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The differential acoustic diagnosis between two *Pseudopaludicola* sister species (Anura, Leptodactylidae, Leiuperinae)

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The dwarf swamp frogs, genus *Pseudopaludicola*, include 21 species, which occur throughout South America (Frost 2016). *Pseudopaludicola* is recognized as monophyletic, supported by a set of osteological, morphological, and molecular characters (Veiga-Menoncello *et al.* 2014, and cites therein). Veiga-Menoncello *et al.* (2014) recognized a clade (“clade II” there), which included two species that share karyotypes with $2n = 20$: *P. ameghini* (Cope, 1887) and *P. ternetzi* Miranda-Ribeiro, 1937. However, historically these two sister species have undergone many taxonomic changes and so far they do not have a clear taxonomic diagnosis one from the other (Haddad & Cardoso 1987; Lobo 1996; Fávero *et al.* 2011; Pansonato *et al.* 2013; Cardozo & Toledo 2013, and cites therein).

Fávero *et al.* (2011) proposed that differences in chromosome morphology of the pair 7 (subtelocentric in *P. ameghini* and submetacentric in *P. ternetzi*) and in the distribution patterns of the heterochromatic C bands could distinguish *P. ternetzi* from *P. ameghini*. However, Cardozo *et al.* (2016) stated that the pair 7 of *P. ternetzi* also presents a metacentric morphology, and that chromosome data on this species should be revisited. Pansonato *et al.* (2013) reported the absence of a light vertebral stripe, as on some specimens of *P. ternetzi* (Miranda-Ribeiro 1937), on the analyzed individuals of *P. ameghini* by them, but they did not access the type series of the latter. In addition, Roberto *et al.* (2013) assumed *P. ameghini* as undiagnosed, and Pansonato *et al.* (2013) drew attention for the need of a recharacterization of *P. ternetzi*. Cardozo & Toledo (2013) proposed that *P. ameghini* and *P. ternetzi* can be discriminated each other by their calls, and according to Pansonato *et al.* (2013), also by the warty dorsum of *P. ameghini* (slightly warty in *P. ternetzi*). In Cardozo & Toledo (2013) the data on call were restricted to one recording from Uberlândia (state of Minas Gerais), besides no acoustic comparison between these two species was provided. Pansonato *et al.* (2014) and Andrade *et al.* (2016) highlighted the low divergence between conspecific lineages of *Pseudopaludicola* species, including *P. ameghini* and *P. ternetzi*, with around 2% of genetic distance.

Bioacoustics has been proved as a relevant approach to solve taxonomic issues within *Pseudopaludicola* (e.g. Magalhães *et al.* 2014; Carvalho *et al.* 2015a; Andrade *et al.* 2016; Pansonato *et al.* 2016, and cites therein), given the morphological conservatism among component species and the high intraspecific variation on color patterns and body size which have often originated misleading identifications. Herein we carry out a specimen analysis and a deeper acoustic comparison based on a sample from these two sister species with complex taxonomic history, and suggest an acoustic diagnosis for them. We also compare the acoustic traits of *P. ternetzi* and *P. ameghini* with other congeners, trying to provide an accurate acoustic diagnosis.

In the field, we recorded the calls with the digital recorders Marantz PMD 671, Boss 864 (both coupled to Sennheiser ME67/K6 microphones) and M-audio Microtrack II (Sennheiser ME66/K6). Recorders were set at 44.1 or 48.0 kHz sampling rates, and at 16-bit resolution. Forty four adult males of *P. ternetzi* were recorded: 18 males from Ituiutaba (MG), eight males from Minaçu (GO), and 18 males from Pontal do Araguaia (MT). All recordings

were taken from 16:40–20:59 h and between 13 September 2011 and 26 November 2014. Eight males of *P. ameghini* were recorded: two males from Chapada dos Guimarães (MT; type locality), two males from Santo Antonio do Leverger (MT), and four males from Cáceres (MT). These recordings were taken from 18:02–20:35 h and between 14 December 2008 and 06 January 2016. We also analyzed three males (FNJV 4579–4581) from municipality of Chapada dos Guimarães (MT) recorded by Adão J. Cardoso. These recordings were made with a tape recorder Uher 4000 IC (coupled to an Uher M538 microphone) between 17:00–19:30 h on 14–15 October 1982 and 13 January 1984, respectively; and were digitalized at 48.0 kHz sampling rates, and at 16-bit resolution. See analyzed specimens, labels of the recordings and call voucher specimens for both species in Appendix 1. Specimens of *P. ternetzi* from Minaçu are especially important because they are close from its type locality.

