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Bioacoustics of the False Toad *Pseudobufo subasper* Tschudi, 1838 from Southeast Asia

Renaud Boistel[1,*], Olivier S.G. Pauwels[2], and Amadeus Plewnia[3]

True toads (Bufonidae) are one of the most species-rich amphibian families, with a nearly global distribution. They are naturally absent only from Australia and Madagascar, where human-mediated introductions of alien bufonids have subsequently filled this biogeographic gap (Pramuk et al., 2008; Shine, 2010; Moore et al., 2015; Frost, 2025). Today, 658 species in 55 genera have been described from this remarkable radiation (Frost, 2025). However, the majority of true toads, particularly paleotropical taxa, have received little attention. As a result, their evolutionary history remains poorly understood (Liedtke et al., 2016; Pereyra et al., 2021; Othman et al., 2022). Data on adult and larval morphology, bioacoustics, ecology, evolution, and natural history are sorely needed to advance integrative bufonid systematics as the baseline for the group's conservation (Padial et al., 2010).

An example is the monotypic genus *Pseudobufo*, for which almost all of this information is lacking, while its phylogenetic position remains debated (Smart et al., 2017; Chan and Grismer, 2019; Portik et al., 2023). *Pseudobufo subasper* is a large, exclusively aquatic toad with extensively webbed feet that occurs in Borneo, Peninsular Malaysia, and Sumatra (Manthey and Grossman, 1997; Inger et al., 2017; Smart et al., 2017; IUCN SSC Amphibian Specialist Group, 2020). While several authors have mentioned observations of calling males (Boulenger, 1912; Inger, 1966; Smart et al., 2017), the species' advertisement call has not been described in detail. We here fill this knowledge gap by providing detailed descriptions of the calls of *P. subasper* based on a captive specimen.

[1] Laboratory Mecadev UMR 7179 CNRS/MNHN, Département Adaptations du Vivant, Bâtiment d'Anatomie Comparée, 55 rue Buffon, 75005 Paris, France.

[2] Royal Belgian Institute of Natural Sciences, Rue Vautier 29, B-1000 Brussels, Belgium.

[3] Biogeography Department, Trier University, Universitätsring 15, 54296 Trier, Germany.

[*] Corresponding author. E-mail: rboistel@gmail.com

Materials and Methods

Call recordings. An adult male (snout–vent length 75.7 mm, measured with digital callipers to the nearest 0.1 mm; Fig. 1) from Kalimantan, Borneo was obtained through the pet trade (La Ferme Tropicale, Paris) and kept in a terrarium in the live animal collection at the Muséum National d'Histoire Naturelle, Paris, France (MNHN) following established husbandry standards (Poole and Grow, 2012). We observed calling activity at night, when the toad was active. Calls were recorded at a distance of 15 cm using omnidirectional microphones (Voice Technologies VT401 and VT 500W, Microtech Gefell MM220) connected to a Nagra Seven digital audio recorder with the following settings: sampling rate 48 kHz, 24-bit dynamic range, frequency response range 20–20,000 Hz.

Call description. The description is based on seven calls, with additional recordings analysed to assess within-individual variation. These original recordings are available at the sonothéque of the MNHN (https://sonotheque.mnhn.fr; accession numbers: MNHN-SO-2024-3531–3541) and in the FONOZOO repository (Fonoteca Zoológica, 2025; code: 14921–31). All recordings were analysed using SASLab Pro v5.3.2 (Avisoft) using the following specifications: FFT (Hamming window size 1024 points; filter bandwidth 46.9 Hz, T = 1/F = 21.3 ms; zero padding at eight and overlap at 95%), spectrum (FFT of call; Hamming window size 32,769 points; filter bandwidth 1.46 Hz, T = 1/F = 683 ms) and FFT of pulse (Hamming window size 2049 points; filter bandwidth = 24.4 Hz, T = 1/F = 42.67 ms), Hilbert transformation for envelope and instantaneous frequency. We visualised oscillograms and spectrograms using Seewave v2.2.3 (Sueur et al., 2008) in the R environment (R Core Team, 2025).

