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# Revisiting Features of the Advertisement Call of Three Species of the *Rhinella granulosa* Group (Anura: Bufonidae): Taxonomic Implications

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**Abstract.—The *Rhinella granulosa* group currently comprises 13 species distributed from Panama to South America. Descriptions of anuran calls have provided significant taxonomic information in studies involving problematic species groups. Herein, we revisit acoustic features of the advertisement calls in three of these species: *Rhinella major*, *Rhinella bergi* and *Rhinella dorbignyi*. In addition, we analyzed release calls of *R. bergi* and *R. azarai*. We discuss geographical variation in calls, comment on their frequency band structure, and compare calls with those described previously. To search for discrimination among populations of *R. major,* we used the random forest model, multidimensional scaling analysis, and Wilcoxon-Mann-Whitney rank sum tests. Advertisement calls consisted of a long-lasting trill of a variable pulse number (2–8) distinctive to each species, with the presence of one harmonic in *R. bergi*. Release calls consisted of multiple notes, pulsed or not, with the presence of harmonics. This marks the first time that harmonics have been reported for that species. Additionally, we found that temporal characteristics (e.g., note duration) varied among populations of *R. major*, whereas dominant frequency remained a stereotyped property.**

The *Rhinella granulosa* species group was taxonomically reviewed by Narváes and Rodrigues (2009) and Pereyra et al. (2021). Currently, 13 taxa are recognized and distributed in open habitats from Panama to southern South America (Pereyra et al., 2021). The taxonomic history of the species group has been long and occasionally challenging (Pereyra et al., 2016) due to morphological conservatism. As a result, some synonymizations have occurred (Narváes and Rodrigues, 2009; Pereyra et al., 2021). These facts highlight the necessity of employing lines of evidence beyond morphology, such as molecular and acoustic characterizations, to reveal hidden diversity within the group.

Advertisement and release calls have been employed as important taxonomic tools to diagnose morphologically cryptic anuran species (Köhler et al., 2017), especially within the *R. granulosa* species group, in which combinations of acoustic characters such as note duration, pulses per note, and dominant frequency allow for distinguishing a species (São-Pedro et al., 2011; Guerra et al., 2011; Bernardes et al., 2015; Murphy et al., 2017; Giaretta et al., 2018). Here, we revise the advertisement calls of *Rhinella major*, *R. bergi*, and *R. dorbignyi*, aiming to provide further details for each species and discuss geographical variations. Release calls of *R. bergi* and *R. azarai* are also described.

## Materials and Methods

*Study Sites.*—Analyzed calls were from seven localities in Argentina including the Dry Chaco (dry forests, savannas, and xerophytic vegetation) and the Wet Chaco (forests of quebracho and algarrobo and other areas prone to flooding), as well as three human-disturbed localities in Brazil around the Amazon Forest (riverbanks and forest borders) (Fig. 1). The call sample consisted of advertisement calls from 31 males (*R. major, R. bergi, and R. dorbignyi*) and release calls from two males (*R. azarai* and *R. bergi*). Males emitted release calls while being held by the recorder with two fingers on the axillae. Sound files from Argentina are housed in the Fonoteca Zoológica de la Universidad Nacional del Nordeste (FZ-UNNE). Voucher specimens and calls from Brazil are housed in the frog collection at Museu de Biodiversidade do Cerrado, Universidade Federal de Uberlândia (MBC–UFU). Further details on recordings, including dates, time, sound files, and voucher specimens, are in Appendix Table 1. Because calls from Macapá (Amapá state), Vitória do Xingu, and Rurópolis (Pará state) were essentially similar (with no significant differences and all measured characteristics overlapping in range, results not shown), these samples were pooled as Brazil samples, all less than 1,000 km apart and close to the Amazon River. The species studied here were identified based on their diagnostic characteristics as specified in Narváes and Rodrigues (2009).

*Acoustics.*—For the Brazilian sample, calls were recorded using a Marantz digital recorder (PMD 670 and PMD 671) set at a sampling rate of 48 kHz and a 16-bit resolution, coupled to a Sennheiser ME67/K6 directional microphone. In Argentina, calls were recorded with an M-audio Microtrack II digital recorder coupled to a Sennheiser ME66/K6 microphone. Calls were analyzed with the software Raven Pro 32-bit version 1.6.3 (Bioacoustics Research Program, 2022) with the following settings: window size = 256 samples, window type = Hann, 3 dB filter bandwidth = 270 Hz, overlap = 85% (locked), DFT size = 1,024 samples, grid spacing = 43.1 Hz). Oscillogram and spectrogram illustrations were generated using the Seewave v.1.6 package (Sueur et al., 2008) on the R version 4.2.1 platform (R Development Core Team, 2022). Analyzed call definitions followed Köhler et al. (2017) and included the following parameters: call duration, note duration, call rate, call interval, note interval, number of notes per call, number of pulses per note, dominant frequency (using "Peak Frequency" function), minimum and maximum frequency (using "Frequency 5%" and "Frequency 95%" functions, respectively), and pulse period when possible. Finally, to distinguish harmonics from sidebands in recorded calls, we checked if frequency peaks were a multiple integer of fundamental frequency, and if so, they were identified as harmonics.

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Fig. 1. Geographic distribution of populations of the *Rhinella granulosa* group studied in this work. Numbers indicate how many individuals were recorded at each locality. For more details, see Appendix Table 1.

*Statistics*.—We classified calls among *R. major* populations (Miraflores, Parque Nacional Chaco, and Brazil) using the random forest model with the package 'randomForest' version 4.6-12 in.R (Liaw and Wiener, 2002) and discriminant analysis of principal components with the package adegenet version 2.0.1 (Jombart, 2008; Jombart et al., 2010). The random forest (RF) analysis provided a distance measure among objects subject to multidimensional scaling that can be plotted using the ''proximity-Plot'' function of the package rfPermute v.2.1.5 (Archer, 2016). Discriminant analysis of principal components (DAPC) analysis was conducted in an exploratory context and used to assess the congruence of results with those obtained from RF discrimination. Finally, we tested all acoustic variables for statistically significant differences among populations with the exact Wilcoxon-Mann-Whitney rank sum test using the package coin (resampling statistics, function "wilcox_test," Hothorn et al., 2008). Because these tests were done between population pairs, we adjusted significance levels ("$\alpha$") according to the number of pairings using the Holm method ("p.adjust" function in R). We assumed significance when $P \leq 0.05$. Statistical analyses were not applied to data from other species.

## Results

*Morphological Identification*.—Identification was based on the diagnostic features listed in Narváes and Rodrigues (2009). We distinguished *R. bergi* from *R. major* by features such as presence of a parietal crest, a long infraorbital crest, and mostly continuous crests, especially in localities where both species could occur. We differentiated *R. azarai* from *R. major* by the presence of a parietal crest, from *R. bergi* by its smaller eye diameter relative to eye–nostril distance, and from both species by a maxillary crest that was visible in dorsal view and inclined anteriorly upward in lateral view. It is worth noting that individuals of *R. bergi* did not exhibit a longitudinal dorsal stripe.

*Advertisement Calls of R.* major.—Males of *R. major* from Argentina and Brazil were observed calling in chorus at night around temporary pools. As a general pattern, the call (Table 1; Fig. 2) consisted of a long series of a single type of pulsed notes, emitted at irregular intervals. In all cases, calls rapidly reached maximum amplitude toward the end.

In Brazilian populations, advertisement calls lasted 1.4–11.6 s, spaced by intervals between 0.25–56.9 s. Calls had 31–248 notes with a duration of 32.5–41.3 ms. Dominant (i.e., fundamental) frequency peaks ranged from 2,497 to 3,186 Hz. In specimens from Parque Nacional Chaco (PN Chaco), Argentina, calls were shorter, lasting 2.9–6.9 s with intervals ranging 1.9–12.1 ms. Calls had 41–94 notes with a duration of 48–55.7 ms. Dominant (i.e., fundamental) frequency peaks ranged from 2,239 to 2,928 Hz. Calls of specimens recorded in Miraflores, Argentina had a duration of 1.4–14.9 s and were separated by intervals between calls of 0.58–42.26 s. Calls had 24–249.92 notes with a duration of 30.89–43.56 ms. Dominant (i.e., fundamental) frequency peaks ranged from 2,325 to 2,928 Hz. Further details can be found in Table 1.

Multivariate analyses (RF and DAPC) allowed for separation of the PN Chaco population from those from Brazil, as well as between PN Chaco and Miraflores (Fig. 3; see Appendix Fig. 1 for a comparison of the species of the granulosa group), which were supported by statistically significant differences (exact Wilcoxon rank sum test). However, samples from Brazil and Miraflores broadly overlapped in the multidimensional scaling analysis. In the RF multivariate approach, we found no (0%) classification error in PN Chaco males, whereas males from other localities had classification errors (Brazil = 15%, Miraflores = 83%). Similarly, DAPC revealed essentially the same discrimination pattern among samples (results not shown).

