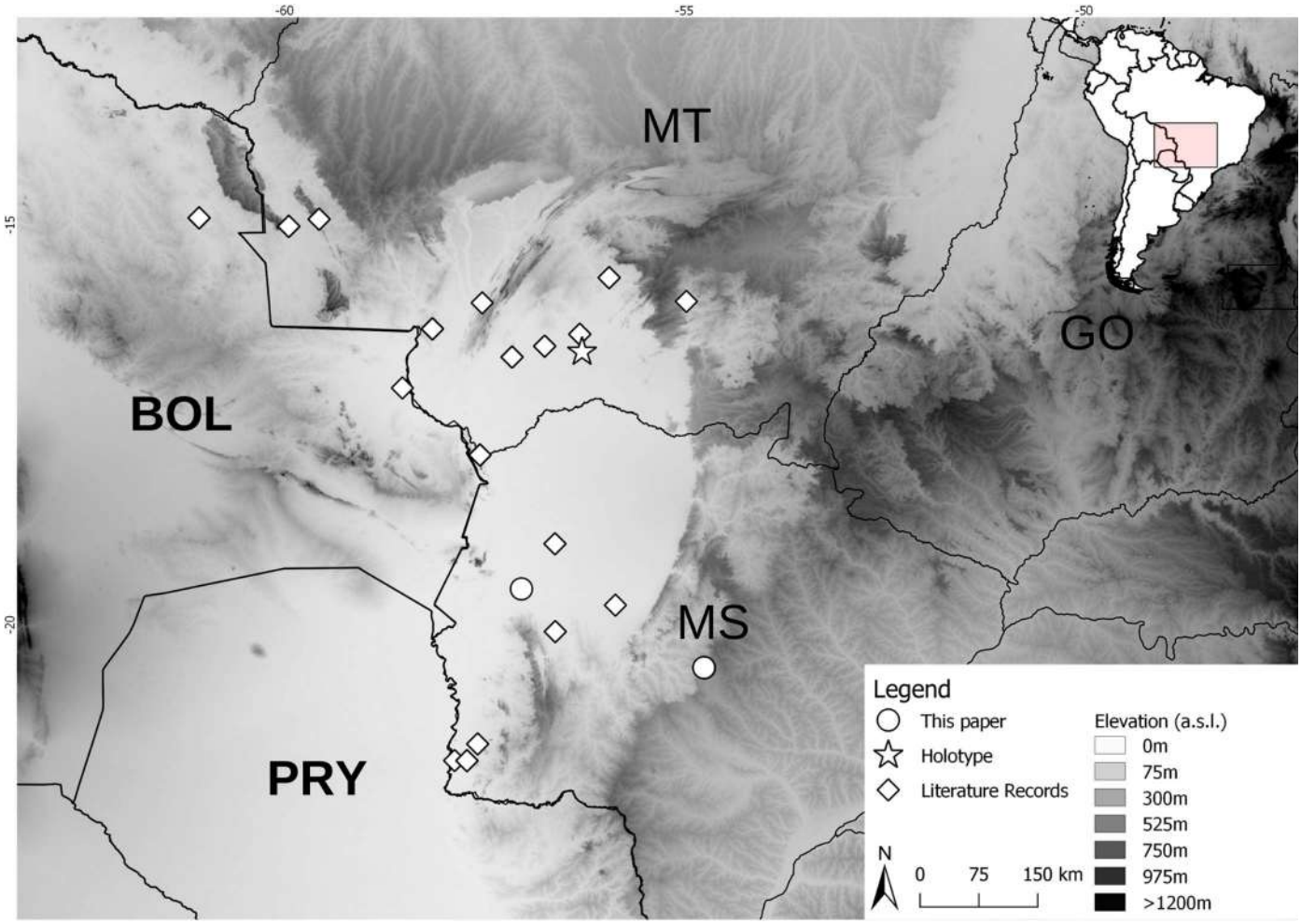

FIGURE 1—Distribution map based on point records (see Tab. 1) of the *Pseudopaludicola motorzinho*. Abbreviations of the Brazilian states: GO = Goiás; MS = Mato Grosso do Sul; MT = Mato Grosso; and coutries. BOL = Bolivia; PRY = Paraguay.