Terminology follows Boistel (2003) and Boistel et al. (2011). Temporal parameters include number of calls (with calls consisting of several tonal pulses), call duration (duration of pulsed calls in ms), inter-call interval (time between calls within a recording in s), number of pulses (single tonal elements of a call),

Figure 1. Adult male *Pseudobufo subasper*. Photo by Renaud Boistel.

pulse duration (duration of tonal pulses in ms), inter-pulse interval (time between pulses within a call in s), call rate and pulse rate (number of calls or pulses per minute respectively), and tempo (overall ratio between call/pulse duration and inter-call/inter-pulse interval in %). Spectral parameters include dominant frequency (frequency that carries the maximal energy in the spectrum in Hz), spectral centroid (weighted mean of the spectrum by magnitude in Hz), energy < 1500 Hz (fraction of total energy allocated in the lower frequency range in %), quality factor (frequency selectivity estimated by the ratio between dominant frequency and bandwidth at –3 dB, no unit), and number of harmonics (count of multiple frequency components above the fundamental frequency). Measurements are provided as mean ± standard deviation with range in brackets.

Results and Discussion

Basic parameters. The recordings showed that the vocalization consists of 1–12 calls (Table 1, Fig. 2). Calls are pulsed with large variation in the number of pulses, ranging from 2–75 (Figs. 2, 3). Within calls, pulses are either of equal intervals or are clustered in groups of 2–6 (Fig. 2). In addition, amplitude shows high variation across calls, being either stable, ascending over the first pulses followed by a decrease, descending over the entire call, or slowly ascending over the entire call.

Call analysis. To assess within-individual variation of the advertisement calls, we analysed 2135 pulses in 60 calls from 11 recordings in detail (Tables 1, 2). The recordings analysed showed a sequence duration of 0.624 s at 4:40 min. The mean call duration was 1.25 ± 0.78 s (range 0.05–3.87) with calls consisting of 34.2 ± 14.9 pulses (2–75). The call rate was 0.20 ± 0.24 calls per minute (0.02–0.76) with a rhythm of 24.9%, separated by inter-call intervals of 16.4 ± 37.0 s (2.4–112.4). Approximately 95.8% of the acoustic energy was concentrated below 1500 Hz, indicating a spectral focus in the lower frequency range. The carrier frequency had a quality factor (Q) of 11 ± 8 (4–60), indicating that it functions as a resonator with minimal low-frequency selectivity. The spectral centre of gravity, representing the energy-weighted mean frequency, was located at 1609 ± 272 Hz (1436–3576). Seven calls, consisting of 209 pulses and a dominant frequency of 1226 ± 111 Hz (656–1934), showed an increase in signal amplitude from first to last call (Fig. 2).

Table 1. Acoustic parameters of the advertisement call of *Pseudobufo subasper*. Column headings include the duration of a call series (Ser Dur) in seconds, the number of calls in that series (Call N°), the average duration of calls in these series (Call Dur) in milliseconds, the inter-call interval (Int Call) in seconds, the number of pulses in the calls (N° Puls), the average pulse duration (Puls Dur) in milliseconds, the inter-pulse interval (Int Puls) in seconds, the call rate (Call Rate) in notes/min, the pulse rate (Puls Rate) in pulses/min, the dominant frequency (Dom Fq) in Hz, the spectral centroid (Spec Cen) in Hz, and the number of harmonics (N° Harm). F1 and F2 indicate harmonic multiples of the fundamental frequency. Values are shown as mean ± standard deviation with ranges in brackets. Recording numbers (Rec No.) are the last four digits of accession codes to the 2024 sonothéque records kept at the Muséum National d'Histoire Naturelle (MNHN-SO-2024).