Acoustic terminology follows Köhler *et al.* (2017). We calculated means and standard deviations considering mean values of individual males, whereas the range (variation) encompasses the minimum and maximum values for all call samples. For each advertisement call, we analyzed ten notes and all pulse/interpulse intervals that comprise these notes. Pulse rate was calculated as pulses per second. Air temperature was taken for each recorded male with a handheld digital thermometer. Calls were analyzed using Raven Pro 1.5, 64-bit version (Bioacoustics Research Program 2014) with the following settings: window type = Hann, window size = 256 samples, 3 dB filter bandwidth = 248 or 270 Hz, brightness = 50%, contrast = 50%, overlap = 85% (locked), DFT size = 1024 samples (locked), and a grid spacing (spectral resolution) = 43.1 or 46.9 Hz, color map = Cool. Temporal traits were measured directly in the oscillogram, and spectral ones were measured directly in the spectrogram. Raven Pro 1.5 obtained the peaks of dominant frequency and other frequency bands automatically through the “Peak Frequency (Hz)” function. We generated call figures using Seewave v.1.6 package (Sueur *et al.* 2008) on the R (version 3.3.1) platform (R Core Team 2016). Seewave settings were Hanning window, 85% overlap and 256 points resolution (FFT). We also assessed the within-male call variation through the coefficients of variation [$CV = (SD/mean) \times 100$]. The average was calculated for each trait from individual male CV values. Acoustic traits with low within male CV (usually less than 5%) were classified as static and traits with higher values (usually greater than 12%) as dynamic (Gerhardt 1991). As it is only possible to establish only one note per minute measurement for each analyzed male, we check the variability of this temporal trait through between-male coefficient of variation. Gerhardt (1991) reported that between-male coefficients of variation of static acoustic properties were less than 11%, whereas coefficients of variation of dynamic properties exceeded 15%.

Multivariate normality assumption was verified through of the “mardiaTest” function on R (Package ‘MVN’; Korkmaz *et al.* 2014), and it was not applied to our acoustic data (result = data are not multivariate normal). Considering the (multivariate) acoustic dataset, we searched for discrimination between *P. ternetzi* and *P. ameghini* by applying the Random Forests model (Breiman 2001), through of the Package ‘randomForest’ on R (further details in Liaw & Wiener 2002; and Andrade *et al.* 2016). The RF results include an estimate of distances among the objects, which can be subject to a Multidimensional Scaling Analysis (MDS) with the “proximity.plot” function of the Package ‘rfPermute’ version 2.0.1 (Archer 2016), which also allows display the results graphically. The directly or indirectly packages related to the application of the discriminant functions were done in R (R Core Team 2016).

For the multivariate, multidimensional analysis/plots and statistical tests, we used the following acoustic traits: pulse duration, interpulse interval, pulse rate, note duration, internote interval, notes per minute, pulses per note, peak of dominant frequency, air and water temperatures. As in anurans it is well known that environmental variables can influence the acoustic traits, we incorporate this issue in our study through the inclusion of air and water temperatures as two continuous variables in our multivariate analyzes. We tested these traits for statistical significance differences between species through the Exact Wilcoxon Mann Whitney Rank Sum Test using the Package ‘coin’ (Resampling Statistics model) (Hothorn *et al.* 2008; function “wilcox_test”) on R. Significance was considered when $p < 0.01$. Given that we could not get access to raw call data from other *Pseudopaludicola* species to test for normality and homoscedasticity, we decided to refrain from conducting statistical analyses. Therefore, we restricted interspecific acoustic comparisons with other *Pseudopaludicola* species to call type and range values; we took the averages when there were no range values in works describing calls.

Adult males of *P. ternetzi* from the studied populations ranged in SVL from 14.0 to 18.1 mm, while adult males of *P. ameghini* ranged from 16.1 to 18.7 mm (see Appendix 1). We observe a high intraspecific variation in the dorsal color pattern of this two species, especially regarding presence or absence of a clear dorsal lateral stains on back. In addition, only two out 77 specimens of *P. ternetzi* possesses a light vertebral stripe (AAG-UFU 5019 from

Ituiutaba (MG), and AAG-UFU 3463 from Pontal do Araguaia (MT)), this feature being absent in all specimens of *P. ameghini*.

Pseudopaludicola ternetzi emits a long advertisement call (4.8–223 s), which consists of series of pulsed notes (1–11 series of notes per call) that lasts 0.5–192 s, separated by intervals of 0.093–1.4 s (Figure 1A). Notes last 32–80 ms separated by intervals of 19–88 ms, and are released at a rate of 606–921 notes per minute; notes have a slightly increase in amplitude from the first to the second pulse (= midpoint of note), followed by a decrease in amplitude regarding the second and third (see oscillogram in Figure 1B). Notes are composed of 3–6 (mean=3) non-concatenated pulses (Figure 1B). Pulses vary from 1–16 ms, separated by intervals of 1–14 ms, and are released at a rate of 61–139 pulses per second (Figure 1B). Dominant (= fundamental) frequency peaks between 3516–4500 Hz. Notes can possess up to two harmonics; the second from 6802–7430 Hz (Figure 1B). Air temperature of recorded calls varied from 21.5–29.0°C, and water varied from 25–31°C. Quantitative traits are summarized in Table 1.

The advertisement call of *P. ameghini* also consists of a long series of pulsed notes (Figure 1C and D), as well in *P. ternetzi*. Quantitative acoustic traits of *P. ameghini* are also summarized in Table 1. However, *P. ternetzi* is distinguished from *P. ameghini* by its higher pulse rate (*P. ternetzi*: 61–139; *P. ameghini*: 40–56 pulses per second), higher note rate (*P. ternetzi*: 606–921; *P. ameghini*: 348–452 notes per minute), and shorter note duration (*P. ternetzi*: 32–80; *P. ameghini*: 60–121 ms). We also found significant differences (with $p < 0.01$) between *P. ternetzi* and *P. ameghini* in all other temporal traits: internote interval, pulses per note, pulse duration, interpulse interval. The two species are indistinguishable in their peaks of dominant frequency ($p = 0.6$).