Temporal traits were significant in discriminating geographic samples. Compared to Brazil and Miraflores, PN Chaco exhibited a longer note duration ($Z = -2.94$, $P = 0.00084$ and $Z = -2.56$, $P = 0.00952$, respectively), longer pulse period ($Z = -2.83$, $P = 0.0017$ and $Z = -2.56$, $P = 0.0095$), shorter note rate ($Z = 2.94$, $P = 0.00084$ and $Z = 2.35$, $P = 0.019$), pulse rate ($Z = 2.94$, $P = 0.00084$ and $Z = 2.56$, $P = 0.0095$), and a greater number of pulses per note ($Z = -3.30$, $P = 0.00042$ and $Z = -2.39$, $P = 0.024$). Note duration was the most informative trait for distinguishing the PN Chaco population (Fig. 4). Calls from Miraflores did not differ significantly from those from Brazil.

*Advertisement Calls of R.* bergi.—We observed males calling at margins of temporary ponds next to roads or forest fragments

Table 1. Temporal and spectral features of advertisement calls in *R. major* populations evaluated in this study and compiled from the literature. For populations recorded in Argentina and Brazil in this study, values of summary statistics are presented as mean ± 1 SD (range). *n* = number of recorded males (analyzed calls). For other populations, values were transcribed as provided in the original publications. EBB = Estación Biológica del Beni, VX = Vitória do Xingu.

| | Köhler et al. (1997) Bolivia (EBB) *n* = 1 (5) | Guerra et al. (2011) Argentina (Vera) *n* = 4 (21) | Bernardes et al. (2015) Brazil (Monte Dourado) *n* = 4 (21) | Present study: Brazil (Macapá, Rurópolis, VX) *n* = 13 (67) | Present study: Argentina (PN Chaco) *n* = 4 (14) | Present study: Argentina (Miraflores) *n* = 6 (58) |
|---|---|---|---|---|---|---|
| Air temp. (°C) | 25 | 20 | 28 | 25–29 | 25 | 25–30 |
| Water temp. (°C) | - | - | - | 27–30 | - | 27–30 |
| Call duration (s) | 5.3 ± 1.4 (4.1–7.5) | 5.1 ± 1.6 (3.6–7.2) | 4.2 ± 0.96 (1.4–6.7) | 4.7 ± 1.7 (1.4–11.6) | 5.2 ± 1.2 (2.9–6.9) | 5.1 ± 2.9 (1.4–14.9) |
| Call rate (calls/min) | - | - | - | 5.8 ± 2.7 (1.6–10.3) | 5.2 ± 1.7 (3.8–7.3) | 6.5 ± 2.8 (2.9–10.8) |
| Call interval | - | - | - | 7.7 ± 10.4 (0.3–56.9) | 6 ± 3.6 (1.9–12.1) | 4.8 ± 6.7 (0.6–42.3) |
| Frequency 5% (Hz) | - | - | - | 2,381 ± 110 (2,110–2,540) | 2,310 ± 407 (1,076–2,583) | 2,282 ± 133 (2,067–2,497) |
| Frequency 95% (Hz) | - | - | - | 3,232 ± 130 (3,000–3,445) | 3,079 ± 102 (2,971–3,229) | 2,966 ± 208 (2,670–3,230) |
| Dominant frequency (Hz) | 2,960 (2,500–3,900) | 2,726 ± 179 (2,500–2,936.7) | 2.67 ± 0.14 (2.44–3.09) | 2,864 ± 177 (2,497–3,186) | 2,707 ± 206 (2,239–2,928) | 2,656 ± 212 (2,325–2,928) |
| Notes per call | 96.4 ± 23.5 (75–133) | 84.3 ± 18.5 (61.4–106) | 84.0 ± 17.5 (28–137) | 95.7 ± 35.1 (31–248) | 73 ± 15.2 (41.3–94) | 91.1 ± 50.9 (24–250) |
| Note duration (ms) | 42.7 ± 1.5 (40–48) | 45 ± 4.7 (42–52) | 40.4 ± 0.7 (32–47) | 36.4 ± 2.2 (32.5–41.3) | 52.9 ± 2.3 (48–55.7) | 38.2 ± 2.7 (31–43.6) |
| Note interval (ms) | - | 15 ± 2.2 (12–18) | 9.5 ± 1.9 (4–18) | 11.7 ± 2.5 (7.5–18) | 17.8 ± 1.6 (15.8–21.7) | 16 ± 2.6 (11.3–20.7) |
| Note rate/s | 18.3 ± 0.4 (17.7–18.7) | 16.8 ± 1.8 (14.3–18.6) | 20.3 ± 0.6 (19.2–21.8) | 20.4 ± 1.3 (17–24.3) | 15.5 ± 4.9 (13.6–32.4) | 19 ± 7.2 (4.1–60.4) |
| Pulses per note | 6 | 6.6 ± 0.6 (6–7.3) | 5.8 ± 0.2 (5–6) | 6.1 ± 0.4 (5–7) | 7.6 ± 0.5 (7–8) | 6.7 ± 0.6 (5–7) |
| Pulse rate/s | 139.5 ± 4.8 (134.5–145.8) | - | 145.1 ± 4.6 (116.3–187.5) | 166 ± 8.4 (150.4–179.5) | 140.4 ± 19.4 (75.7–155.3) | 174.1 ± 10.8 (146.9–203.7) |
| Pulse period | - | - | - | 5.5 ± 0.3 (5–6.7) | 6.4 ± 0.31 (5.9–7) | 5.3 ± 0.2 (4.9–5.7) |
| Pulse duration | - | 4 ± 0.5 (4–5) | - | - | - | - |
| Pulse interval | - | 3 ± 07 (2–3) | - | - | - | - |

between 21:00–23:00 h. Advertisement calls of *R. bergi* were recognized as regular series of pulsed notes (trills), with two pulses per note (Figs. 5, 6). Calls reached maximum amplitude quickly and remained constant until the end as in other species of the *R. granulosa* group. We report for the first time the presence of harmonics in calls of *R. bergi* between 6 and 7.5 kHz in all populations evaluated. This feature has not been documented previously in calls of the *R. granulosa* group. Advertisement calls of *R. bergi* had an amplitude modulation (ascending at the beginning and descending at the end), with the second pulse having the highest amplitude in each note. Similarly, a slight modulation was observed in fundamental frequencies (Fig. 6).

Males from San Luis del Palmar emitted calls with an average duration of 18 s (9.4–51.2) and mean note duration of 65 ms (60–74). The dominant frequency ranged from 3,143 to 3,488 Hz. Frequency peak in harmonics ranged from 6,198 to 6,809 Hz. From one individual in this locality, we obtained an exceptional recording of a long call lasting 51 s and consisting of 540 notes. Specimens recorded in La Leonesa had an average call duration of 14 s (5.4–32.9) and mean note duration of 64 ms (61.1–66.9). The dominant frequency ranged from 3,656 to 4,031 Hz. Frequency peak in harmonics ranges from 7,351 to 7,842 Hz. Calls from the type locality (Corrientes) had an average call duration of 14 s (11.5–17.3) and mean note duration of 44.2 ms (43.6–44.8). The dominant frequency was 3,316 Hz. Frequency peak in harmonics ranged from 6,522 to 6,528 Hz. *R. bergi* from Paso de la Patria emitted calls with an average duration of 10 s (8–12.07) and mean note duration of 40.7 ms (40.5–40.9). Dominant frequency was 3,359 Hz. Frequency peak in harmonics ranged from 6,587–6,598 Hz. Further details can be found in Table 2.

*Advertisement Calls of R.* dorbignyi.—A male (SVL = 52.5 mm) was calling in chorus and found alongside temporary pools at night after heavy rain showers. Air and water temperature at the time of recording were 19 °C and 24 °C, respectively. The advertisement call consisted of a long sequence of pulsed notes with maximum amplitude reached from the beginning and