Rec. No.	Ser Dur	Call Nº	Call Dur	Int Call	Nº Puls	Puls Dur	Int Puls	Call Rate	Puls Rate	Dom Fq	Spec Cen	Nº Harm	F1	F2
3531	1.2	1	1.2		40	9.0 ± 1.2 (6.8–13.6)	29.0 ± 25.7 (8.1–151.8)		34.5	1309	2076	6.0	573	
3532	1.3	1	1.3		37	1.6 ± 4.8 (0.6–29.8)	60.0 ± 338.1 (1.0–20032.2)		27.5	1416	1634	4.0	558	
3533	0.62	1	0.62		28	4.4 ± 0.6 (3.6–6.8)	22.7 ± 8.1 (5.9–45.3)		44.8	1255	1501	8.0	675	802
3534	4.0	3	0.70 ± 0.32 (0.49–1.1)	0.99 ± 0.57 (0.53–1.3)	27.3±18.8 (15–49)	11.7 ± 19.7 (4.8–186.4)	25.6 ± 14.25 (9.3–89.7)	0.76	36.6 ± 8.3 (30.5–46.1)	1277 ± 29 (1257–1311)	1577 ± 7 (1570–1583)	6.0	663 ± 35 (639–688)	
3535	34.2	5	0.41 ± 0.41 (0.05–1.0)	8.0 ± 14.6 (0.5–29.9)	14.4±15.1 (2–39)	1.2 ± 1.9 (0.6–15.8)	71.2 ± 401.5 (0.9–2682.8)	0.15	35.5 ± 3.9 (28.8–38.5)	1256 ± 54 (1225–1352)	1923 ± 924 (1485–3576)	6.0 ± 1.4 (5–8)	625 ± 93 (500–700)	839 ± 115 (719–995)
3536	158.6	6	1.2 ± 0.2 (0.9–1.4)	30.3 ± 18.2 (4.0–51.6)	43.0±5.3 (37–53)	10.1 ± 5.4 (6.3–63.6)	47. 5± 170.5 (9.7–2159.7)	0.04	37.7 ± 4.9 (31.8–42.9)	1323 ± 15 (1305–1343)	1582 ± 33 (1526–1614)	5.0 ± 0.9 (4–6)	572 ± 10 (559–588)	879 ± 81 (822–936)
3537	58.6	6	1.0 ± 0.3 (0.7–1.3)	10.5 ± 12.6 (0.4–29.0)	45.3±11.7 (31–62)	6.1 ± 1.3 (3.7–13.0)	21.5 ± 15.4 (8.0–195.1)	0.10	46.1 ± 5.9 (38.5–52.5)	1276 ± 66 (1221–1400)	1545 ± 31 (1518–1597)	8.7 ± 2.4 (4–11)	576 ± 89 (477–704)	834 ± 33 (806–873)
3538	279.7	6	1.1 ± 0.3 (0.7–1.5)	54.6 ± 85.8 (0.4–198.6)	52.8±12.5 (39–75)	8.7 ± 1.7 (5.3–15.0)	20.4 ± 9.5 (7.9–128.9)	0.02	50.3 ± 8.8 (40.4–63.9)	1275 ± 35 (1224–1309)	1631 ± 36 (1597–1697)	11.7 ± 1.0 (10–13)	582 ± 23 (554–619)	823 ± 15 (812–846)
3539	31.5	8	0.72 ± 0.20 (0.55–1.2)	3.7 ± 7.5 (0.60–20.7)	32.5±10.7 (15–53)	6.6 ± 1.6 (4.0–14.4)	18.6 ± 11.3 (6.1–111.4)	0.25	45.0 ± 9.3 (23.7–54.5)	1254 ± 30 (1225–1306)	1499 ± 34 (1436–1557)	8.6 ± 1.2 (6–10)	630 ± 55 (562–691)	818 ± 16 (801–839)
3540	205.7	11	0.94 ± 0.43 (0.55–2.0)	19.5 ± 34.7 (0.5–106.1)	34.5±11.0 (18–52)	8.9 ± 3.2 (3.7–21.3)	26.9±28.4 (5.9–494.6)	0.05	38.9 ± 8.4 (26.3–49.9)	1276 ± 63 (1205–1441)	1608 ± 32 (1565–1647)	7.7 ± 0.8 (6–9)	480 ± 44 (398–574)	841 ± 66 (752–948)
3541	51.4	12	1.1 ± 1.1 (0.4–4.2)	3.5 ± 8.0 (0.7–27.6)	29.3 ± 11.4 (14–58)	8.0 ± 3.8 (2.9–53.2)	43.8 ± 127.1 (9.0–1379.0)	0.23	36.7 ± 12.7 (4.8–54.9)	1263 ± 32 (1204–1329)	1559 ± 52 (1495–1661)	11 ± 1 (9–12)	575 ± 87 (477–699)	832 ± 50 (719–877)
Means	91.6±99.0 (0.62–279.7)	5.4 ± 3.9 (1–11)	1.3 ± 0.78 (0.05–3.9)	15.7 ± 34.2 (0.4–198.6)	34.2 ± 14.9 (2–75)	8.3 ± 3.4 (2.9–63.6)	30.3 ± 82.0 (5.9–2159.7)	0.2 ± 0.24 (0.2–0.76)	29.7 ± 11.0 (4.2–45.3)	1277 ± 50 (1204–1441)	1609 ± 272 (1436–3576)	11 ± 3 (4–16)	576 ± 79 (468–704)	747 ± 111 (719–995)

