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Vocalization of the Sullen toad, *Scythrophrys sawayae* (Anura: Leptodactylidae: Paratelmatobiinae), from near its type locality, Paraná, Brazil

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The genus *Scythrophrys* LYNCH, 1971 belongs to the subfamily Paratelmatobiinae within the family Leptodactylidae. It currently is a monotypic genus for the species *Scythrophrys sawayae* (COCHRAN, 1953) (FROST 2020), although SANTOS et al. (2020) recently mentioned a putative second species, *Scythrophrys* sp. from the Serra do Tabuleiro, in Santa Catarina, southern Brazil. To date, *S. sawayae* is known from the Serra do Mar in the Brazilian states of Paraná and Santa Catarina (LOURENÇO et al. 2008, FROST 2020, SANTOS et al. 2020).

Scythrophrys sawayae was described from a single specimen collected in Banhado, Paraná, Brazil, in July 1945, apparently by a student (B. HERTEL) of PAULO SAWAYA from the University of São Paulo. DORIS COCHRAN, herpetologist at the United States Museum of Natural History, upon receiving some frogs from PAULO SAWAYA, noticed a specimen "apparently new to science" and described and named it *Zachaenus sawayae*. The genus *Scythrophrys* was later proposed to accommodate this species (as *Scythrophrys sawayae*), with the name being derived from Greek *scythros* and *phryne*, meaning "sullen toad" (LYNCH 1971).

The locality of Banhado, Paraná, specified by COCHRAN (1953) corresponds to the railway station close to Marumbi, between Piraquara and Morretes, in the state of Paraná (BOKERMANN 1966). This railway line was opened in 1885 and is still used today, connecting the city of Curitiba to the port city of Paranaguá. Banhado Station is located at about 860 m a.s.l. (25°26'17" S, 48°58'43" W).

In the present work, we describe for the first time the vocalizations of *S. sawayae*, which consist of an advertisement call and an aggressive call. Recordings were made in Mananciais da Serra (25°29'46" S, 48°58'58" W, 1000 m a.s.l.), municipality of Piraquara, Paraná, six kilometres in

a straight line from the type locality at Banhado railway station. Mananciais da Serra is a protected area of Atlantic Forest with a transition between Araucaria Forest and Dense Rain Forest (REGINATO & GOLDENBERG 2007).

Vocalizations were recorded from ten males between September and November 2019 with a digital recorder Tascam DR-40 (sampling rate 44.1 kHz, 16-bit resolution, wav format), coupled to a Yoga CSR HT81 microphone, from about 50 cm from the calling male. We endeavoured to locate precisely each male before starting the recording, and collect the calling male. We did not use recordings that could not be attributed to a specific calling male. Eight males were collected as voucher specimens. After each recording session, the snout-vent lengths (SVL) and masses of the collected males were taken with digital callipers (precision \pm 0.05 mm) and a digital scale (precision \pm 0.01 g), and air temperature was measured with a digital thermo-hygrometer (precision \pm 0.1°C). Male specimens were euthanized with 5% xylocaine, fixed in 10% formalin, preserved in 70% ethanol, and later deposited in the Amphibian Collection of the Universidade Tecnológica Federal do Paraná, Francisco Beltrão (collection numbers: RLUTF 430, 431, 432, 472, 491, 492, 730 and 745). Recordings were deposited in the Fonoteca Neotropical Jacques Vielliard, Unicamp, Campinas, Brazil (collection numbers: FNJV 45445-45454).