topography) with the resolution of 5 km (Amatulli et al., 2018), mean annual evapotranspiration with the resolution of 30 arc seconds (Trabucco and Zomer, 2019) and Annual Mean UV-B (2004–2013) with spatial resolution of 15 arc-minutes (Beckmann et al., 2014). These layers were resampled to a resolution of 15 arcmin using the nearest neighbor interpolation. To avoid overprediction and low specificity, we cropped the bioclimatic layers to span from latitude −70 to −40 and longitude −30 to −10 (values in decimal degrees) (Werneck, 2011).

To reduce autocorrelation among occurrence data and potential for overfitting we eliminated one of each pair of records falling within single grid cells (~3 km) using the package 'spThin' (Aiello-Lammens et al., 2015). To remove problems related to multicollinearity of the environmental explanatory variables, we calculated the Variance Inflation Factor (VIF) values for variables to each species. All values that were highly correlated (VIF > 10) were removed through a stepwise procedure, using 'usdm' package (Naimi, 2013). Thus, we retained nine from 22 climatic variables that were used throughout this study (Bio2 – mean diurnal range, Bio8 - mean temperature of wettest quarter, Bio9 - mean temperature of driest quarter, Bio13 - precipitation of wettest month, Bio14 - precipitation of driest month, Bio18 - precipitation of warmest quarter, Bio19 - precipitation of coldest quarter, slope, and mean annual evapotranspiration).

We performed ENM using nine different algorithms implemented in the biomod2 package (Thuiller et al., 2016) in R, including the following: three regression methods [GAM: general additive model (Hastie and Tibshirani, 1990), GLM: general linear model (McCullagh and Nelder, 1989), MARS: multivariate adaptive regression splines (Friedman, 1991)]; three machine learning methods [GBM: generalized boosting model (Ridgeway, 1999), MAXENT: Maximum Entropy (Phillips et al., 2006), RF: random forest (Breiman, 2001)], two classification methods [CTA: classification tree analysis (Breiman, 1984), FDA: flexible discriminant analysis (Hastie et al., 1994)], and one envelope model [SRE: Surface Range Envelop (Busby, 1991)]. To meet the criteria of having absence (or pseudo-absence) data for most of these models (except SRE), we generated two equal-sized (to the true presence records) sets of random pseudo-absence (PA) points across the model background. The models were calibrated