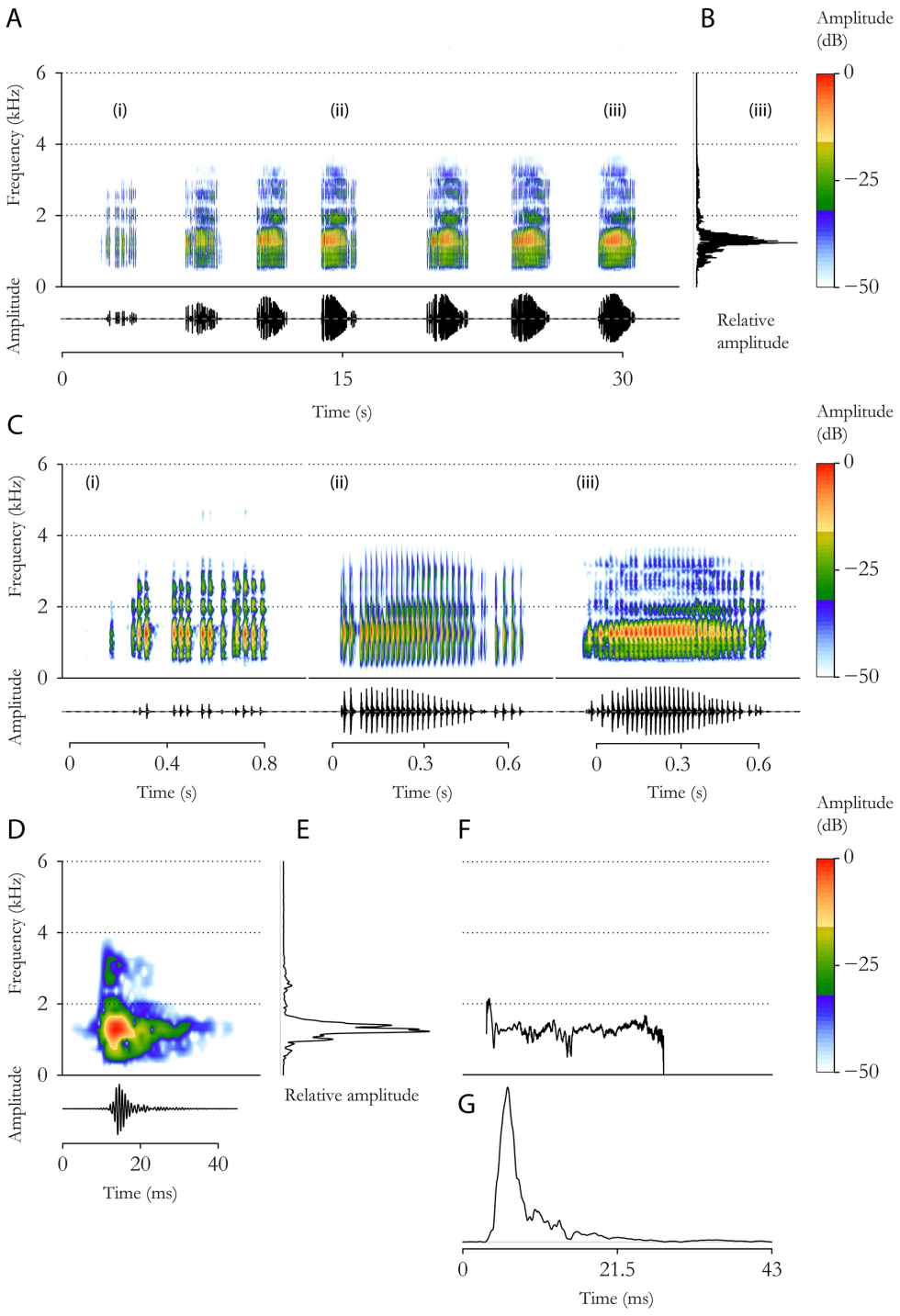

Figure 2. Advertisement call (MNHN-SO-2024-3546/FZ-SOUND-CODE 14929) of *Pseudobufo subasper*. (A) Spectrogram and oscillogram (FFT 1024 points, 46.9 Hz, 21.3 ms; zero padding at 8 points and overlap at 95%). (B) Spectrum of the last call (iii) (FFT 32,769 points, 1.46 Hz, 683 ms). (C) Detailed spectrogram and oscillogram of three calls from (A), indicated by i), ii) and iii). (D) Spectrographic and oscillographic representations of a single pulse extracted from call (iii) (same FFT as A). (E) Spectrum of the same pulse (FFT 2049 points, 24.4 Hz, 42.67 ms). (F) Instantaneous frequency and (G) envelope of a single pulse.

