The Herpetological Bulletin 163, 2023: 31–34
SHORT NOTE

https://doi.org/10.33256/hb163.3134

After 160 years of 'silence': the advertisement call of the frog *Ischnocnema verrucosa*

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INTRODUCTION

Anurans may communicate using different signals (e.g. acoustic, chemical, seismic, tactile and visual) in isolation or in a multimodal context (Starnberger et al., 2014). Vocal communication is a conspicuous trait displayed in reproductive, defensive and agonistic social contexts (Toledo et al., 2015; Ferreira et al., 2019a). Advertisement calls are species specific and are emitted for mate attraction, thus making them useful as a taxonomic tool (Köhler et al., 2017). However, basic information about the vocal repertoire of many species of anurans is still lacking, which hampers species delimitation.

Ischnocnema verrucosa Reinhardt & Lütken, 1862, is endemic to Brazil and restricted to the south-east of the country. *Ischnocnema* comprises 39 species in six species series (*I. guentheri*, *I. lactea*, *I. parva*, *I. ramagii*, *I. venancioi* and *I. verrucosa*), there are three species unassigned to any series (*I. manezinho*, *I. nanahallux* and *I. sambaqui*), and many unnamed species (Canedo & Haddad, 2012; Taucce et al., 2018; Thome et al., 2020). The genus is a taxonomic problem with many populations not identified to the species level (e.g. Mascarenhas et al., 2015; Santos-Pereira et al., 2016; Comitti, 2017; Fiorillo et al., 2018; Ferreira et al., 2019b; Zornosa-Torres et al., 2020; Monteiro & Cremer, 2021).

These identification uncertainties within *Ischnocnema* usually relate to poor species diagnoses, the cause of which includes a lack of bioacoustics information. Indeed, 14 (36 %) species of *Ischnocnema* still lack description of their vocal repertoire. Among species belonging to the *I. verrucosa* series, *I. abdita*, *I. bolbodactyla*, *I. juipoca*, and *I. penaxavantinho* have known calls (Sazima & Cardoso, 1978; Haddad et al., 1988; Pombal & Cruz, 1999; Giaretta et al., 2007; Bastos et al., 2015; Rocha et al., 2017; Assis et al., 2018). However, *I. octavioi*, *I. surda* and *I. verrucosa* have unknown calls and taxonomic problems. Canedo et al. (2010) did not find a morphological character to differentiate *I. octavioi* from *I. verrucosa*. According to Silva et al. (2013), the specific status between *I. surda* and *I. verrucosa* needs to be reassessed because the main diagnostic character seems to vary in a clinal manner. Furthermore, Canedo and Haddad (2012) found *I. verrucosa* paraphyletic with respect to *I. octavioi*, reinforcing doubts about the specific limits in this species complex.

The type locality of *I. verrucosa* is the Municipality of Juiz de Fora, State of Minas Gerais, south-eastern Brazil and is also the species' southernmost locality. In addition, *I. verrucosa* has been reported from the western portion of Minas Gerais state (Moura et al., 2012; Silva et al., 2013; Zornosa-Torres et al., 2020), southern and central Espírito Santo state (Tonini et al., 2010; Montesinos et al., 2012; Ferreira et al., 2019b), and southern Bahia state (Orrico, 2010; Freitas et al., 2011; 2019). Herein we describe the advertisement call of *I. verrucosa* for the first time, 160 years after its morphological description, and compare vocal traits across *Ischnocnema* species.

MATERIALS & METHODS

We analysed 49 calls emitted by four males of *Ischnocnema verrucosa* recorded at the Augusto Ruschi Biological Reserve, Municipality of Santa Teresa, State of Espírito Santo, south-eastern Brazil: MBML 11814 recorded after playback stimuli at 09:55 h, 6 July 2019, 19° 53'54'' S, 40° 32'42'' W, air temperature 18 °C; MBML 11685 and two unvouchered specimens recorded without playback stimuli at ca. 07:00 h, 27 September 2019, 19° 54'27'' S, 40° 32'12'' W, air temperature 19 °C.