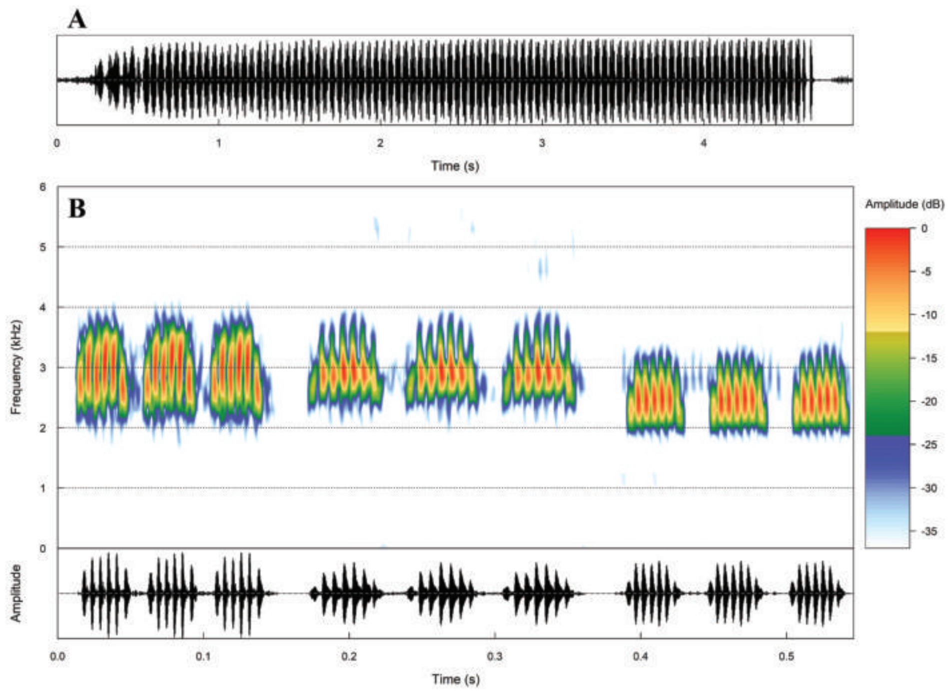


FIG. 2. (A) Oscillograms of the advertisement call of *R. major* from Macapá, state of Pará, Brazil. A complete call (Rhinella_majorMacapaAP6aAAGm661MK2). (B) Audiospectrograms and corresponding oscillograms of three notes from the middle portions of the advertisement calls of males from the three studied populations of *R. major* depicting six or seven pulses. The first three notes correspond to Macapá (Pará, Brazil), the next three to PN Chaco, and the last three to Miraflores, both in Chaco Province, Argentina.

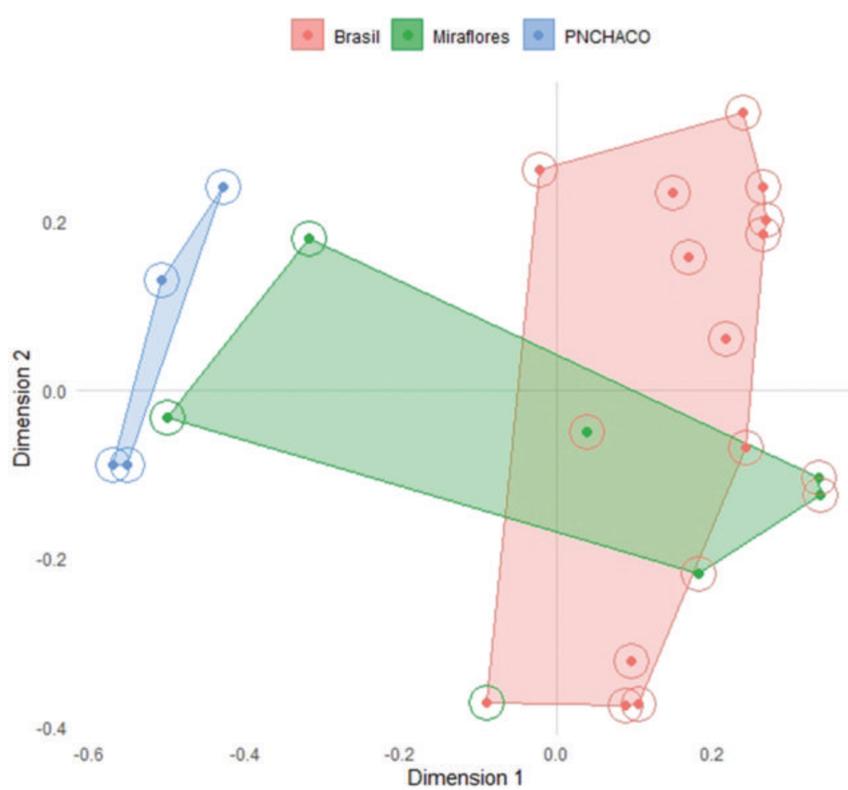


FIG. 3. Plot of the two first axes of a multidimensional scaling analysis on the random forest result for the acoustic data of *R. major* from Brazil, PN Chaco, and Miraflores. Note the complete discrimination between PN Chaco and Brazil populations. Discrimination was mostly due to differences in note duration and pulses per note (see main text).

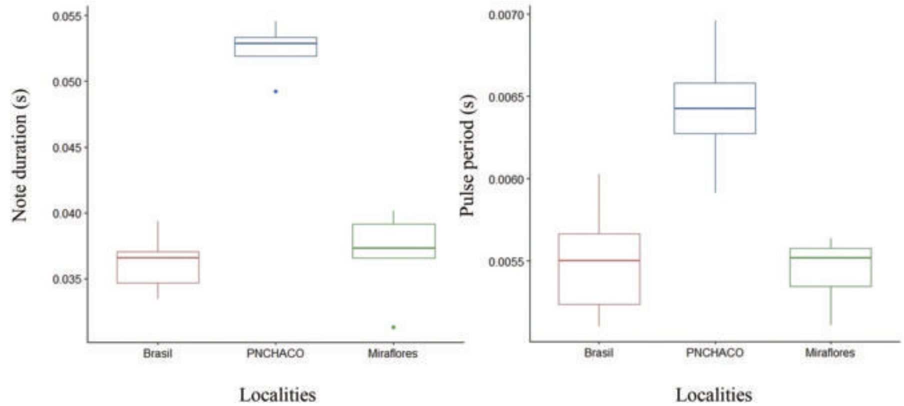


FIG. 4. Call features identified as important in the discrimination of the three studied *R. major* populations (Brazil, PN Chaco, and Miraflores).

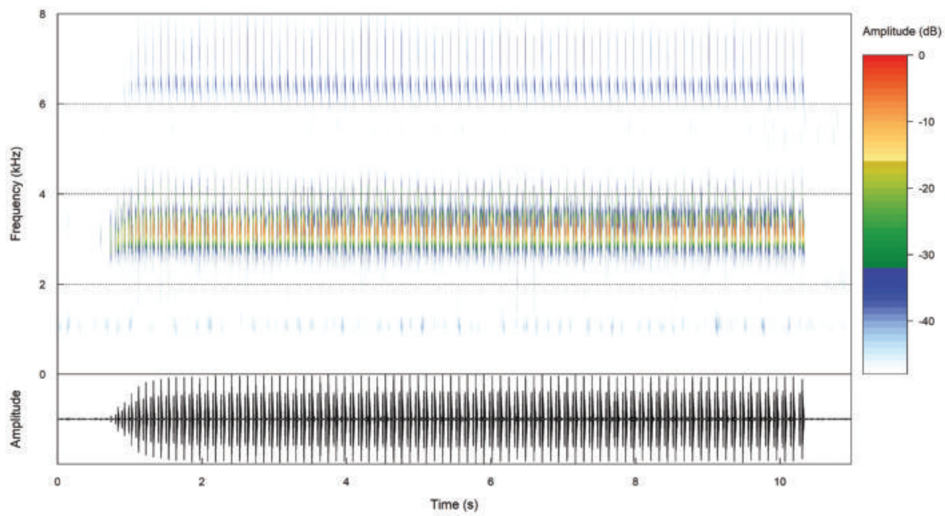


FIG. 5. Audiospectrograms and respective oscillograms of the advertisement call of *R. bergi* from San Luis del Palmar, Corrientes province, Argentina. A complete call (FZ-UNNE 0137, UNNEC 14184).

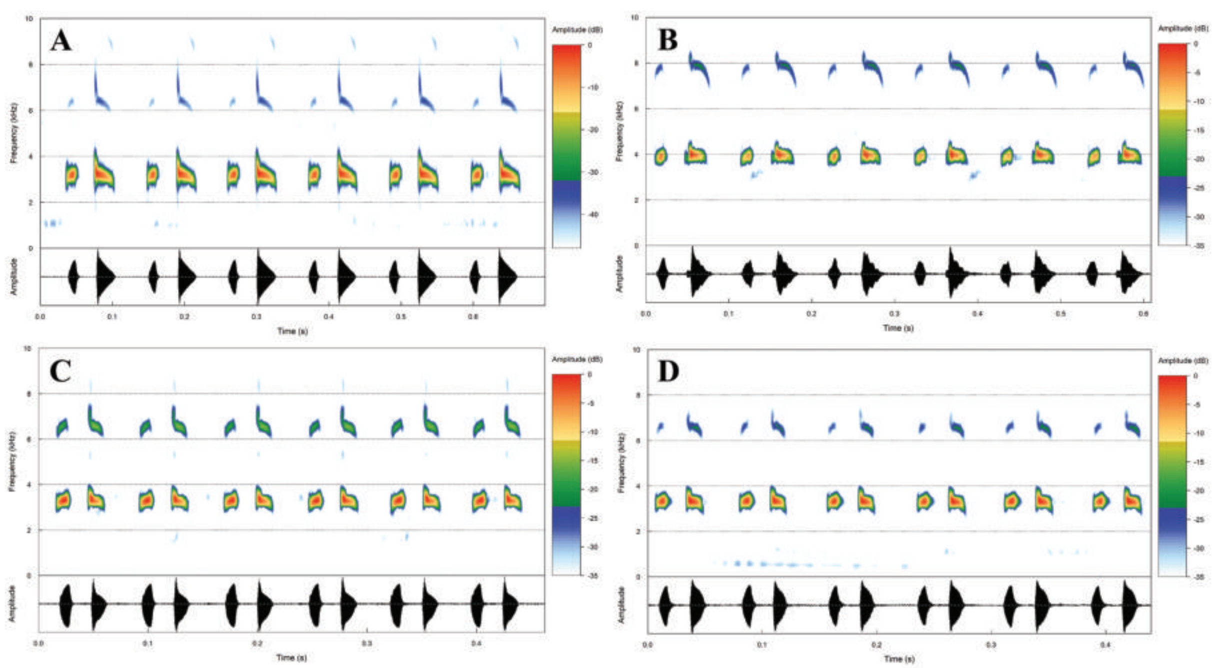


FIG. 6. Audiospectrograms and corresponding oscillograms of six notes from the middle portions of the advertisement calls of males from the four studied populations of *R. bergi*. (A) San Luis del Palmar, (B) La Leonesa, (C) Corrientes, and (D) Paso de la Patria, Argentina.