We analysed 95 advertisement calls and 10 aggressive calls of *S. sawayae* (Supplementary Table S1). Calls were analysed in Raven Pro 1.6 for Windows (Cornell Lab of Ornithology – Bioacoustics Research Program 2013), using FFT (Fast Fourier Transformation) = 256 window width, without filters. For the advertisement calls, the following call parameters were assessed: minimum frequen-

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cy (Hz), maximum frequency (Hz), dominant frequency (Hz), call duration (s), inter-call interval (s), call rate (calls/ min), number of pulses (un), pulse duration (s), pulse rate (pulse/s), and inter-pulse interval (s). For aggressive calls, the same call parameters were measured, except for intercall interval, call rate and pulse rate. The spectral measurements were obtained by selecting three variables in the "choose measurements" option in Raven 1.6: (1) low frequency, (2) high frequency, and (3) peak frequency. Sound figures were obtained using the TuneR version 1.0 (LIGGES et al. 2014) and Seewave version 1.7.3 (SUEUR et al. 2008) of the R platform, version 3.6.3 (R Developmental Core Team, 2016). Oscillograms and spectrograms of advertisement calls were produced with a window size of 512 samples, 90% overlap, and DFT of 512 samples. As for the aggressive call, generating the oscillogram and spectrogram in R with a clear representation of the call structure necessitated the amplification of the original audio (multiplied by two), and a band-pass filter was applied at 1 to 7 kHz to improve the visualization. To assess whether there are correlations between the acoustic parameters and SVL, mass and air temperature, we used Pearson's correlation test, with level of significance of p ≤ 0.05 , performed in PAST version 4.01. All acoustic parameters measured for the advertisement call were tested with SLV, mass and air temperature. We used a call-centred approach, adopting the terminology and definitions suggested by KÖHLER et al. (2017).

Scythrophrys sawayae is a small, terrestrial leaf litter frog. Mean snout–vent length of recorded males was $14.6 \pm$ 0.7 mm (13.6–15.8 mm; N = 8) and mean mass was 0.31 ± 0.07 g (0.2–0.4 g; N = 8). Air temperatures, measured after each recording, varied from 14.4 to 20.7°C, with an average of 16.2 ± 2.3°C.

Advertisement calls were the most frequently emitted calls, and aggressive calls were emitted by males in re-



Figure 1. Spectrogram and oscillogram of advertisement call of *Scythrophrys sawayae*. Male recorded on 18 November 2019, voucher RLUTF 730, 0.2 g, 13.7 mm, 23:25 h, 14.9°C.

Table 1. Numerical parameters of the vocalizations of *Scythrophrys sawayae* at Mananciais da Serra, Paraná, Brazil.

Call parameter	Advertisement call	Aggressive call
	$X \pm SD$	$X \pm SD$
	(Min-Max)	(Min-Max)
	calls / males	calls / males
Minimum frequency (Hz)	3020.45 ± 391.61	2424.00 ± 356.82
	(2296.88-3701.42)	(1948.96-3085.86)
	95 / 10	10 / 4
Maximum frequency (Hz)	5245.83 ± 244.30	4971.66 ± 301.40
	(4926.55-5830.65)	(4168.62-5305.51)
	95 / 10	10 / 4
Dominant frequency (Hz)	4293.20 ± 238.28	3807.79 ± 234.47
	(3979.34-4806.21)	(3789.84-4306.64)
	95 / 10	10 / 4
Call duration (s)	0.24 ± 0.05	0.29 ± 0.129
	(0.15 - 0.30)	(0.162-0.492)
	95 / 10	10 / 4
Call rate (calls/min)	68 ± 19	
	(40-103)	_
	10	
Inter-call interval (s)	0.80 ± 0.30	
	(0.41 - 1.26)	_
	95 / 10	
Pulse number	7.51 ± 1.78	58.9 ± 20.78
	(3-13)	(19-80)
	30 / 10	10 / 4
Pulse duration (s)	0.007 ± 0.001	0.0041 ± 0.0008
	(0.004 - 0.008)	(0.002-0.006)
	30 / 10	10 / 4
	10.83 ± 3.9	
Pulse rate (pulse/s)	(6.10-17)	
	30 / 10	-
Inter-pulse interval (s)	0.04 ± 0.03	0.004 ± 0.001
	(0.02 - 0.11)	(0.001 - 0.01)
	30 / 10	10 / 4

sponse to advertisement calls from other males in dense aggregations. Since we do not have more observations on the social context of these aggressive calls, and as there are no previous studies on territoriality or physical combat, we abstain from speculating on more detailed functions of these calls. Further studies should be conducted to confirm the function of the call we here refer to as the aggressive call (compare KÖHLER et al. 2017)

