

Geographical distribution extension and notes on vocalisations of *Ischnocnema penaxavantino* Giaretta, Toffoli & Oliveira, 2007 (Anura: Brachycephalidae)

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INTRODUCTION

The frog genus *Ischnocnema* Reinhardt & Lütken, 1862 comprises 33 species (Frost, 2014), of which 31 are distributed in four species series (*I. guentheri*, *I. lactea*, *I. parva* and *I. verrucosa*). Two further species (*I. manezinho* and *I. sambaqui*) are not allocated in the series (Padial et al., 2014). Currently, there is a gap in knowledge about the vocalisations of frogs in the genus *Ischnocnema*, in which less than 30% have an advertisement call described (Sazima & Cardoso, 1978; Castanho & Haddad, 2000; Giaretta et al., 2007; Oliveira et al., 2008; Conte et al., 2010; Martins & Haddad, 2010; Pombal, 2010; Taucce et al., 2012; Berneck et al., 2013) and only two species have aggressive calls known (Conte et al., 2010; Berneck et al., 2013).

Ischnocnema penaxavantino Giaretta, Toffoli & Oliveira, 2007 is a species from the Cerrado domain and it was previously reported only from the State of Minas Gerais (Giaretta et al., 2007). It is closely related to *I. juipoca* and both may be confounded (Giaretta et al., 2007). *I. juipoca* has been recorded in the Floresta Nacional de Silvânia, State of Goiás, Brazil (Bastos & Pombal, 2001; Bastos et al., 2003; Morais et al., 2012). However, following taxonomy in Giaretta et al. (2007), these specimens may represent *I. penaxavantino*, and not *I. juipoca*. *I. juipoca* and *I. penaxavantino* can be differentiated by size and vocalisation (Giaretta et al., 2007). *I. penaxavantino* is smaller than *I. juipoca*, and its advertisement call is longer, has more pulses, and has higher pulse repetition rate (Giaretta et al., 2007). Herein, we present data about the vocalisations of a specimen collected at the Floresta Nacional de Silvânia, which also allows us to identify specimens in this reserve as *I. penaxavantino*.

METHODS

We studied *I. penaxavantino* (Fig. 1A) at the Floresta Nacional (Flona) de Silvânia (16°39'32" S, 48°36'29" W; about 900 m a.s.l.), Municipality of Silvânia, State of Goiás, Central Brazil. We recorded vocalisations (wav file; 44 kHz; 16 bit) of an *I. penaxavantino* male in April 2009 using a Marantz PMD 660 recorder coupled with a Sennheiser ME66 microphone.

The vocalisations were analysed with Cool Edit 96 and Avisoft-SASLab Lite® software. Frequency information was obtained through Fast Fourier Transformation (FFT) (width, 1,024 points). The audiospectrograms and oscillograms were created in overlap (90%) and Window (Flat Top) with the use of package R 3.0.1, Seewave 1.6.4 (Sueur et al., 2008).

We recorded two types (A and B) of vocalisations emitted by *I. penaxavantino* from Flona de Silvânia. Call descriptions followed Gerhardt (1998), Gerhardt & Huber (2002), and Wells (2007). The following acoustic variables were measured: call duration (s), pulse number (pulse/call), pulse duration (ms), dominant frequency (Hz), time interval between calls (s), and call repetition rate (calls/min). We used a Student's t-test to compare the acoustic parameters (call duration, pulse number, pulse duration, and dominant frequency) of the type A and B calls of *I. penaxavantino* from the Flona de Silvânia. We tested for normality within data and homogeneity of variances prior to statistical analysis using Levene's test and, where necessary, calculated t-tests with separate variance estimates (Zar, 1996). We collected the recorded individual (collecting permit number: 15377-4 / Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio) and housed at the Coleção Zoológica da Universidade Federal de Goiás (ZUFG 5267).