Table 2. Temporal and spectral features of the advertisement calls of *R. bergi* populations from Argentina evaluated in this study and reviewed in literature. For populations recorded in this study, values of summary statistics are presented as mean ± 1 SD (range). *n* = number of recorded males (analyzed calls). For the remaining population, values were transcribed as provided in the original publications.

| | Guerra et al. (2011) | Present study | | | |
|---|---|---|---|---|---|
| | Vera *n* = 3 (12) | San Luis del Palmar *n* = 4 (30) | La Leonesa *n* = 2 (10) | Corrientes *n* = 1 (2) | Paso de la Patria *n* = 1 (2) |
| Air temp. (°C) | 21–23 | 18–22 | 23 | 27 | 25 |
| Water temp. (°C) | | 22–23 | 22 | 27 | 30.7 |
| Call duration (s) | 15 ± 5.6 (9.9–21.1) | 18.2 ± 10.7 (9.4–51.2) | 14.3 ± 8.2 (5.4–32.9) | 14.4 ± 4.1 (11.5–17.3) | 10.1 ± 2.9 (8–12.1) |
| Call rate (calls/min) | - | 2.7 ± 1.7 (1.2–6) | 3.3 ± 0.8 (2.7–4.4) | 2.9 | 3.5 |
| Call interval | - | 9.6 ± 6.5 (2.7–25.2) | 7.8 ± 5.2 (3.3–16.9) | 4 | 9.2 |
| Frequency 5% (Hz) | - | 3,069 ± 134 (2,885–3,229) | 3,623 ± 96 (3,468–3,703) | 3,057 | 3,100 |
| Frequency 95% (Hz) | - | 3,664 ± 388 (3316–4,780) | 6,023 ± 1,863 (3,890–7,828) | 3,596 ± 31 (3,574–3,617) | 3,617 |
| Dominant frequency (Hz) | 3,828.8 ± 151 (3,653.5–3,923) | 3,350 ± 111 (3,143–3,488) | 3,904 ± 128 (3,656–4,031) | 3,316 ± 61 (3,273–3,359) | 3,359 |
| Peak frequency (Hz) (harmonics) | - | 6,555 ± 209 (6,198–6,809) | 7,746 ± 175 (7,351–7,842) | 6,525 ± 4 (6,522–6,528) | 6,592 ± 7 (6,587–6,598) |
| Notes per call | 144.2 ± 47 (101–194.2) | 181.6 ± 118.1 (84.5–540.3) | 156.1 ± 69.6 (80.9–301.6) | 194.7 ± 55.3 (155.6–233.8) | 136.9 ± 43.1 (106.4–167.4) |
| Note duration (ms) | 62 ± 2.4 (61–65) | 65.1 ± 3.5 (60–74) | 64.1 ± 1.9 (61.1–66.9) | 44.2 ± 0.9 (43.6–44.8) | 40.7 ± 0.3 (40.5–40.9) |
| Note interval (ms) | 40 ± 3.4 (36–43) | 40.8 ± 8 (29.9–56.2) | 40.7 ± 2 (37.9–43.6) | 30.7 ± 0.5 (30.3–31) | 33.7 ± 2.6 (31.9–35.5) |
| Note rate/s | 9.7 ± 0.5 (9.2–10.2) | 9.4 ± 1 (6.7–10.8) | 9.6 ± 0.2 (9.2–9.8) | 13.4 ± 0.05 (13.4–13.5) | 13.6 ± 0.4 (13.3–13.9) |
| Pulse per note | 2 | 2 | 2 | 2 | 2 |
| Pulse rate | - | 31.3 ± 1.3 (28.1–32.8) | 31.2 ± 1 (29.9–32.7) | 45.3 ± 0.9 (44.6–45.9) | 49.2 ± 0.3 (48.9–49.4) |
| Pulse duration | 13 ± 2.9 (11–17) | 20.3 ± 3.3 (15.5–29) | 19.8 ± 1.2 (18.6–21.56) | 14.7 ± 0.8 (14.1–15.2) | 12.3 ± 1.1 (11.5–13.1) |
| Pulse interval | 27 ± 7.5 (18–33) | 32.5 ± 7.1 (17.2–44.3) | 32.5 ± 1.1 (30.5–33.8) | 22.92 ± 0.9 (22.3–23.5) | 23.5 ± 1.9 (22.1–24.8) |

maintained until the end. Four calls were analyzed and found to have, on average, a duration of 9.51 s (± 0.4), spaced by intervals of 23.2 s (± 6.4), and emitted at a rate of 1.85 calls/min. Calls were composed of 387.1 notes (± 21.4) per call with a duration of 19.1 ms (± 0.2), spaced by intervals of 5.1 ms (± 0.1). Notes involved three well-defined pulses and had an amplitude modulation (ascendant at beginning and descendant at end) along their extent, with the central pulse having the highest amplitude in each note (see Fig. 7); the period of the pulse had a duration of 5.8 ms (± 0.2). The dominant frequency was 2,067 Hz, and the 5% and 95% frequencies were 1,722 Hz and 2,433 Hz, respectively.

*Release Call of R.* bergi.—We found and measured the first known harmonics in this type of call for this species (Fig. 8). The release call had an average duration of 5.7 s with an interval of 2.7–3.8 s and was composed of 19–63 non-pulsed notes. Notes had a duration of 20–26 ms, spaced by intervals of 106–132 ms. The dominant frequency was 2,449 Hz. On average, the frequencies of harmonics I and II were 4,895 Hz and 7,501 Hz, respectively.

*Release Call of R.* azarai.—We found calls consisting of groups of notes or isolated notes, including both non-pulsed and pulsed notes (Figs. 9, 10). Additionally, we present the first report of harmonics in the release call of *R. azarai*. Call duration ranged from 0.02–3.7 s, with intervals between 0.5 and 4.1 s. Notes varied from 21.6 to 73 ms in duration, separated by 150–272 ms intervals. Notes had slightly ascending frequency modulation over their duration and irregular amplitude. Pulsed notes were composed of 2–8 non-concatenated pulses. Pulses lasted 4.6–12.6 ms, separated by intervals of 3.8–13 ms. Peaks in dominant frequency ranged between 1,908 and 2,131 Hz. Another frequency band was recognizable at a higher frequency, peaking between 3,572 and 4,286 Hz (3,922 ± 199 Hz).

## Discussion

*Advertisement Call of R.* major.—We found that populations grouped as "Brazil," despite being located in different habitats (river banks and forest borders), exhibited call characteristics that overlapped, with no significant differences between them. Only one individual from Macapá showed an unusually higher