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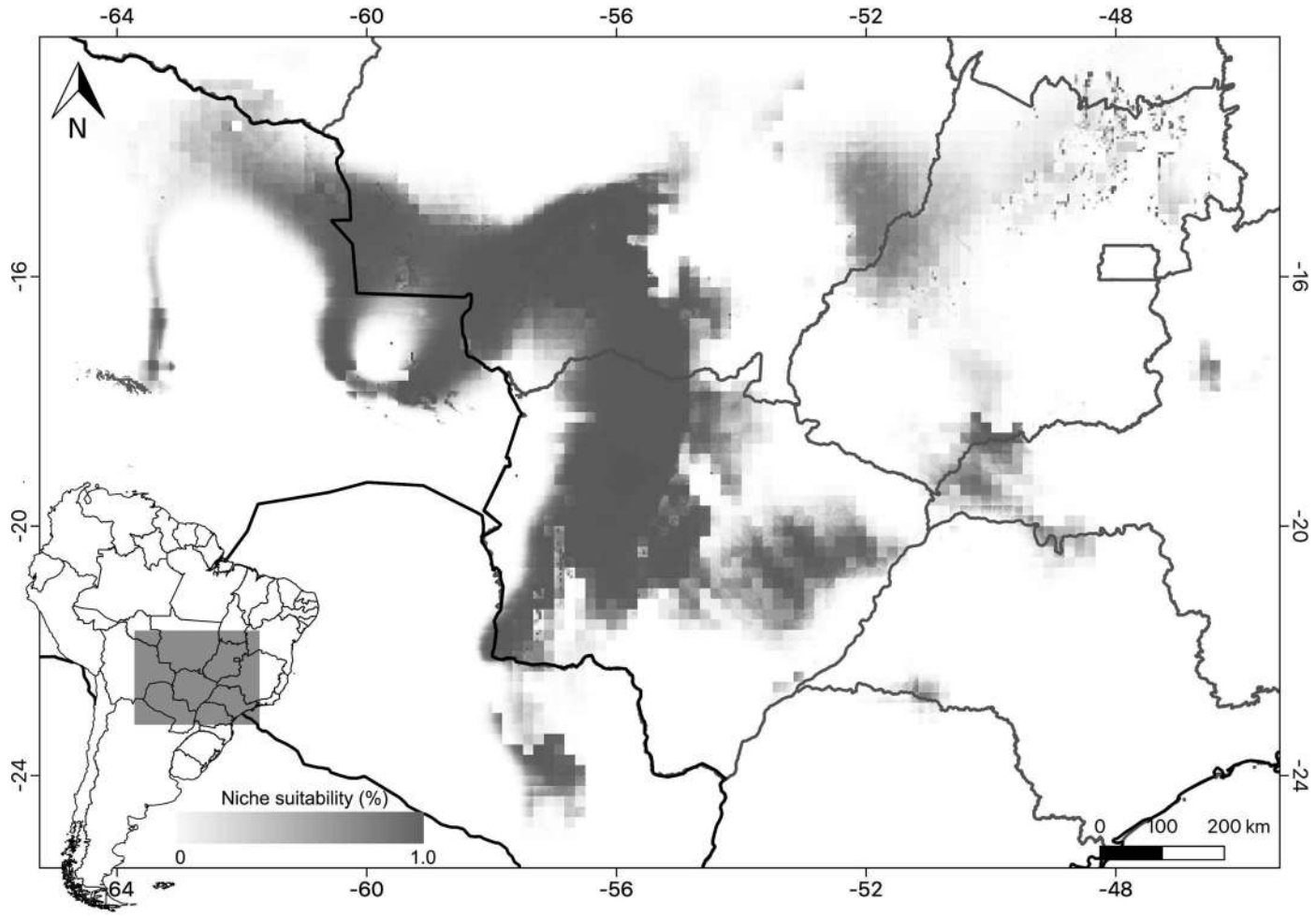

FIGURE 2—Predicted distribution of *Pseudopaludicola motorzinho* based on environmental suitability. Occurrence localities can be found in Table 1 and Fig. 1.

using 70% of randomly selected data. The other 30% of data were used for intrinsic model evaluation.

Individual model performance was evaluated using two metrics—true skill statistic (TSS) and the area under the curve of receiver operating characteristics (ROC) implemented in the biomod2 package. TSS is calculated as "sensitivity + specificity -1" and ranges from -1 to +1, where +1 indicates perfect agreement, a value of 0 implies agreement expected by chance, and a value of less than 0 indicates agreement lower than expected by chance. Models with high predictive accuracy (TSS > 0.7; ROC > 0.8) were used for projection of *P. motorzinho* distribution. We constructed ensemble maps based on the median of two runs of all the selected models in which individual accuracy had TSS value equal to or greater than 0.8 (Table S1).

RESULTS—The species was recorded in a total of 21 localities, occurring at altitude between 78 and 506 m a.s.l. (Table 1, Fig. 1). The new records resulted in an increase of 127 m of altitudinal range and 140 km in a straight line in southeastern to the nearest record. The resulting ensemble models based on nine ENM algorithms (Fig. 2) gave averages of TSS = 0.98 and ROC = 0.99. The predicted distribution of *P. motorzinho* remained adjusted to the occurrence points in Brazil and Bolivia, plus a projected possible occurrence to the eastern toward central portions of Bolivia (Fig. 2). The variable bio8 (mean temperature of wettest quarter) was the most import variable (48% of explication), followed by mean annual evapotranspiration (35% of explication) to *P. motorzinho* distribution, and bio9 (mean temperature of driest quarter, 28% of explication). Other climate predictors (Bio13 and Bio14) helped to explain the *P. motorzinho* ecological niche models, though to a lesser extent. The advertisement call of our records consists of a series of 62–195 notes per series; series duration was 8.62–11.49 s, note duration varies between 9–25 ms; pulses per note varies 2–5; and peak frequency varies 4565–4823 Hz (Fig. 3, Table 2).