RESULTS AND DISCUSSION

The type A call is similar to advertisement calls (Fig. 1B) described by Giaretta et al. (2007). These calls were emitted at irregular intervals that varied from 15.63 to 149.14 s (45.07 ± 47.88 s; N = 8 calls) in which two calls were emitted per minute (N = 1 male). The amplitude of calls increased gradually. The average pulse per call varied from 15 to 18 (17.16 ± 1.33 ; N = 8 calls) with a pulse duration ranging from 0.014 to 0.032 ms (0.0215 ± 0.008 ms; N = 24 pulses). Call duration and dominant frequency varied, respectively, from 0.63 to 0.845 s (0.793 ± 0.068 s; N = 8 calls) and 3385 to 3564 Hz (3455.83 ± 60.35 Hz; N = 8 calls).

We recorded a second type of call (Type B call) emitted for *I. penaxavantino* males. This vocalisation was emitted in response to other males. The type B call (Fig. 1B) is shorter

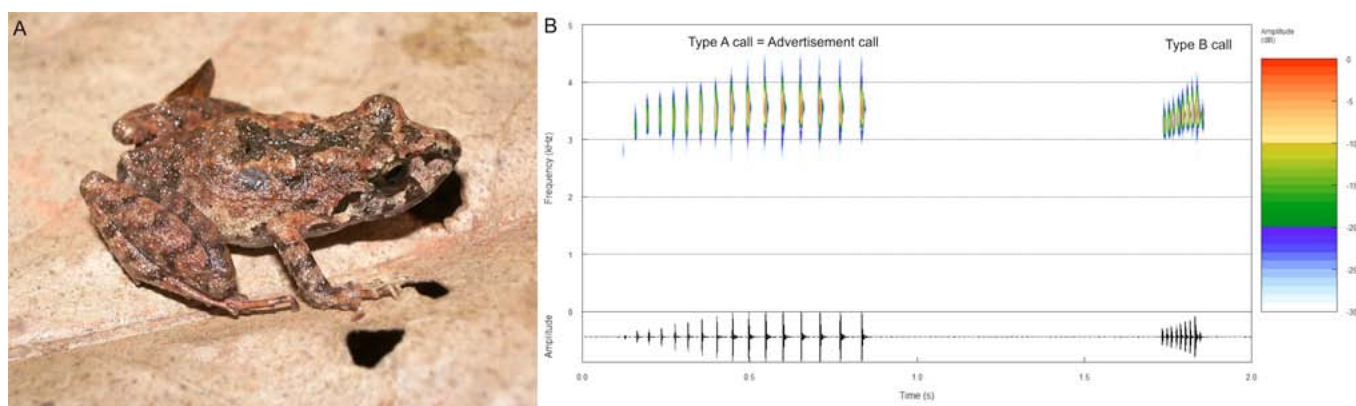


Figure 1. A) *I. penaxavantino* from the Floresta Nacional de Silvânia, Municipality of Silvânia, State of Goiás, Central Brazil. Photograph by Robson Ávila. B) Audiospectrogram (top) and oscillogram (bottom) of the type A (advertisement call) and B calls of *I. penaxavantino* from the Floresta Nacional de Silvânia, Municipality of Silvânia, State of Goiás, Central Brazil. SVL = 15.9 mm; Mass = 0.4 g.

than the type A (advertisement call) with a call duration ranging from 0.108 to 0.147 s (0.127 ± 0.012 s; $N = 8$ calls). This call was also emitted at irregular intervals ranging from 2.013 to 130 s (16.88 ± 42.42 s; $N = 8$ calls). The type B call is composed, on average, of 8.5 ± 0.5 pulses (8–9 pulses; $N = 8$ calls), with a duration ranging from 0.011 to 0.018 ms (0.014 ± 0.002 ms; $N = 32$ pulses). The mean dominant frequency varied from 3246 to 3421 Hz (3359 ± 61.35 Hz; $N = 8$ calls), and the call repetition rate is three calls per minute ($N = 1$ male). We observed that call duration (tsepar. var. = 23.67; $df = 5.25$; $p < 0.001$), pulse number (tsepar. var. = 15.08; $df = 6.22$; $p < 0.001$), and dominant frequency ($t = 2.94$; $df = 12$; $p < 0.05$) are different between the type A and B calls of *I. penaxavantino*. Only the pulse duration (tsepar. var. = 2.15; $df = 5.91$; $p = 0.07$) did not vary between these calls.