The multidimensional approach (randomForest) on acoustic data highlighted a full segregation between *P. ternetzi* and *P. ameghini* (Figure 2), with zero classification error. Distinct clustering were chiefly promoted (Figure 2A) by notes per minute, note duration and pulse rate, which are the main sources of variation in both variable importance measurements (Figure 2B). On the other hand, air and water temperatures and peak of dominant frequency were the variables with the lowest importance measures as produced by randomForest (Figure 2B). The confusion matrix using Random Forests model (settings: number of tree permutations = 1000; number of variables tried at each split = 3.0) corroborate discrimination (error rate = 0.0 %) in the classification of adult males of *P. ternetzi* ($n = 44$ adult males) and *P. ameghini* ($n = 11$ adult males). It is noteworthy that the air and water temperatures overlapped at the time of the field recordings for both species (Table 1), and are among the variables that least explained the variation of the dataset, with low importance measures as produced by randomForest (Figure 2B). Therefore, we do not attribute the found acoustic differences to the influence of the air and water temperatures.

Traits that were classified as static (within-male CV < 5%) to both species were pulse rate (*P. ternetzi*: 2.5 ± 2.8 [0–7.8], *P. ameghini*: 3.0 ± 0.9 [1.2–3.9]), peak of dominant frequency (*P. ternetzi*: 0.8 ± 0.2 [0–4], *P. ameghini*: 0.8 ± 0.9 [0–3.2]), minimum reached dominant frequency (*P. ternetzi*: 2.0 ± 0.5 [0.7–7.5], *P. ameghini*: 2.2 ± 1.6 [1.3–6.7]) and maximum reached dominant frequency (*P. ternetzi*: 1.2 ± 0.1 [0.5–2.9], *P. ameghini*: 1.0 ± 0.3 [0.5–1.5]). The other traits of both species were classified as dynamics (within-male CV: *P. ternetzi* 7.3–70.4%; *P. ameghini* 7.0–70.4%). Additionally, the between-male coefficients of notes per minute were equal to 10.4% in *P. ternetzi*, and 8.7% in *P. ameghini*. These low variabilities among males of both species allowed us to classify this trait as static. Gerhardt (1991) classified acoustic properties as static (CV < 5%, low variation coefficients of variation) and dynamic (CV > 12%). The spectral and fine scale temporal traits are usually important for species recognition, whereas variable temporal traits may be important for mate choice (Gerhardt 1991). Therefore, the two sister species differ from one another in temporal traits which is expected to be associated with their species recognition.

Pseudopaludicola ternetzi and *P. ameghini* are promptly diagnosed from *P. canga* Giaretta & Kokubun, 2003 (Carvalho *et al.* 2015a and cites therein), *P. giarettai* Carvalho, 2012 (Carvalho *et al.* 2015b and cites therein), *P. hyleaustralis* Pansonato, Morais, Ávila, Kawashita-Ribeiro, Strüssmann & Martins, 2012 (Pansonato *et al.* 2012), *P. facureae* Andrade & Carvalho, 2013 (Carvalho *et al.* 2015a and cites therein) and *P. parnaiba* Roberto, Cardozo & Ávila, 2013 (Roberto *et al.* 2013; Carvalho *et al.* 2015a) by possessing a pulsed note structure (Figure 1), whereas these five species have calls composed of non-pulsed notes. The advertisement call of *P. ternetzi* and *P. ameghini* easily distinguishes them from other congeners with pulsed note structure by the quantitative traits highlighted in Table 2.

TABLE 1. Acoustic traits of *Pseudopaludicola ameghini* and *P. ternetzi*. Mean \pm standard deviation (minimum–maximum). n = number of males recorded (number of analyzed pulsed notes). Trait abbreviations: CD = call duration; SND = series of notes duration; ISI = interseries interval; SC = series per call; ND = note duration; II = internote interval; PD = pulse duration; IPI = interpulse interval; NM = notes/minute, PR = pulse rate; PN = pulses per note; PDF = Peak of dominant frequency; MiDF = Minimum reached dominant frequency; MaDF = Maximum reached dominant frequency; AT = Air temperature; and WT = Water temperature.