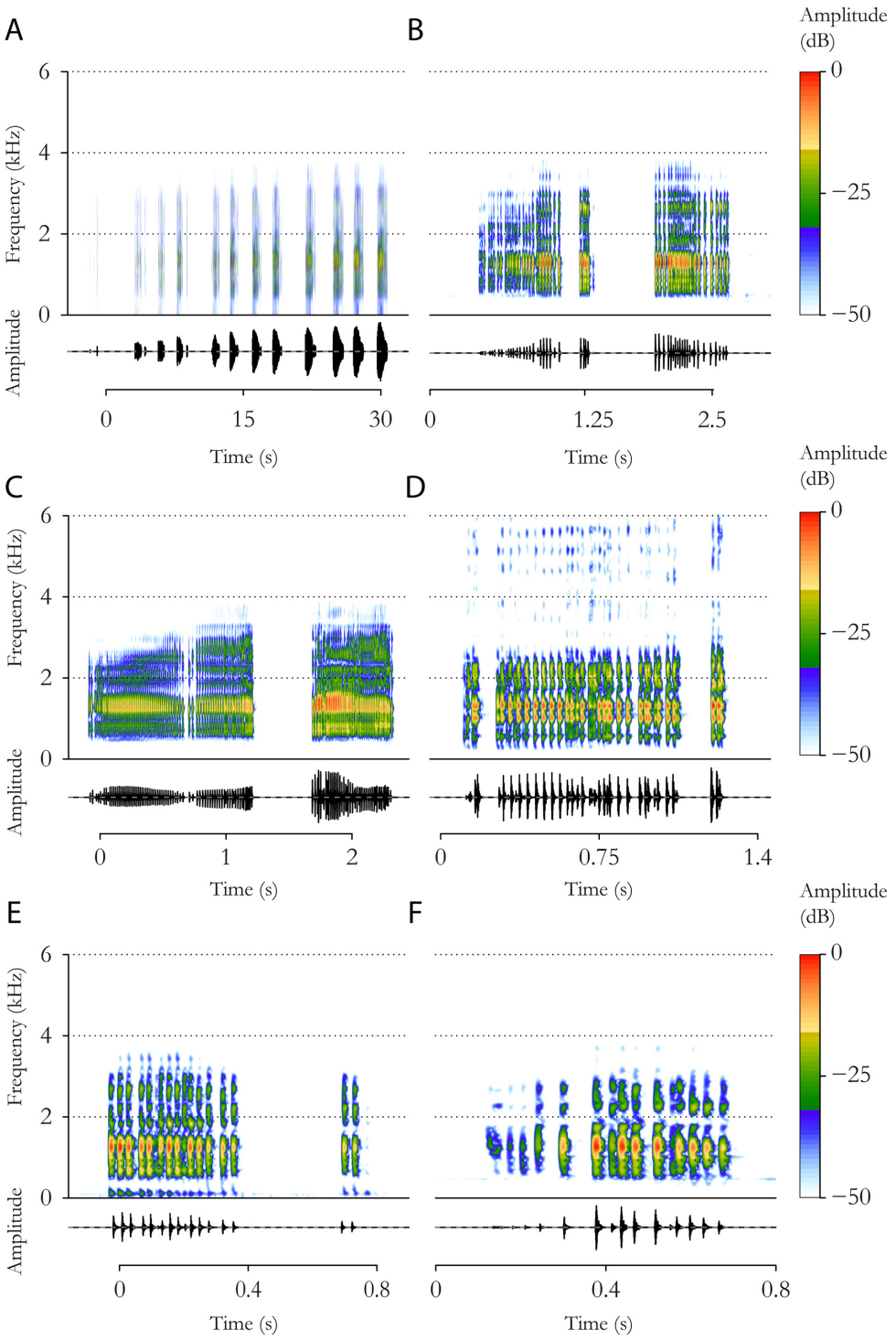

Figure 3. Variation of the advertisement call of *Pseudobufo subasper*. (A–F) Spectrograms (FFT 1024 points, 46.9 Hz, 21.3 ms) and oscillograms with stepwise increased temporal resolution. (A, E) MNHN-SO-2024-3537/FZ-SOUND-CODE 14931. (B) MNHN-SO-2024-3534/FZ-SOUND-CODE 14930. (C) MNHN-SO-2024-3539/FZ-SOUND-CODE 14929. (D) MNHN-SO-2024-3531/FZ-SOUND-CODE 14921. (F) MNHN-SO-2024-3536/FZ-SOUND-CODE 14924).