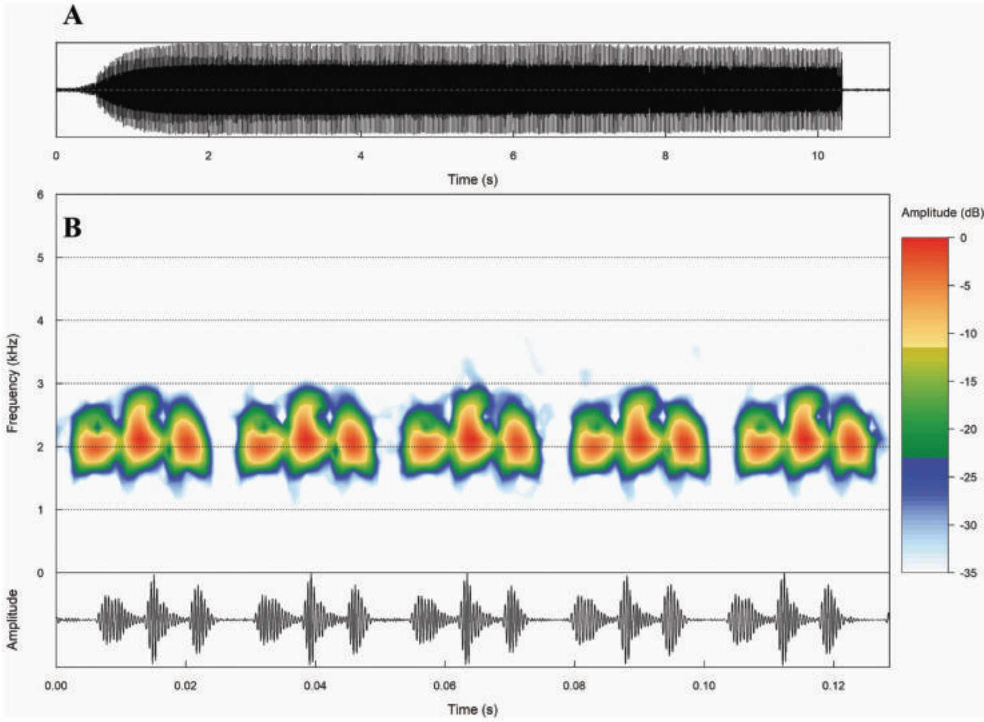


FIG. 7. (A) Oscillograms of the advertisement call of *R. dorbignyi* from San Luis del Palmar, Corrientes province, Argentina. A complete call (FZ-UNNE 0136, UNNEC 14136). (B) Audiospectrograms and corresponding oscillograms of five notes from the middle portion of the advertisement call from Figure 6A, depicting five notes with three pulses per note.

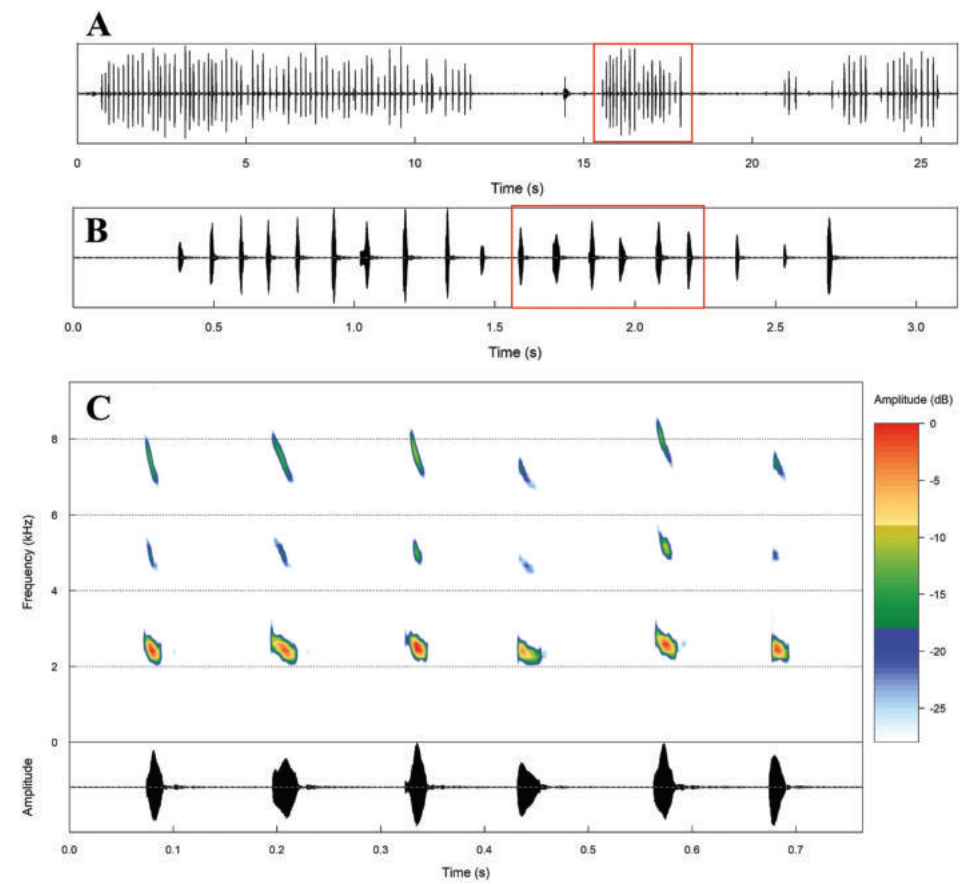


FIG. 8. (A) Oscillogram of three release calls of *R. bergi* from San Luis del Palmar, Corrientes province, Argentina. (B) Oscillogram of an entire release call of the stretch highlighted in red in Figure 7A, detailing 19 notes non-pulsed. (C) Spectrogram (top) and corresponding oscillogram of the note highlighted in Figure 7B, detailing six notes with its harmonics (FZ-UNNE 0139, UNNEC 14183).

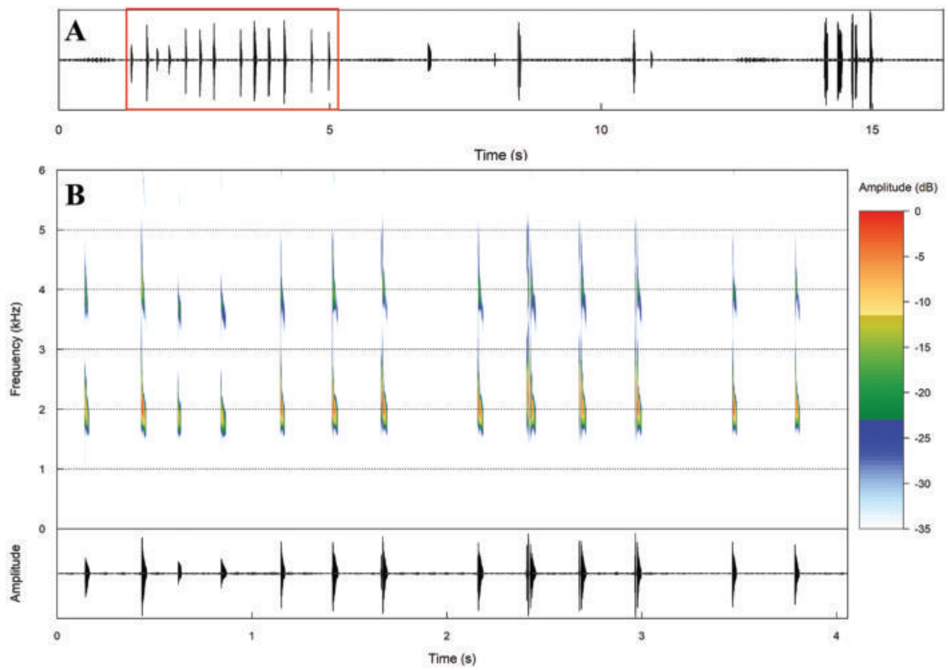


FIG. 9. (A) Oscillogram of a release call of *R. azarai* from Isla Apipé, Corrientes province, Argentina, detailing the existence of isolated notes during the call. (B) Spectrogram (top) and corresponding oscillogram of one release call highlighted in Figure 8A, detailing 13 notes with its harmonics (FZ-UNNE 0149, UNNEC 14189).

interval between calls compared to other records. Previously described calls of *R. major* were similar to ours in terms of call duration, dominant frequency, note interval, and pulses per note (Köhler et al., 1997; Guerra et al., 2011; Bernardes et al., 2015). Note duration and pulse period allowed us to differentiate populations from PN Chaco from our samples from Miraflores and Brazil (Table 1). Despite the relatively short geographic distance (200 km) between Miraflores and PN Chaco, we found statistically significant differences between these populations in five temporal variables. Surprisingly, samples from Brazil, which are geographically more distant (approximately 2,000 km) from both PN Chaco and Miraflores were more similar to the