Traits	<i>Pseudopaludicola ameghini</i>							<i>Pseudopaludicola ternetzi</i>						
	Chapada dos Guimarães n = 5 (50)	Santo Antonio do Leverger n = 2 (20)	Cáceres n = 4 (40)	Average n = 11 (110)	Pontal do Araguaia n = 18 (240)	Ituiutaba n = 19 (190)	Minaçu n = 7 (70)	Average n = 44 (500)						
CD (s)	20.7 \pm 10.6 (7.7–33.2)	28.6 \pm 18.1 (15.8–41.4)	30.4 \pm 13 (21.8–49.6)	25.7 \pm 12.3	57.8 \pm 62.8 (4.8–222.9)	39.3 \pm 24.9 (8.5–76.9)	39.8 \pm 33.4 (7.8–88.5)	47.1 \pm 45.4						
SND (s)	6.4 \pm 5.9 (0.4–22.9)	6 \pm 5.6 (1–17.9)	3.3 \pm 0.3 (0.2–12)	5.6 \pm 4.8	30.1 \pm 46 (0.5–192.5)	22.2 \pm 9.3 (0.5–46.8)	12.5 \pm 9.9 (5.9–29.4)	25.6 \pm 37.5						
ISI (s)	0.6 \pm 0.4 (0.2–1.6)	1 \pm 0.6 (0.3–2.5)	1 \pm 0.1 (0.2–2.7)	0.8 \pm 0.4	0.348 \pm 0.186 (0.093–1.4)	0.336 \pm 0.185 (0.202–1.1)	0.347 \pm 0.15 (0.126–0.604)	0.345 \pm 0.171						
SC	4.2 \pm 2 (4–6)	4.5 \pm 0.7 (4–5)	5.5 \pm 0.7 (5–6)	4.6 \pm 1.6	3.1 \pm 2.1 (1–8)	3.2 \pm 1.3 (2–5)	4.8 \pm 3.6 (1.4–11)	3.4 \pm 2.3						
ND (ms)	92 \pm 11 (72–121)	102 \pm 2 (80–114)	87 \pm 4 (60–111)	92 \pm 9	42 \pm 4 (32–73)	43 \pm 3 (35–80)	45 \pm 3 (34–77)	43 \pm 3						
II (ms)	55 \pm 5 (42–87)	65 \pm 12 (52–109)	54 \pm 3 (48–69)	57 \pm 7	33 \pm 4 (19–73)	46 \pm 3 (37–88)	45 \pm 4 (32–67)	41 \pm 7						
PD (ms)	11 \pm 2 (6–16)	9 \pm 1 (7–12)	12 \pm 1 (7–17)	11 \pm 2	9 \pm 1 (5–16)	8 \pm 1 (4–12)	7 \pm 1 (4–11)	8 \pm 1						
IPI (ms)	12 \pm 3 (1–23)	16 \pm 0 (9–22)	13 \pm 2 (5–18)	13 \pm 3	5 \pm 1 (1–11)	8 \pm 1 (1–14)	9 \pm 1 (4–13)	7 \pm 2						
NM	407 \pm 44.8 (348.1–452.5)	372.8 \pm 18.1 (360–385.6)	410.1 \pm 24.9 (378–438)	401.9 \pm 35.1	766.5 \pm 74.6 (654–921)	667.1 \pm 36.3 (606–726)	665.7 \pm 35.3 (612–712.2)	707.6 \pm 73.4						
PR	48.2 \pm 3.6 (39.6–55.6)	45.8 \pm 1.6 (43.9–50)	46.5 \pm 1.2 (42.6–50)	47.2 \pm 2.6	79.8 \pm 7.8 (61.2–138.9)	75.7 \pm 6.6 (62.5–105.3)	74.2 \pm 2.4 (64.5–88.2)	77.1 \pm 6.9						
PN	4.4 \pm 0.2 (4–5)	4.7 \pm 0.1 (4–5)	4 \pm 0.1 (3–5)	4.3 \pm 0.3	3.4 \pm 0.3 (3–6)	3.3 \pm 0.2 (3–5)	3.3 \pm 0.2 (3–5)	3.3 \pm 0.3						
PDF (Hz)	3549.9 \pm 246.5 (3140.6–3832.9)	4028.9 \pm 334.8 (3703.1–4312.5)	3866 \pm 115.3 (3703.1–3984.4)	3751.9 \pm 283.7	3998.6 \pm 348.1 (3609.4–4500)	3733.2 \pm 126.3 (3515.6–4078.1)	3746.6 \pm 141.1 (3515.6–4125)	3843.9 \pm 272.7						
MiDF (Hz)	2350.4 \pm 129 (2112.5–2631.6)	2752.6 \pm 149.7 (2564.5–3017.4)	2843.8 \pm 102.4 (2702–3071.1)	2602.9 \pm 267.6	2383.6 \pm 154.9 (1563.1–2826.9)	2454.8 \pm 247.5 (2156.8–3101.1)	2795.3 \pm 140 (2483.5–3173.1)	2479.9 \pm 241.3						
MaDF (Hz)	4638.3 \pm 418.4 (4153.8–5170.5)	5441.7 \pm 125.1 (5315.2–5700.3)	4808.8 \pm 155.2 (4602.7–5052.7)	4846.4 \pm 414.6	5213.9 \pm 441.8 (4544.3–6100)	4762.7 \pm 272.4 (4242.9–5393.7)	4660.9 \pm 214.5 (4258.7–5007.9)	4931.1 \pm 415.3						
AT (°C)	20–26.3	23	25	–	26–27.5	21.5–25	22.5–29	–						
WT (°C)	23–28.2	–	29	–	29–31	25–26	28	–						

TABLE 2. Comparison of the acoustic traits among *Pseudopaludicola* species with pulsed note structure. Values in bold highlight the variables that distinguish *P. ternetzi* and *P. ameghini* from their other congeners. The species with concatenated pulses (= lack interpulse interval; sensu Magalhães *et al.* 2014) were classified as absence in interpulse interval trait. (minimum–maximum).