The advertisement call consists of a single pulsed note with ascending frequency modulation. It is a short call, with a duration of 0.24 ± 0.05 s, a high call rate of $68 \pm$ 19 calls/min, and inter-call intervals of 0.80 ± 0.30 s. Each note consists of 7.51 \pm 1.78 pulses, with a pulse duration of

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 0.007 ± 0.001 s, inter-pulse intervals of 0.04 ± 0.03 s, and a pulse rate of 10.83 ± 3.9 pulses/s. Regarding its spectral parameters, the call has a high pitch, with a dominant frequency of 4293.20 \pm 238.28 Hz, and a bandwidth of 2296–



Figure 2. Spectrogram and oscillogram of aggressive call of *Scythrophrys sawayae*. Male recorded on 25 September 2019, voucher RLUTF 432, 0.3 g, 15.4 mm, 20:39 h, 14.4°C.

5831 Hz (Table 1, Fig. 1). None of the measured acoustic parameters was correlated with SVL, mass or air temperature (p > 0.05).

Only four recorded males sporadically emitted aggressive calls. Aggressive calls consist of a multipulsed note with ascending frequency modulation, ascending amplitude modulation, and a strong terminal increase in amplitude on the last two or three pulses. Duration of the call is 0.29 ± 0.13 s. Each note consists of 58.9 ± 20.78 pulses, with a pulse duration of 0.0041 ± 0.0008 and an interpulse interval of 0.004 ± 0.001 . Inter-call interval, call rate and pulse rate were not measured because these calls were emitted only sporadically and amid advertisement calls. Their dominant frequency was 3807.79 ± 234.47 Hz, with a bandwidth of 1948.96-5305.51 Hz.

Males of *Scythrophrys sawayae* were observed vocalizing in the leaf litter, usually from under leaves (Fig. 3), which sometimes renders it difficult to locate them. they vocalized at night, in choruses of 10 to more than 30 individuals. As mentioned above, their advertisement calls have a high call rate, and vocalizations could be heard almost continuously throughout the night.

Temperature information, mass and size of recorded males constitute crucial information to be added in the descriptions of vocalizations, as these variables may strongly influence various acoustic parameters. (e.g., GUIMA-



Figure 3. Adult male Scythrophrys sawayae on leaf litter in its natural habitat.

RÃES & BASTOS 2003, LINGNAU & BASTOS 2007, TOLEDO & HADDAD 2009, FORTI et al. 2017). Particularly in the case of cryptic species, it is important that a large number of recordings, obtained under different environmental conditions, be available for analysis (e.g., PADIAL et al. 2008, GLAW et al. 2010, MONTEIRO et al. 2014).

Scythrophrys is a monotypic genus in the subfamily Paratelmatobiinae, limiting the options for comparisons with calls of related species. In addition to *Scythrophrys*, this subfamily includes *Crossodactylodes* (5 species), *Paratelmatobius* (7 species), and *Rupirana* (1 species) (FROST 2020, SANTOS et al. 2020). Recently, SANTOS et al. (2020) constructed a phylogeny of the subfamily based on three mitochondrial and five nuclear markers, recovering Paratelmatobiinae and each of its four genera as monophyletic. Our description of the vocalizations of *S. sawayae* may help, in the future, to distinguish it from newly discovered cryptic species.

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Supplementary data

The following data are available online:

Supplementary Table S1. Raw dataset of call parameters of each individual *Scythrophrys sawayae* recorded in the field.