Table 2. Additional acoustic parameters of the advertisement call of *Pseudobufo subasper*. Column headings include the call tempo (Call Temp) in %, the pulse tempo (Puls Temp) in %, the energy level at < 1500 Hz (E_{1500}) in %, and the quality factor (Qual F). F3–F5 indicate harmonic multiples of the fundamental frequency. Recording numbers (Rec No.) are the last four digits of accession codes to the 2024 sonothéque records kept at the Muséum National d'Histoire Naturelle (MNHN-SO-2024).

Rec No.	Call Temp	Pulse Temp	E_{1500}	Qual F	F3	F4	F5
3531		1.5	90.0	4.0	1019		1310
3532		2.8	94.2	5.6		1172	
3533		20.2	97.9	6.4	996		1255
3534	112.4	38.8 ± 1.0 (38–39.9)	96.9 ± 0.3 (96.5–97.1)	10.9 ± 6.5 (6.8–18.4)	1081 ± 66 (1010–1139)		1285 ± 38 (1263–1329)
3535	6.4	44.8 ± 46.4 (0.6–122)	94.5 ± 7.9 (80.5–98.1)	18.6 ± 23.1 (5.1–59.6)	1055 ± 43 (1017–1103)		1256 ± 54 (1256–1352)
3536	4.6	11.8 ± 6.1 (4.3–19.6)	94.6 ± 0.8 (93.8–95.9)	6.7 ± 1.0 (5.4–7.9)			1323 ± 15 (1305–1343)
3537	11.4	29.7 ± 5.5 (20.8–35.2)	96.7 ± 0.7 (96.2–97.8)	12.5 ± 3.8 (7.7–18.5)	1023 ± 8 (1011–1027)		1232 ± 15 (1214–1247)
3538	2.4	43.2 ± 5.8 (35.5–51.5)	97.4 ± 0.5 (96.8–98.1)	8.7 ± 6.8 (4.8–22.4)	941 ± 34 (907–995)		1280 ± 54 (1217–1360)
3539	22.5	32.6 ± 3.8 (26.1–36.3)	98.2 ± 0.2 (97.8–98.4)	9.5 ± 4.5 (6.2–18.4)	1011 ± 43 (979–1082)	1143 ± 44 (1092–1197)	1250 ± 24 (1225–1280)
3540	5.5	36.2 ± 15.2 (23.2–74.4)	95.7 ± 0.4 (95.3–96.5)	9.8 ± 3.1 (5.2–14.8)	1039 ± 33 (1012–1077)		1261 ± 64 (1205–1441)
3541	33.7	29.0 ± 11.1 (6.4–37.8)	97.5 ± 0.2 (97.2–97.9)	10.2 ± 3.4 (5.9–19.7)	1064 ± 96 (971–1228)		1261 ± 29 (1204–1302)
Means	24.9 ± 37.0 (2.4–112.4)	35.7 ± 17.0 (6.4–122.0)	96.4 ± 2.6 (80.5–98.4)	10.2 ± 7.6 (4–59.6)	1025 ± 80 (907–1228)	1158 ± 43 (1092–1197)	1271 ± 52 (1204–1441)

The instantaneous frequency showed rapid variation (Fig. 2G). In addition, there was considerable variation in call patterns (amplitude, time, frequency) over the course of the calls (Fig. 3).

The pulse envelope was asymmetrical, with a particularly brief attack of 3.15 ms. The shape of the envelope was fairly homogeneous. The mean pulse duration was 8.3 ± 3.4 ms (2.9–63.6), emitted at a rate of 29.7 ± 11.0 pulses/s (4.2–45.3) and a rhythm of 26.1%, and separated by inter-pulse intervals of 30.3 ± 82.0 ms (5.9–2159.7).

Future studies with larger sample sizes could elucidate whether the calls of *P. subasper* exhibit individual or population-level variation, providing further insight into intraspecific bioacoustic signatures. Given the patchy but large distribution of *P. subasper* and the lack of a spatially broad molecular sampling, additional bioacoustic information may help to elucidate the potential presence of uncharacterised diversity in this yet monotypic genus.

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