	Note duration (ms)	Pulses per note	Pulse rate (pulses/sec)	Internote interval (ms)	Interpulse interval (ms)	Peak of dominant frequency (Hz)	Literature
<i>P. ternetzi</i>	32–80	3–5	61.2–138.9	19–88	1–14	3515.6–4500	Present study
<i>P. ameghini</i>	60–121	3–6	39.6–55.6	42–109	1–23	3140.6–4312.5	Present study
<i>P. falcipes</i>	-	2	-	70	30	4200–5800	Haddad & Cardoso 1987
<i>P. mineira</i>	40±4	2	-	100 ± 40	20±2	4306–4823	Pereira & Nascimento 2004
<i>P. pocoto</i>	126–397	2–3	7.6–18	136–507	43–187	4734–6373	Magalhães <i>et al.</i> 2014 Andrade <i>et al.</i> 2017
<i>P. atragula</i>	300–700	9–36	-	40–900	2–64	3617.6–4263.6	Pansonato <i>et al.</i> 2014
<i>P. saltica</i>	30–108	2–6	13–28	80–198	1–48	4478.9–5531.2	Andrade <i>et al.</i> 2016
<i>P. murundu</i>	27–126	2–6	10.8–25	30–184	1–39	5081.8–6375.0	Andrade <i>et al.</i> 2016, and cites therein
<i>P. Jaredi</i>	56–178	2–7	17.1–25.7	84–184	1–46	5081.8–5986.2	Andrade <i>et al.</i> 2016
<i>P. mystacalis</i>	30–60	12–20	-	40–200	absence	4478.9–5340.2	Pansonato <i>et al.</i> 2014
<i>P. boliviana</i>	16–32	2–7	-	2–14	absence	3789.8–5250	Durè <i>et al.</i> 2004 Pansonato <i>et al.</i> 2016
<i>P. ibisoroca</i>	25–50	3–12	-	67–585	absence	3617.6–4651.2	Pansonato <i>et al.</i> 2016
<i>P. motorzinho</i>	5–25	2–6	-	29–200	absence	4306.6–5684.8	Pansonato <i>et al.</i> 2016