We recorded using a Tascam DR-40 (with internal microphone) at a sampling rate of 48 kHz and 24 bit resolution. We analysed spectral and temporal parameters using Raven Pro 1.6.1 software from the Cornell Laboratory of Ornithology (Center for Conservation Bioacoustics, 2019). Spectral information was accessed with Fast Fourier Transformation (FFT) size of 512 and overlap of 85.2 %. Sound figures were produced using seewave and tuneR packages in R (R Core Team, 2020): hamming window type, FFT size of 512 and overlap of 90 %. We followed the bioacoustics terminology and the call-centered approach

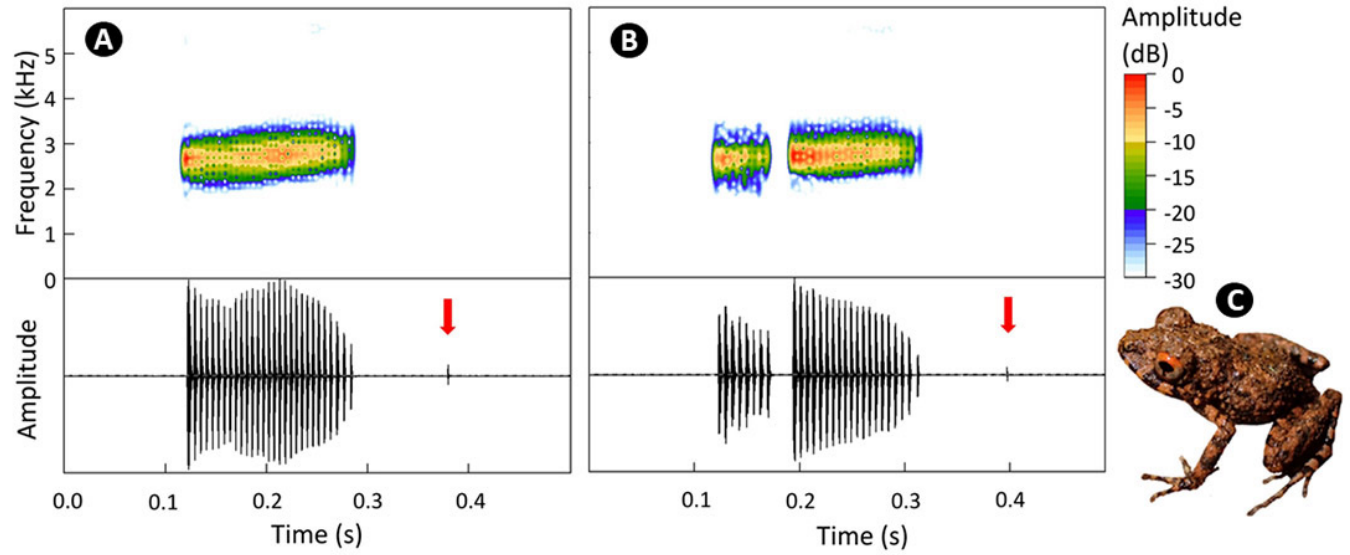

Figure 1. Spectrogram (top) and oscillogram (bottom) of the advertisement call of *Ischnocnema verrucosa* recorded at the Municipality of Santa Teresa, State of Espírito Santo, south-eastern Brazil - **A.** Note with pulses not arranged in pulse groups, **B.** Note arranged in two pulse groups, and **C.** A recorded male (MBML 11685 / FNJV 59028). Red arrow indicates isolated pulses with lower amplitudes.

from Köhler et al. (2017) (i.e. uninterrupted units are treated as calls whenever they are isolated by long silent intervals). We considered the calls as a single note with pulses arranged in pulse groups when the silence between them was more than twice as long as the pulse duration. Values are presented as minimum–maximum (mean ± standard deviation).

Our research protocol was approved by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, Permit Number: 63575–5). Vocal recordings were deposited at the Fonoteca Neotropical Jacques Vielliard (FNJV 59028 and 59029).

RESULTS

The call of *Ischnocnema verrucosa* (Fig. 1) consists of one note with a duration of 0.097–0.319 s (0.19 ± 0.04; n = 49), 14–62 pulses (34 ± 9.6; n = 49) arranged in 1–3 pulse groups per note (1.8 ± 0.6; n = 49) and emitted at a rate 135.5–193.9 pulses/s (171.8 ± 15.9; n = 49). Calls had a peak frequency of 2583.9–3014.6 Hz (2709.6 ± 88.8; n = 48), frequency 5 % 2239.4–2497.8 Hz (2338.9 ± 60.8; n = 45) and frequency 95 % 2928.5–5770.9 Hz (3366.3 ± 658.8; n = 48). An isolated pulse with lower amplitude was emitted after 0.01–0.12 s (0.07 ± 0.02; n = 48) in 48 of the 49 analysed calls (Fig. 1).