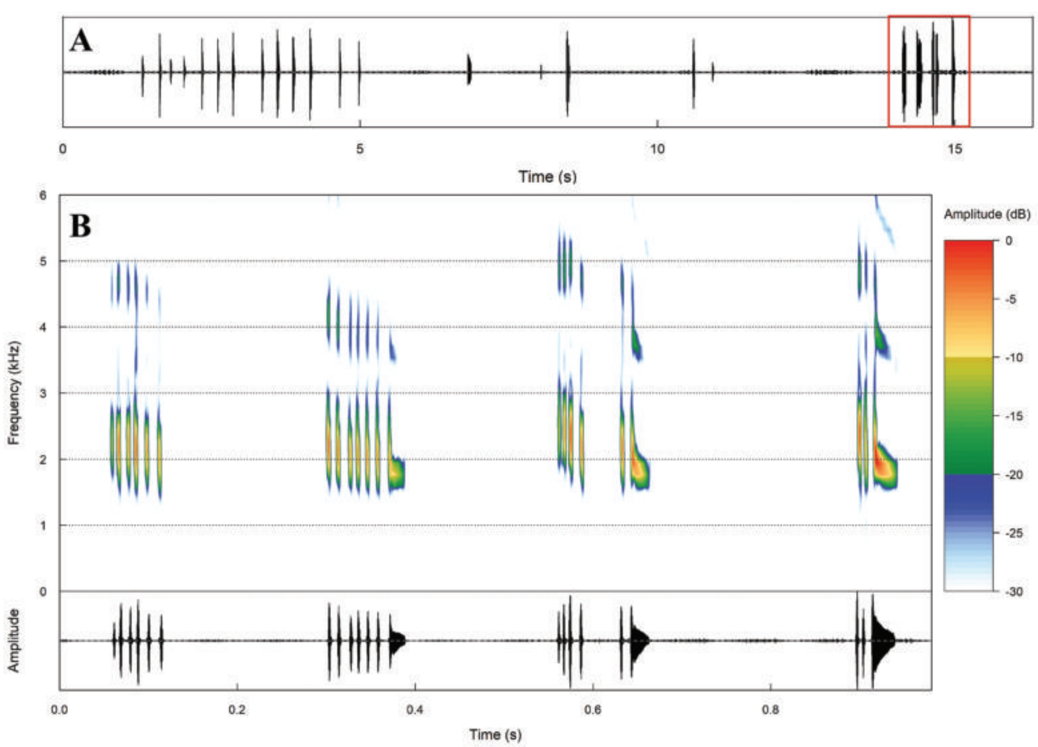


FIG. 10. (A) Oscillogram of a release call of *R. azarai* from Isla Apipé, Corrientes province, Argentina. (B) Spectrogram (top) and corresponding oscillogram of one release call highlighted in A, detailing five notes pulsed (FZ-UNNE 0149, UNNEC 14189).

Table 3. Advertisement call variables of *Rhinella granulosa* group species obtained from this study and the literature. Values of summary statistics are presented as mean ± 1 SD (range). *n* = number of recorded males (analyzed calls). T = temperature, CD = call duration, DF = dominant frequency, ND = note duration, NI = note interval, P/N = pulses per note, PD = pulse duration, PI = Pulse interval. (a) Guerra et al. (2011); (b) Zweifel et al. (1965); (c) Salas et al. (1998); (d) Giaretta et al. (2018); (e) São-Pedro et al. (2011); (f) Torres-Suárez (2013); (g) Zimmerman et al. (1983); (h) Morais et al. (2012); (i) de Carvalho et al. (2013); (j) Köhler et al. (1997); (k) Bernardes et al. (2015); (l) Moreno et al. (2025).

| Species | SVL | T (°C) | CD(s) | DF (Hz) | Notes/call | ND (ms) | NI (ms) | Note/s | P/N | PD (ms) | PI (ms) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *R. azarai* (a) *n* = 2 (9) | 41.6 | 16–21 | 19.3 ± 1.1 (18.6–20.1) | 2,499 ± 37 (2,473–2,525) | 264.4 ± 51.4 (228–301) | 51 ± 15 (40–62) | 24 ± 3.7 (21–26) | 13.7 ± 3.4 (11–16) | 3 | – | – |
| *R. bergi* (a) *n* = 3 (12) | 35.2–36 | 21–23 | 15 ± 5.6 (9.9–21.1) | 3,828.8 ± 151 (3,653.5–3,923) | 144.2 ± 47 (101–194.2) | 62 ± 2.4 (61–65) | 40 ± 3.4 (36–43) | 9.7 ± 0.5 (9.2–10.2) | 2 | 13 ± 2.9 (11–17) | 27 ± 7.5 (18–33) |
| *R. bergi* *n* = 8 (44) | – | – | 16.8 ± 9.9 (5.4–51.3) | 3,475 ± 259.7 (3,143–4,031) | 175 ± 104.5 (80.9–540.3) | 61.9 ± 7 (40.5–71.2) | 40.5 ± 8.6 (21–57.3) | 10 ± 1.4 (8.4–13.9) | 2 | 19.6 ± 3.5 (11.5–29) | 31.6 ± 6.6 (17.2–44.3) |
| *R. centralis* (a) *n* = 1 (2) | – | – | 5.2 ± 0.3 (4.9–5.5) | 2,541 ± 45 (2,500–2,587) | 171.5 ± 11.2 (160–182) | 21 ± 0.7 (20–23) | 9 ± 0.8 (7–10) | 33 ± 0.2 (32.9–33.2) | 4 | 4 ± 0.7 (2–5) | 2 ± 0.5 (1–3) |
| *R. centralis* (b) *n* = 2 (17) | – | 24 | 4.3 (3.5–5.3) | 2,200–3,300 | – | – | – | 32.7 | – | – | – |
| *R. dorbignyi* *n* = 1 (4) | – | – | 9.51 ± 0.36 (9.16–9.89) | 2,067.18 | 387.1 ± 21.4 (367.1–407.1) | 19.1 ± 0.16 (18.9–19.2) | 5.13 ± 0.14 (4.98–5.1) | 40.7 ± 0.7 (40.1–41.3) | 3 | – | – |
| *R. dorbignyi* (a) *n* = 13 (56) | 44.6–55 | 16–20.9 | 8.3 ± 1.5 (6–10.8) | 2,128 ± 104 (1,952–2,349) | 291.7 ± 58.1 (219.2–396.6) | 23 ± 1.4 (20–25) | 5 ± 2.2 (3–10) | 35 ± 2.9 (29.1–37.8) | 3 | 5 ± 0.4 (5–6) | 3 ± 0.6 (3–4) |
| *R. dorbignyi* (a) *n* = 12 (49) | – | 16-23.5 | 8.1 ± 2.8 (5.6–12.8) | 2,043 ± 122 (1,840–2,338) | 313.4 ± 60.6 (183.7–386.4) | 21 ± 4.2 (15–27) | 5 ± 2.2 (2–8) | 40.6 ± 8.1 (29.5–56.5) | 3 | 5 ± 0.4 (5–6) | 3 ± 0.6 (3–4) |
| *R. dorbignyi* (c) *n* = 2 (31) | – | 19–28 | 3.9 ± 0.5 | 2,400 (2,100–3,200) | – | – | – | – | – | – | – |
| *R. granulosa* (d) *n* = 12 (41) | 49.3 | 21–27 | 5.9 ± 1.7 (3.7–9.6) | 2,640 ± 112 (2,472–2,809) | 187 ± 49.7 (121–283) | 21 ± 2 (19–25) | 10 ± 2 (7–13) | 31.8 ± 2.2 (29.2–34.7) | 4.1 ± 0.3 (4–5) | 5 ± 1 (4–7) | |
| *R. granulosa* (e) *n* = 7 (7) | 43.4 | 27–28.6 | 4 ± 0.6 (2.4–6.8) | 2,906 ± 93 (2,497–3,186) | 149 ± 21 (89–278) | – | – | – | – | – | – |
| *R. humboldti* (f) *n* = 6 (18) | 48.7 | – | 2.2 ± 1.9 (1.5–4.8) | 3,153 ± 167 (2,928–3,445) | 100.3 ± 27.1 (49–145) | 22 ± 1 (21–23) | 9 ± 1 (8–11) | – | 4 | 4 ± 0 (3–4) | 2 |
| *R. humboldti* (g) *n* = 1 | 25.4 | 22–25 | – | 2,100 | 135 | – | – | – | – | – | – |
| *R. major* (k) *n* = 4 (21) | – | 28 | 4.2 ± 0.96 (1.4–6.7) | 2.67 ± 0.14 (2.44–3.09) | 84.0 ± 17.5 (28–137) | 40.4 ± 0.7 (32–47) | 9.5 ± 1.9 (4–18) | 20.3 ± 0.6 (19.2–21.8) | 5.8 ± 0.2 (5–6) | – | – |
| *R. major* *n* = 23 (139) | – | 25–30 | 4.9 ± 2.2 (1.4–14.9) | 2,762 ± 218 (2,239–3,186) | 91.5 ± 41.5 (24–250) | 38.9 ± 5.4 (30.9–55.7) | 14.1 ± 3.4 (7.5–21.7) | 19.34 ± 5.2 (4.1–60.4) | 6.5 ± 0.7 (5–8) | – | – |
| *R. major* (j) | 34.5 | 24.5 | 5.3 (4.1–7.5) | 2,960 (2,500–3,900) | 75–133 | | | 18.3 | 6 | – | – |
| *R. major* (a) *n* = 4 (21) | 49.2–55 | – | 5.1 ± 1.6 (3.6–7.2) | 2,725 ± 178 (2,500–2,936) | 84.3 ± 18.5 (61.4–106) | 45 ± 4.7 (42–52) | 15 ± 2.2 (13–18) | 16.8 ± 1.8 (14.3–18.6) | 6.6 ± 0.6 (6–7.3) | 4 ± 0.5 (4–5) | 3 ± 0.7 (2–3) |
| *R. merianae* (d) *n* = 5 (20) | 45.1 | 26–27 | 5.4 ± 1.1 (4–7.1) | 3,036 ± 95 (2,959–3,189) | 193 ± 29.3 (153–217) | 19 ± 1 (17–20) | 7 ± 1 (7–8) | 38.5 ± 0.7 (37.7–39.5) | 3.8 ± 0.4 (3–4) | 5 ± 0 (5–6) | |
| *R. merianae* (a) *n* = 1 (3) | – | 25 | 5.4 ± 1.5 (4–7.1) | 2,315 ± 112 (2,220–2,436) | 213 ± 64.8 (149–283) | 18 ± 1.5 (16–22) | 7 ± 1.3 (4–9) | 39.1 ± 1.1 (27.2–39.9) | 4 | 3 ± 0.9 (2–6) | 1 ± 0.4 (1–2) |
| *R. mirandaribeiroi* (l) *n* = 25 (195) | – | 20–28 | 4.8 ± 1.2 (1.3–8.7) | 2,823 ± 164 (2,250–3,359) | 147.3 ± 37.9 (35–232.5) | 23 ± 3 (17–32) | 10 ± 1 (8–14) | 29 ± 3.5 (20.2–35.3) | 4.1 ± 0.3 (4–5) | – | – |
| *R. mirandaribeiroi* (h) *n* = 2 (10) | 60.5 | 20.4 | 5.3 ± 0.9 (4.2–6.4) | 2,462 ± 67 (2,306–2,538) | 146.3 ± 31.28 (110–194) | 33 ± 3 (29–38) | – | – | 4 | 8 ± 0.9 | – |
| *R. pygmaea* (i) *n* = 1 (8) | 37.7 | 24.9 | 3.9 ± 1.2 (2.1–5.9) | 2,593 ± 43 (2,519–2,643) | 92.3 ± 27.7 (56–114) | 27.6 ± 1.2 (25–32) | 10.7 ± 1.1 (9–13) | 25.9 ± 0.4 (25–26) | 3 | – | – |