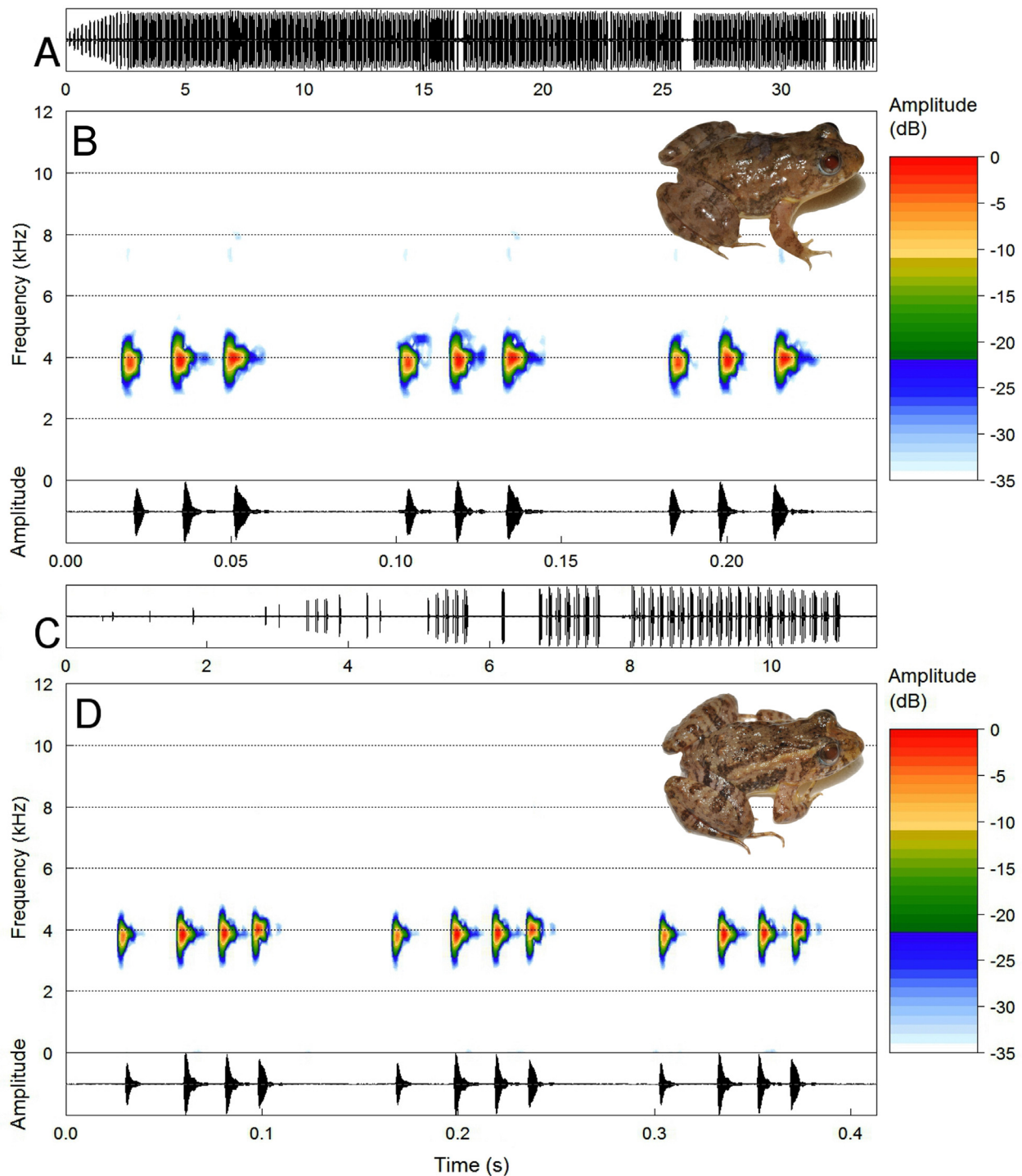


FIGURE 1. (A) Oscillogram of the entire advertisement call with five series of pulsed notes; and (B) audiospectrogram (above) and corresponding oscillogram (below) detailing three pulsed notes of *P. ternetzi* from Minaçu (GO); inset: an adult male of *P. ternetzi* (AAG-UFU 5021, SVL=15.3 mm), call voucher of this recording; and (C) Oscillogram of an entire advertisement call with five series of pulsed notes; and (D) audiospectrogram (above) and corresponding oscillogram (below) detailing three pulsed notes of *Pseudopaludicola ameghini* from Chapada dos Guimarães (MT) (type-locality), Brazil; inset: an adult male of *P. ameghini* from Cáceres (MT) (AAG-UFU 5367, SVL=17.0 mm). Sound file in (A) and (B): Pseudop_ternetziMinaçuGO9cLBM_AAGmt; recorded at 18:20 h, on 19 November 2014, air temperature 24°C. Sound file in (C) and (D): Pseudop_ameghiniChapadaGuimaraesMT2aBFVTmt; recorded at 18:52 h, on 14 December 2008, air temperature 23.9°C; unvouchered recording.

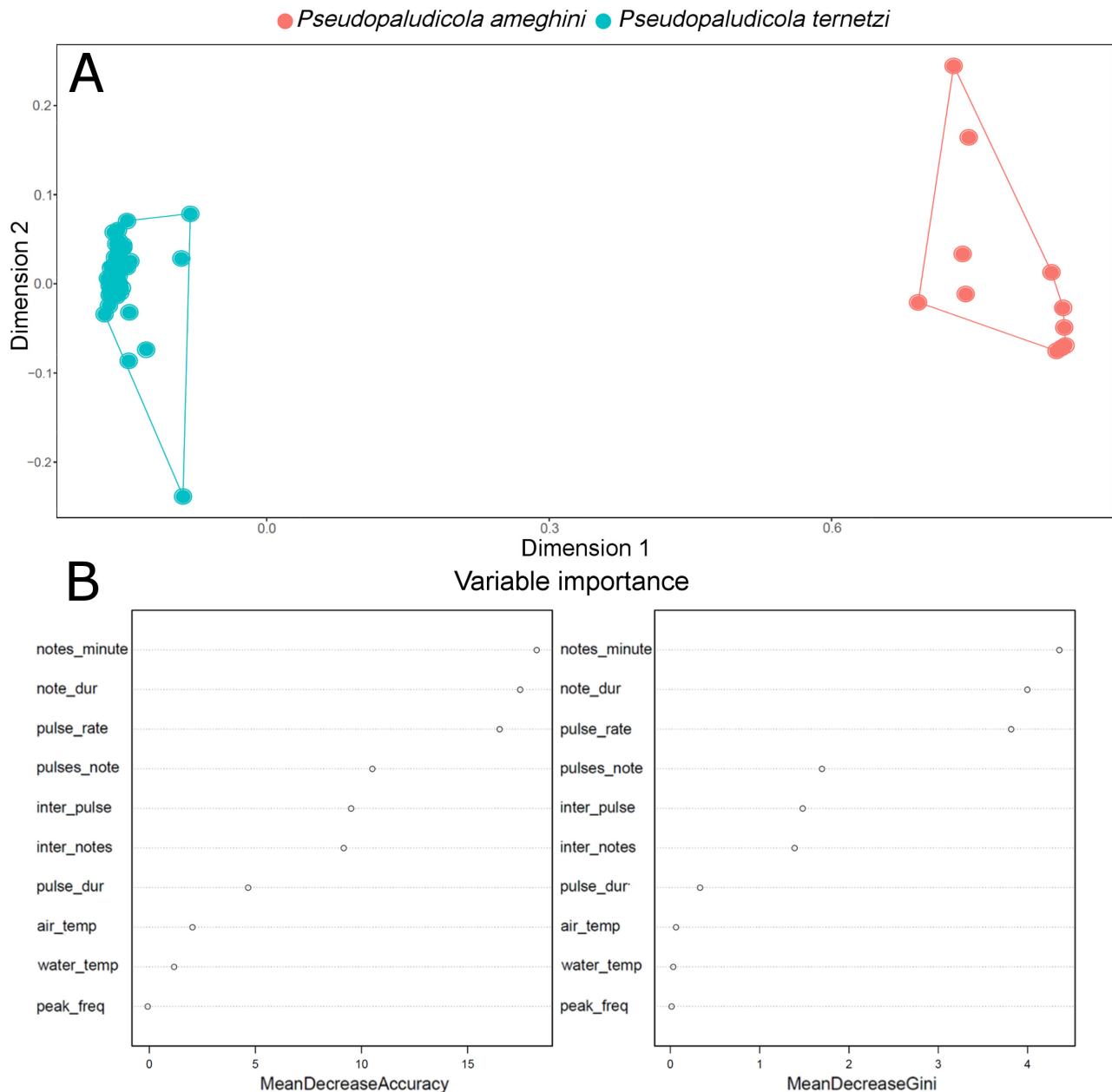


FIGURE 2. (A) First and second dimensions of the Multidimensional scaling of the proximity scores from Random Forest considering acoustic traits of 44 males of *Pseudopaludicola ternetzi* (blue circles), and eleven males of *P. ameghini* (red circles). (B) Dotchart of variable importance score as indicated by the Random Forest analysis.