DISCUSSION—Regarding the distribution of *P. motorzinho*, it occurs in four different ecoregions, Pantanal, Cerrado, Chiquitano Dry Forest, and Humid Chaco (*sensu* Dinerstein et al., 2017), which the last two occur only in the border of them. Two of our records are in Cerrado from Mato Grosso do Sul state and increased the altitudinal

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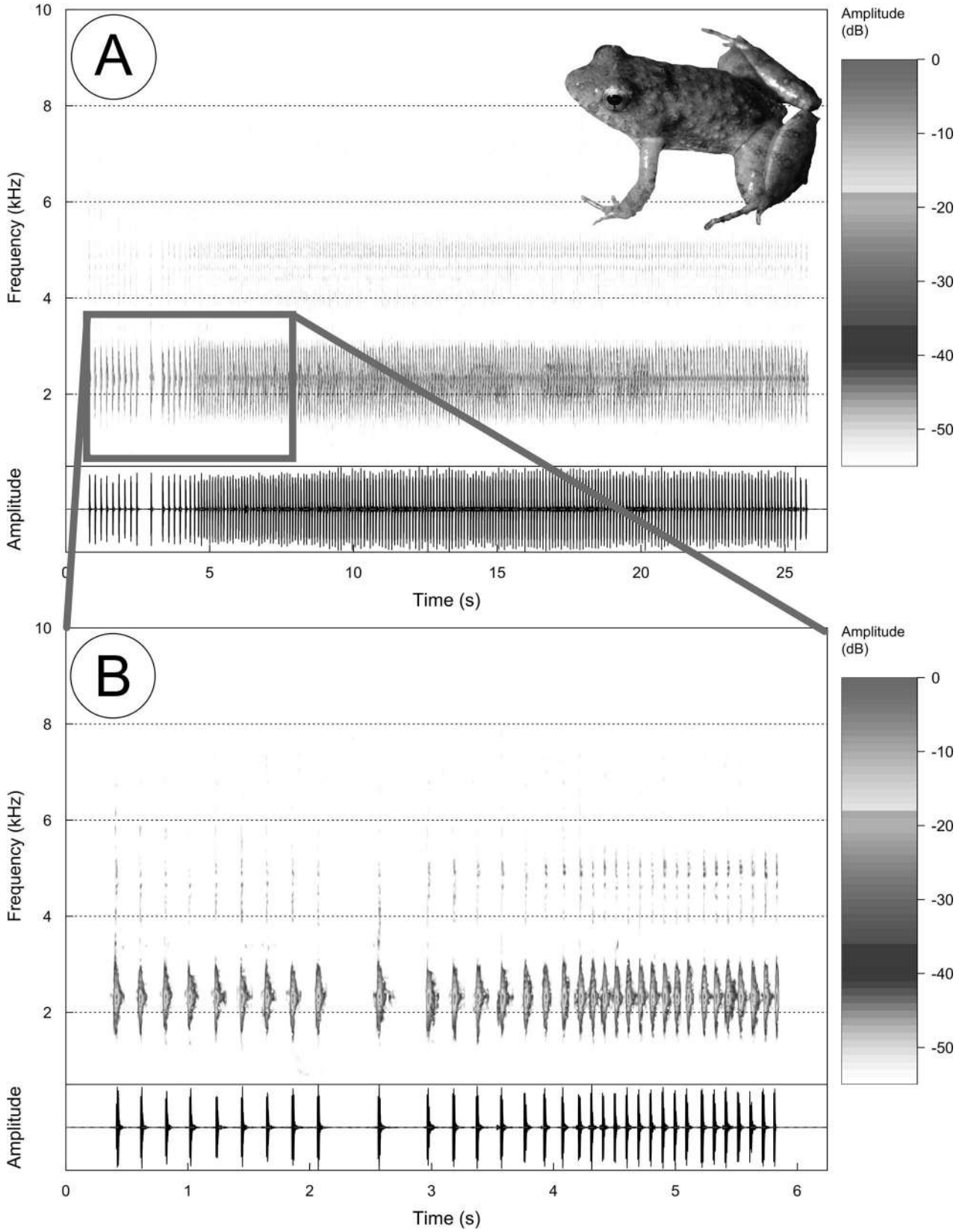