DISCUSSION

The Municipality of Santa Teresa is ca. 350 km north-east from the type locality (Municipality of Juiz de Fora) of *Ischnocnema verrucosa*. Although the calls herein reported were not recorded from topotype specimens, they belong to the same population sampled to recover the phylogenetic position of *I. verrucosa* by Canedo & Haddad (2012). They included three specimens of *I. verrucosa* to investigate the molecular phylogeny of *Ischnocnema* (same as Taucce et al., 2018) of which two were from our location at Santa Teresa (MNRJ 34899 and 34900) and one from the Municipality of Camacan, State of Bahia (CFBH 23685); therefore, the phylogenetic position of topotypes of *I. verrucosa* has not been tested. Neves et al. (2017) recently reported *I. verrucosa* from five different sites at the type locality. However, after sampling these areas for five years (2006–2011), *I. verrucosa* was not observed calling (Neves & Varela-Rios, pers. comm).

Vocal parameters have been widely used for diagnosing species within *Ischnocnema* (Taucce et al., 2012; 2018). However, the genus still lacks acoustical evaluation in an evolutionary approach to improve homology criteria. Bioacoustic traits have not been used to diagnose any of the six species series. Rocha et al. (2017) stated that a general bioacoustical pattern for the *I. verrucosa* series is unclear. However, within the *I. verrucosa* series, acoustic traits seem to relate to genetic lineages and morphological similarities. The advertisement call of *I. verrucosa* differs from those of the *I. juipoca* + *I. penaxavantinho* lineage by its lower number of notes per call (5–19 notes per call combining vocalisation of *I. juipoca* and *I. penaxavantinho*; see Giaretta et al., 2007; Bastos et al., 2015; Assis et al., 2018) and from those of the *I. abdita* + *I. bolbodactyla* lineage by its lower number of pulses per note (2–4 pulses per note combining calls of *I. abdita* and *I. bolbodactyla*; see Pombal & Cruz, 1999; Rocha et al., 2017).

Among the other species of *Ischnocnema*, the advertisement call of *I. verrucosa* differs from *I. sambaqui*, *I. manezinho*, *I. randorum*, *I. nigriventris*, *I. bocaina*, *I. lactea*, *I. parnaso*, *I. colibri*, *I. feioi*, *I. garciai*, *I. oea*, *I. gualteri*, *I. guentheri*, *I. henselii*, *I. izecksohni*, and *I. nasuta* by its shorter duration (0.5–41.9 s combined values from these species). The advertisement call of *I. verrucosa* differs from *I. vizottoi* and *I. melanopygia* by its longer duration (0.01–0.072 s combined values from these species). The advertisement call of *I. verrucosa* differs from *I. sambaqui*, *I. manezinho*, *I. nigriventris*, *I. vizottoi*, *I. bocaina*, *I. concolor* and *I. melanopygia* by its pulsed structure (unpulsed structure in these species). The advertisement call of *I. verrucosa* differs from *I. parnaso*, *I. parva* and *I. colibri* by its higher pulse rate (16.55–44.87 pulses/s combined values from these species). Finally, the advertisement call of *I. verrucosa* differs

from *I. sambaqui*, *I. manezinho*, *I. randorum*, *I. nigriventris*, *I. bocaina*, *I. feioi*, *I. garciai*, *I. oea*, *I. gualteri*, *I. guentheri*, *I. henselii*, *I. izecksohni* and *I. nasuta* by its lower number of notes (2–170 notes per call in these species). For further information on the advertisement call of these species, see Heyer (1985), Castanho & Haddad (2000), Martins & Haddad (2000), Berneck et al. (2013), Andrade et al. (2017), Silva-Soares et al. (2018), Taucce et al. (2018a;b), Forti et al. (2019), and Taucce et al. (2019).

Among species of the *I. verrucosa* series, only *I. octavioi* and *I. surda* still lack descriptions of their vocalisation. *Ischnocnema octavioi* is the sister taxon of *I. verrucosa* (Canedo & Haddad, 2012; Taucce et al., 2018) and *I. surda* is tentatively closely related to this lineage based on morphological similarities (see Canedo et al., 2010; Dantas & Ferreira, 2010; Silva et al., 2013; Holer et al., 2017). A taxonomic review dealing with these three species is required to access whether they represent distinct valid species.

ACKNOWLEDGEMENTS

We thank the Projeto Bromélias for logistical support sponsored by Rufford Foundation. Instituto Nacional da Mata Atlântica (INMA) and Reserva Biológica Augusto Ruschi for logistical support. APA thanks Coordenação de Aperfeiçoamento de Pessoa de Nível Superior (CAPES), JVAL (380682/2019–2 and 300962/2022-3), CZ (300929/2022-6), and RBF (317799/2021-5) thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for research scholarship.

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Accepted: 5 November 2022