We recognize that *P. ternetzi* and *P. ameghini* are species with a similar external morphology and high intraspecific variation on dorsal color patterns, which may have led to misleading identifications based on their external morphological features. Miranda-Ribeiro (1937) mentioned that some individuals of *P. ternetzi* have on their back “a medial longitudinal stripe”, and Pansonato *et al.* (2013) pointed to the absence of any light vertebral stripe on their specimens of *P. ameghini*. In this study, we observe a light vertebral stripe on only two out of 77 specimens of *P. ternetzi*, and on no specimen of *P. ameghini*. Therefore, this is not a reliable character to distinguish this two species. Cardozo & Toledo (2013) suggested that *P. ternetzi* can be discriminated from *P. ameghini* by having a dorsum slightly warty, but we also were unable to distinguish them based on this character. Recently, Lavilla *et al.* (2016) assigned the Paraguayan specimens, previously *P. ternetzi*, to *P. ameghini* based on the absence of a vertebral line and dorsal surface of the body with planar warts. It is clear that the authors had to give a name to preserved specimens that were housed in Paraguayan collections, what limited them to examine only the external morphology characters. However, we think that a reliable identity of these Paraguayan populations will only be possible after an analysis of their advertisement calls or genetic data.

Pansonato *et al.* (2013) described the advertisement call of *P. ameghini* based on calls of five males from its type locality and other sites in the state of Mato Grosso, and provided acoustic and morphometric evidence for full distinctiveness of *P. ameghini* from *P. mystacalis*, up to that moment considered a synonym according to Haddad & Cardoso (1987). In accordance with Pansonato *et al.* (2013), our results also indicate that the call of *P. mystacalis* described by Haddad & Cardoso (1987) in fact corresponds to the call of *P. ameghini*. Pansonato *et al.* (2013) did not employ the current pulse terminology in their descriptions and not presented an acoustic comparison between *P. ameghini* and *P. ternetzi*. Due to this, and in order to ensure reliability to our discussion, we have based the acoustic comparisons only on our own data.

Cardozo & Toledo (2013) suggested that *P. ternetzi* and *P. ameghini* can be discriminated each other by their advertisement calls. Our acoustic analysis also revealed important results for the full differentiation of *P. ternetzi* from *P. ameghini*, being easily diagnosed by two major non-overlapping temporal traits: pulse rate and notes per minute; and additionally by a significant difference in note duration (Tables 1; Figure 2). On the other hand, spectral traits were unreliable to distinguish them one from each other, what is also valid for other species of *Pseudopaludicola* that are also closely related. For example, both *P. motorzinho* and *P. boliviana* (Pansonato *et al.* 2016), as *P. atragula* and *P. facureae* (Pansonato *et al.* 2014) are diagnosed mainly by their temporal traits.

Our record of *P. ternetzi* from Pontal do Araguaia is the first to the state of Mato Grosso and extends its distribution to ca. 360 km southwest from the type locality. Cardozo & Baldo (2012) stated that Lobo (1996) designated the MNRJ 477 as a paralectotype, but this specimen is a *Physalaemus cuvieri* from Carolina (MA), which was also collected by Carlos Ternetz. Therefore, this specimen does not belong to *P. ternetzi* type series. Lastly, our results corroborate recent papers that proposed the recognition of *P. ameghini* and *P. ternetzi* as full species (Fávero *et al.* 2011; Cardozo & Toledo 2013; Pansonato *et al.* 2013; Veiga-Menoncello *et al.* 2014; Andrade *et al.* 2016; Pansonato *et al.* 2016), and provide elements for the specific recognition of these two sister species.

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APPENDIX 1. Examined specimens and its sound files (wav). The adult specimens and calls are housed in the collection of frogs (AAG-UFU) of the Universidade Federal de Uberlândia, municipality of Uberlândia, state of Minas Gerais, Brazil. Call sections are available at AmphibiaWeb (<http://amphibiaweb.org/>). Voucher numbers are right after the label of their recordings.