FIGURE 3—Audiospectrogram and oscilogram of the advertisement call of *Pseudopaludicola motorzinho* recorded from a male at RPPN Brejo Bonito, Campo Grande municipality, Mato Grosso do Sul state, western Brazil (ZUFMS-AMP11532).

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Table 2—Measurements of acoustic parameters of specimens of *Pseudopaludicola motorzinho*.

Voucher	Notes per serie	Series duration (s)	Note duration (ms)	Pulses per note	Peak Freq. (Hz)	Internote intervals
ZUFMS-AMP11533	134	8.62	15.1 ± 1;1.8 (9–23)	3.6 ± 1;0.5 (2–4)	4823.43	57.5 ± 1.30 (33–110)
ZUFMS-AMP11532	195	11.49	15.1 ± 1;0.9 (11–17)	2.9 ± 1;0.07 (2–3)	4565.03	50 ± 1;31.6 (27–170)
ZUFMS-AMP7589	62	3.59	16.3 ± 1;2.4 (11–25)	3.6 ± 1;0.5 (3–5)	4823.43	32 ± 1;2.44 (25–37)

and easternmost limit for the species, which was the first record in the Upper Paraná Basin. The three males with advertisement calls analyzed agree with the diagnosis of the species for all two records (Table 2 and Fig. 3) (Pansonato et al., 2016). The three advertisement calls that we recorded showed the same pattern (Fig. 3), it starts ascending in the first third (introductory notes), then stabilizes throughout the call. This pattern shows a variation from the original description of the species, which not present this ascending format at the beginning of the call (Pansonato et al., 2016). Anurans exhibit variations in their advertisement calls due to several factors. The acoustic properties of the calls can be affected by the physical environment, including the temperature, humidity, and substrate (Gerhardt and Huber, 2002). Furthermore, ecological factors, such as the presence of predators or competitors, can influence the call characteristics. For instance, some species modify their calls to avoid attracting predators (Ryan, 1985). Moreover, the mating system of the species can also play a role in the variation of the calls. In some species, males compete for mating opportunities, leading to the evolution of complex and diverse calls (Gerhardt, 1991).

The ensemble potential distribution for *P. motorzinho* indicates bio8 (mean temperature of wettest quarter) as the most important variable to species distribution. This variable, plus the third most important (bio9 - mean temperature of driest quarter), can be related to the active period of species, once the activities of ectothermic vertebrates are sensitive to climatic variations due to the effect of temperature and humidity on species physiology (Duellman and Trueb, 1986). In this sense, periods with high temperatures and humidity induces the reproductive period of species, once individuals can track abiotic cues in order to reproduce (Ceron et al., 2020). The mean annual evapotranspiration was the second most important variable to explain the distribution of the species. This variable can be related to the reproductive mode of the species, which lays its eggs directly in open and shallow aquatic habitats (Pansonato et al., 2011) and to the deposition sites (areas with ephemeral ponds), highlighting the importance of areas with high evapotranspiration rate to the breeding of species.

In general, *P. motorzinho* is associated with Pantanal floodplains with their live cycle intimate related to the habitats provided by the floodplain. However, this area has suffered severely in the last years (2019–2022) due to an extreme drought and wildfires (Marengo et al., 2021; Tomas et al., 2021), which may impact directly the populations of *P. motorzinho,* since its narrow distribution depends directly from flooded areas to reproduce (Valério-Brun et al., 2010; Pansonato et al., 2011). Even though other ecoregions may harbor this species, it is extremely important that Pantanal be preserved to maintain the integrity of *P. motorzinho* populations.

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