advertisement call recorded in Miraflores than to that from PN Chaco (Figs. 3, 4). Considering that samples came from specimens living in very different habitats (Amazon Forest border, dry and humid Chaco), our findings indicate the need for further investigation that includes environmental features (e.g., temperature, humidity, vegetation), calling sites, or body size to elucidate whether there is a factor promoting population isolation that could explain call differentiation.

*Advertisement Call of R.* bergi.—Here, we highlight the presence of harmonics in the advertisement call of *R. bergi* from its type locality (Corrientes, Argentina) and surrounding areas. In all calls from our samples, amplitude between the two pulses differed. However, in Corrientes, this difference was minor. Another notable difference was that individuals from La Leonesa exhibited higher average values in spectral traits compared to other locations.

Our data from San Luis del Palmar and La Leonesa aligned with the call description by Guerra et al. (2011) reported from Vera. Coincidentally, the recordings were made at similar air temperatures (18–23°C). In contrast, calls recorded at 25–27 °C (Corrientes and Paso de la Patria) showed shorter note durations (44.2 and 40.7 ms, respectively), higher note rates (13.4 and 13.6), and shorter interval between pulses (22.92 and 23.5 ms) (Table 2). These differences in acoustic features might be attributed to temperature influences (Fonseca and Revez, 2002) but also to other factors such as call display sites, body size, habitat type, and metabolic condition (Ziegler et al., 2016; Muñoz et al., 2020; Bernardy et al., 2024).

*Advertisement Call of R.* dorbignyi.—*R. fernandezae* was recently synonymized with *R. dorbignyi* (Pereyra et al., 2021). Guerra et al. (2011) reported a detailed description of the calls of both species (as they were considered at that time). Although most ranges of the measured variables overlapped, we identified some distinctions. We report shorter note durations (18.9–19.2 ms) and shorter intervals between notes (4.98–5.1 ms) compared to those reported by Guerra et al. (2011). Our results align with this description and extend the known distribution of the species to a new locality: San Luis del Palmar, Corrientes Province.

Advertisement calls of the *R. granulosa* group, as reported in the literature and in this study, are summarized in Table 3. Call duration exhibits minimal variation, with most species producing calls between 5 and 15 s, except for *R. bergi*, which has the longest call (51 s) and the highest variability. Dominant frequency (Hz) shows substantial variation, ranging from 1,800 Hz in *R. dorbignyi* to 4,000 Hz in *R. bergi*. Notable differences are also observed in note duration, with *R. dorbignyi* (18.9–25 ms), *R. bergi* (40.5–71.2 ms), and *R. pygmaea* (25–32 ms) showing distinct values. However, the most reliable variable for differentiating species within the group is the number of pulses per note: *R. bergi* (2), *R. dorbignyi* (3), *R. mirandaribeiroi* (4–5), and *R. major* (5–8). A related issue concerning release calls is that the amount of pressure applied by the recorder to the male axillae is not standardized across studies.

*Release Call of R.* bergi.—Our study offers the first description of release calls in *R. bergi* that reports the presence of two harmonics. We found differences in note duration compared to values reported by Guerra et al. (2011) and Guerra (2020) due to conflicts in terminology between call characterization in our study and those of previous studies. These authors measured release calls in several *Rhinella* species, describing them as consisting of one note per call. In contrast, we describe release calls as consisting of multiple notes. It is worth noting that both our results and those reported previously are based on a single individual. Future studies providing novel acoustic data would be valuable to further clarify the most appropriate terminology to describe the release call of *R. bergi.*

*Release Call of R.* azarai.—Males of this species produced irregular calls with a variable number of notes (1–13) and pulses (1–8), possibly due to pressure differences during amplexus simulation. Previous studies on several *Rhinella* species (Martin et al., 1971; Guerra et al., 2011; Sanabria et al., 2012) also reported a wide variation in the number of pulses. However, our results differ from theirs in temporal traits of calls due to discrepancies in terminology, as discussed earlier. Additionally, we report the presence of harmonics in the release call of *R. azarai.*

Our results show that acoustic data are important tools for identifying species within the *R. granulosa* species group. For instance, the broadly sympatric species *R. bergi*, *R. dorbignyi*, and *R. major* can be clearly distinguished by advertisement call based on the number of pulses per note (two, three, and five to eight, respectively) or the presence of harmonics (only in *R. bergi*). Moreover, release calls may serve as an alternative option for acoustic data that differentiate species. We were able to differentiate the release calls of *R. bergi* and *R. azarai* by the presence of two harmonics and pulsed notes, respectively. In addition, we emphasize the need for further taxonomic investigation that includes environmental factors and morphology to explain intraspecific acoustic variation.

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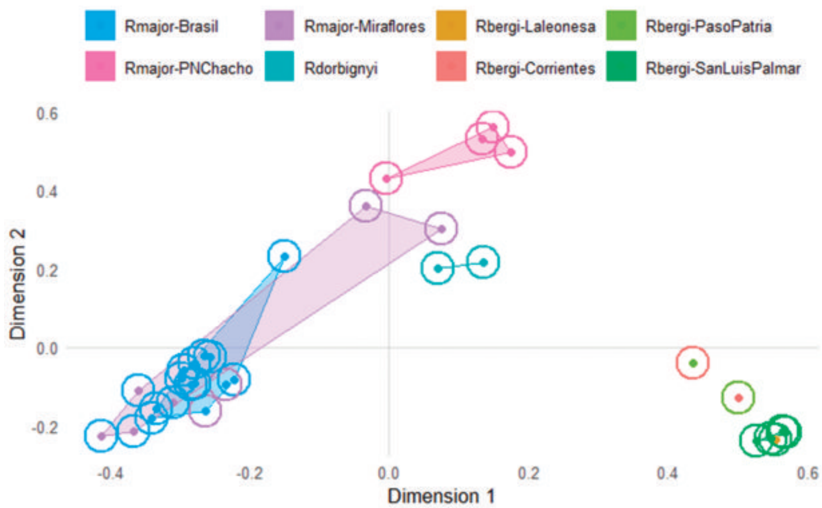
APPENDIX Table 1. List of the analyzed sound files of *R.major, R. bergi, R. dorbignyi,* and *R. azarai*. PA = Pará, AP = Amapá, CH = Chaco, CO = Corrientes, BR = Brazil, AR = Argentina.