Pseudopaludicola ternetzi – BRAZIL: GOIÁS: Serra da Mesa - Minaçu (13°41'56.98"S, 48°16'43.14"W, el. approximately 860 m a.s.l.): AAG-UFU 5021–2; labels of the analyzed recordings: Pseudop_ternetziMinacuGO1aAAGm671, Pseudop_ternetziMinacuGO2aAAGm671; Pseudop_ternetziMinacuGO3aAAGm671; Pseudop_ternetziMinacuGO4aAAGm671; Pseudop_ternetziMinacuGO5aLBM_AAGmt (AAG-UFU 5021); Pseudop_ternetziMinacuGO6aLBM_AAGmt, Pseudop_ternetziMinacuGO7aLBM_AAGmt (AAG-UFU 5022); Serra de Caldas Novas (PESCAN) - Caldas Novas (17°48'30.00"S, 48°42'00.00"W, el. approximately 986 m a.s.l.): AAG-UFU 4322–3, 4343–9, 4353; MATO GROSSO: Pontal do Araguaia (15°58'13.68"S, 52°17'48.44"W, el. 320 m a.s.l.): AAG-UFU 1066–1077, 1086, 1301–7, 1309, 2832, 3122, 3453–61, 3463–5, 5043–4; labels: Pseudop_ternetziPontalAraguaiaMG27aAAGm, Pseudop_ternetziPontalAraguaiaMG27bAAGm, Pseudop_ternetziPontalAraguaiaMG28aAAGm, Pseudop_ternetziPontalAraguaiaMG29aAAGm, Pseudop_ternetziPontalAraguaiaMG30aIAH_AAGmt (AAG-UFU 1304), Pseudop_ternetziPontalAraguaiaMG30bIAH_AAGmt (AAG-UFU 1304), Pseudop_ternetziPontalAraguaiaMG31aIAH_AAGmt, Pseudop_ternetziPontalAraguaiaMG31bIAH_AAGmt, Pseudop_ternetziPontalAraguaiaMG32aIAH_AAGmt (AAG-UFU 1305), Pseudop_ternetziPontalAraguaiaMG33aIAH_AAGmt, Pseudop_ternetziPontalAraguaiaMG33bIAH_AAGmt, Pseudop_ternetziPontalAraguaiaMG34aFSA_AAGb (AAG-UFU 1306), Pseudop_ternetziPontalAraguaiaMG35aFSA_AAGb (AAG-UFU 1307), Pseudop_ternetziPontalAraguaiaMG36aFSA_AAGb, Pseudop_ternetziPontalAraguaiaMG37aFSA_AAGb, Pseudop_ternetziPontalAraguaiaMG38aFSA_AAGb, Pseudop_ternetziPontalAraguaiaMG39aFSA_AAGb (AAG-UFU 3453), Pseudop_ternetziPontalAraguaiaMG39bFSA_AAGb (AAG-UFU 3453), Pseudop_ternetziPontalAraguaiaMG40aFSA_AAGb (AAG-UFU 3454), Pseudop_ternetziPontalAraguaiaMG41aFSA_AAGb (AAG-UFU 3455), Pseudop_ternetziPontalAraguaiaMG42aFSA_AAGb, Pseudop_ternetziPontalAraguaiaMG43aFSA_AAGb, Pseudop_ternetziPontalAraguaiaMG44aFSA_AAGb; MINAS GERAIS: Ituiutaba (19°00'05.52"S, 49°27'24.97"W, el. 600 m a.s.l.): AAG-UFU 0944, 3512–8, 5009–19; labels: Pseudop_ternetziItuiutabaMG8aIAH_AAGmt, Pseudop_ternetziItuiutabaMG9aIAH_AAGmt, Pseudop_ternetziItuiutabaMG10aAAGm671, Pseudop_ternetziItuiutabaMG11aAAGm671, Pseudop_ternetziItuiutabaMG12aAAGm671, Pseudop_ternetziItuiutabaMG13aAAGm671, Pseudop_ternetziItuiutabaMG14aAAGm671, Pseudop_ternetziItuiutabaMG15aAAGm671, Pseudop_ternetziItuiutabaMG16aAAGm671 (AAG-UFU 5009), Pseudop_ternetziItuiutabaMG17aAAGm671 (AAG-UFU 5010), Pseudop_ternetziItuiutabaMG18aAAGm671, Pseudop_ternetziItuiutabaMG19aAAGm671, Pseudop_ternetziItuiutabaMG20aAAGm671, Pseudop_ternetziItuiutabaMG21aAAGm671, Pseudop_ternetziItuiutabaMG22aAAGm671, Pseudop_ternetziItuiutabaMG23aAAGm671, Pseudop_ternetziItuiutabaMG24aAAGm671, Pseudop_ternetziItuiutabaMG25aAAGm671, Pseudop_ternetziItuiutabaMG26aAAGm671; Monte Alegre de Minas (18°52'22.71"S, 48°52'37.38"W, el. 724 m a.s.l.): AAG-UFU 4170, 4607, 4609, 4611; Uberlândia (19°10'55.99"S, 48°23'36.56"W, el. 813 m a.s.l.): AAG-UFU 2624–6, 4670–1.

Pseudopaludicola ameghini – BRAZIL: MATO GROSSO: Cáceres (16°7'2.61"S, 57°40'47.00"W, approximately 130 m a.s.l.): AAG-UFU 5253, 5367–73; labels: Pseudop_ameghiniCaceresMT5aDLB_AAGm670, Pseudop_ameghiniCaceresMT6aDLB_AAGm670, Pseudop_ameghiniCaceresMT7aDLB_AAGm670, Pseudop_ameghiniCaceresMT8aDLB_AAGm670; Chapada dos Guimarães (type-locality; 15°21'23.64"S, 55°49'33.93"W, approximately 369 m a.s.l.): AAG-UFU 4721, labels: Pseudop_ameghiniChapadaGuimaraesMT1aBFVTmt (AAG-UFU 4721), Pseudop_ameghiniChapadaGuimaraesMT2aBFVTmt, FNJV_0004579_Pseudopaludicola_ameghini_Aldeia_Velha_MT_AdaoJoseCardoso.wav, FNJV_0004580_Pseudopaludicola_ameghini_Aldeia_Velha_MT_AdaoJoseCardoso.wav, FNJV_0004581_Pseudopaludicola_ameghini_Aldeia_Velha_MT_AdaoJoseCardoso.wav; Santo Antônio do Leverger (15°49'26.35"S, 55°30'48.48"W, approximately 620 m a.s.l.): AAG-UFU 1443–4; labels: Pseudop_ameghiniSantoAntonioLevergerMT3aTRC_AAGmt (AAG-UFU 1443), Pseudop_ameghiniSantoAntonioLevergerMT4aTRC_AAGmt (AAG-UFU 1444).