| Species | Sound file | Date | Locality (state-country) | Time | Air (°C) | Water (°C) | Voucher specimen |
|---|---|---|---|---|---|---|---|
| *R. major* | Rhinella_majorVitoriaXinguPA1aAAGm661MK2 | 23/1/2020 | Vitória do Xingu (PA-BR) | 20:43 | 27 | - | AAG–UFU 6957 |
| | Rhinella_majorRuropolisPA1aAAGm661MK2 | 23/1/2020 | Rurópolis (PA-BR) | 00:16 | 26 | 27 | Unvouchered |
| | Rhinella_majorRuropolisPA2aPM_AAGm671 | 23/1/2020 | Rurópolis (PA-BR) | 00:52 | 25.5 | 29 | Unvouchered |
| | Rhinella_majorRuropolisPA3aPM_AAGm671 | 23/1/2020 | Rurópolis (PA-BR) | 00:58 | 25.5 | 29 | Unvouchered |
| | Rhinella_majorMacapaAP2aAAGm661MK2 | 26/3/2017 | Macapá (AP-BR) | 20:10 | 26 | 28 | AAG–UFU 5985 |
| | Rhinella_majorMacapaAP3bAAGm661MK2 | 30/3/2017 | Macapá (AP-BR) | 20:38 | 29 | 30 | Unvouchered |
| | Rhinella_majorMacapaAP5aAAGm661MK2 | 31/3/2017 | Macapá (AP-BR) | 19:10 | 28 | 28 | Unvouchered |
| | Rhinella_majorMacapaAP6aAAGm661MK2 | 31/3/2017 | Macapá (AP-BR) | 19:20 | 28 | 28 | Unvouchered |
| | Rhinella_majorMacapaAP7aAAGm661MK2 | 31/3/2017 | Macapá (AP-BR) | 19:28 | 28 | 28 | Unvouchered |
| | Rhinella_majorMacapaAP8aAAGm661MK2 | 31/3/2017 | Macapá (AP-BR) | 19:36 | 28 | 28 | Unvouchered |
| | Rhinella_majorMacapaAP9aAAGm661MK2 | 31/3/2017 | Macapá (AP-BR) | 19:55 | 28 | 28 | Unvouchered |
| | Rhinella_majorMacapaAP10aAAGm661MK2 | 31/3/2017 | Macapá (AP-BR) | 20:04 | 28 | 28 | Unvouchered |
| | Rhinella_majorMacapaAP11aAAGm661MK2 | 31/3/2017 | Macapá (AP-BR) | 20:19 | 28 | 28 | Unvouchered |
| | FZ-UNNE 0359 (Ind. 1) | 13/10/2016 | PN Chaco (CH-AR) | 21:58 | 25 | - | Unvouchered |
| | FZ-UNNE 0362 (Ind. 2) | 13/10/2016 | PN Chaco (CH-AR) | 22:10 | 25 | - | Unvouchered |
| | FZ-UNNE 0363 (Ind. 3) | 13/10/2016 | PN Chaco (CH-AR) | 22:12 | 25 | - | Unvouchered |
| | FZ-UNNE 0364 (Ind. 4) | 13/10/2016 | PN Chaco (CH-AR) | 22:18 | 25 | - | Unvouchered |
| | FZ-UNNE 0687 (Ind. 5) | 8/2/2020 | Miraflores (CH-AR) | 00:01 | 28 | 30 | Unvouchered |
| | FZ-UNNE 0688 (Ind. 6) | 8/2/2020 | Miraflores (CH-AR) | 00:02 | 28 | 30 | Unvouchered |
| | FZ-UNNE 0699 (Ind. 7) | 8/2/2020 | Miraflores (CH-AR) | 00:39 | 28 | 30 | Unvouchered |
| | FZ-UNNE 0942 (Ind. 8) | 1/2/2022 | Miraflores (CH-AR) | 21:13 | 30 | - | Unvouchered |
| | FZ-UNNE 0943 (Ind. 8) | 1/2/2022 | Miraflores (CH-AR) | 21:16 | 30 | - | Unvouchered |
| | FZ-UNNE 0944 (Ind. 8) | 1/2/2022 | Miraflores (CH-AR) | 21:18 | 30 | - | Unvouchered |
| | FZ-UNNE 0945 (Ind. 9) | 1/2/2022 | Miraflores (CH-AR) | 21:28 | 27 | - | Unvouchered |
| | FZ-UNNE 0946 (Ind. 9) | 1/2/2022 | Miraflores (CH-AR) | 21:30 | 27 | - | Unvouchered |
| | FZ-UNNE 0947 (Ind. 9) | 1/2/2022 | Miraflores (CH-AR) | 21:31 | 27 | - | Unvouchered |
| | FZ-UNNE 0949 (Ind. 10) | 1/2/2022 | Miraflores (CH-AR) | 22:08 | 25 | - | Unvouchered |
| | FZ-UNNE 0950 (Ind. 10) | 1/2/2022 | Miraflores (CH-AR) | 22:11 | 25 | - | Unvouchered |
| | FZ-UNNE 0951 (Ind. 10) | 1/2/2022 | Miraflores (CH-AR) | 22:13 | 25 | - | Unvouchered |
| | FZ-UNNE 0952 (Ind. 10) | 1/2/2022 | Miraflores (CH-AR) | 22:16 | 25 | - | Unvouchered |
| *R. bergi* | FZ-UNNE 0138 (Ind. 1) | 22/3/2012 | San Luis del Palmar (CO-AR) | 23:02 | 18 | 23 | UNNEC 14185 |
| | FZ-UNNE 0137 (Ind. 2) | 22/3/2012 | San Luis del Palmar (CO-AR) | 22:49 | 19 | 24 | UNNEC 14184 |
| | FZ-UNNE 0139 (Ind. 3) | 23/3/2012 | San Luis del Palmar (CO-AR) | 09:54 | 25 | - | UNNEC 14183 |
| | FZ-UNNE 0799 | 15/11/2021 | Paso de la Patria (CO-AR) | 21:37 | 25 | 30,7 | Unvouchered |
| | FZ-UNNE 0704 (Ind. 5) | 26/1/2021 | Corrientes (CO-AR) | 21:24 | 27 | 27 | Unvouchered |
| | FZ-UNNE 0292 (Ind. 6) | 19/11/2013 | La Leonesa (CH-AR) | 20:26 | 23 | 22 | UNNEC 14188 |
| | FZ-UNNE 0291 (Ind. 6) | 19/11/2013 | La Leonesa (CH-AR) | 20:26 | 23 | 22 | UNNEC 14188 |
| | FZ-UNNE 0290 (Ind. 6) | 19/11/2013 | La Leonesa (CH-AR) | 20:26 | 23 | 22 | UNNEC 14188 |
| | FZ-UNNE 0288 (Ind. 7) | 19/11/2013 | La Leonesa (CH-AR) | 20:25 | 23 | 22 | Unvouchered |
| | FZ-UNNE 1060 (Ind. 8) | 22/3/2022 | San Luis del Palmar (CO-AR) | 22:17 | 22 | 22 | Unvouchered |
| | FZ-UNNE 1063 (Ind. 9) | 22/3/2022 | San Luis del Palmar (CO-AR) | 22:27 | 22 | 22 | Unvouchered |
| | FZ-UNNE 1064 (Ind. 9) | 22/3/2022 | San Luis del Palmar (CO-AR) | 22:27 | 22 | 22 | Unvouchered |
| | FZ-UNNE 1065 (Ind. 9) | 22/3/2022 | San Luis del Palmar (CO-AR) | 22:29 | 22 | 22 | Unvouchered |
| | FZ-UNNE 1066 (Ind. 9) | 22/3/2022 | San Luis del Palmar (CO-AR) | 22:31 | 22 | 22 | Unvouchered |
| | FZ-UNNE 1067 (Ind. 9) | 22/3/2022 | San Luis del Palmar (CO-AR) | 22:33 | 22 | 22 | Unvouchered |
| | FZ-UNNE 1069 (Ind. 10) | 22/3/2022 | San Luis del Palmar (CO-AR) | 22:41 | 21 | 22 | Unvouchered |
| | FZ-UNNE 1072 (Ind. 11) | 22/3/2022 | San Luis del Palmar (CO-AR) | 22:59 | 21 | 22 | Unvouchered |
| | FZ-UNNE 1073 (Ind. 11) | 22/3/2022 | San Luis del Palmar (CO-AR) | 23:02 | 21 | 22 | Unvouchered |
| | FZ-UNNE 1074 (Ind. 11) | 22/3/2022 | San Luis del Palmar (CO-AR) | 23:05 | 21 | 22 | Unvouchered |
| | FZ-UNNE 1075 (Ind. 11) | 22/3/2022 | San Luis del Palmar (CO-AR) | 23:07 | 21 | 22 | Unvouchered |
| *R. dorbignyi* | FZ-UNNE 0135 | 22/3/2022 | San Luis del Palmar (CO-AR) | 22:38 | 19 | 24 | UNNEC 14135 |
| | FZ-UNNE 0136 | 22/3/2022 | San Luis del Palmar (CO-AR) | 22:43 | 19 | 24 | UNNEC 14136 |
| *R. azarai* | FZ-UNNE 0149 | 19/9/2012 | Isla Apipé (CO-AR) | 20:44 | 10 | 17 | UNNEC 14189 |




Appendix Fig. 1. Plot of the first two axes of a multidimensional scaling analysis on the random forest result for the acoustic data of *R. major* from Brazil, PN Chaco, and Miraflores; *R. bergi* from San Luis del Palmar, Paso de la Patria, Corrientes, La Leonesa; and *R. dorbignyi* from San Luis del Palmar.