The advertisement call of *I. penaxavantino* was described from the type locality, State of Minas Gerais, by Giaretta et al. (2007). The mean values of the acoustic parameters described in this report differ from those presented by Giaretta et al. (2007), but are within the known range for this species. *I. juipoca* was found at the Flona de Silvânia more than 10 years ago (Bastos & Pombal, 2001), but information about vocalisations of individuals in this population was unknown. Giaretta et al. (2007) suggested that such specimens could be assigned as *I. penaxavantino* and also highlighted the importance of requiring bioacoustic analysis.

In this sense, we observed that the dominant frequency and duration of the advertisement call of *I. penaxavantino* from the Silvânia Municipality is similar to that described for *I. juipoca* (0.5 s – Sazima & Cardoso, 1978; 0.7 s – Haddad et al., 1988; 0.51 s, 3300 Hz – Giaretta et al., 2007). However, pulse number seems to be a unique acoustic parameter to strengthen differentiating advertisement calls of *I. juipoca* (10 pulses/call – Sazima & Cardoso, 1978; 9 pulses/call – Haddad et al., 1988; 8 pulses/call – Giaretta et al., 2007) from that within this study (17.16 ± 1.33 pulses/call; range = 15 to 18 pulses/call). Considering these characteristic differences in vocal recordings reinforces our suspicion that specimens of this interesting group found at the Flona de Silvânia are *I. penaxavantino*.

The advertisement calls of some species of the genus *Ischnocnema* have been described in the literature (Castanho

& Haddad, 2000; Giaretta et al., 2007; Oliveira et al., 2008; Conte et al., 2010; Martins & Haddad, 2010; Pombal, 2010; Taucce et al., 2012; Berneck et al., 2013). We observed that the duration of the advertisement call (0.65 to 0.845 s) of *I. penaxavantino* from Flona de Silvânia is lower than that described for *I. hoehnei* (1.3 s – Oliveira et al., 2008), *I. izecksohni* (1.03 to 1.85 s – Taucce et al., 2012), *I. henselii* (20.57 to 26.5 s – Conte et al., 2010), *I. random* (2 to 5 s – Heyer et al., 1990) and *I. guentheri* (9.36 to 13 s – Pombal, 2010). The dominant frequency (3385 to 3564 Hz) of calls in *I. penaxavantino* differed from that described for *I. hoehnei* (2900 Hz – Oliveira et al., 2008), *I. izecksohni* (2250 to 2625 Hz – Taucce et al., 2012), *I. henselii* (2128 to 2501 Hz – Conte et al., 2010), *I. manezinho* (2400 to 2970 Hz – Castanho & Haddad, 2000), *I. sambaqui* (1800 to 2050 Hz – Castanho & Haddad, 2000) and *I. guentheri* (2600 Hz – Pombal, 2010). Herein, we also describe a second type of vocalisation (Type B call) by *I. penaxavantino*. We suggested that this call may have an aggressive function, however further study is necessary to confirm such a hypothesis. In this species, the type B call is shorter than the type A call (advertisement call), with the lowest duration and note number. The aggressive calls of *I. henselii* and *I. nigriventris* have been described in the literature (Conte et al., 2010; Berneck et al., 2013).

In *I. penaxavantino*, the type B call has higher dominant frequency than the aggressive calls of other species of the genus *Ischnocnema* (Conte et al., 2010; Berneck et al., 2013). The call duration (0.108 to 0.147 s), pulse number (8 to 9 pulses/call), and dominant frequency (3246 to 3421 Hz) of the type B call of *I. penaxavantino* differ from those observed for aggressive call of *I. henselii* (0.47 to 0.77 s; 1 to 5 pulses/call; 2214.9 to 3301 Hz, respectively – Conte et al., 2010) and *I. nigriventris* (0.03 to 0.041 s, non-pulsed, 2928 to 3014 Hz, respectively; Berneck et al., 2013).

We conclude that the advertisement call (type A call) of *I. penaxavantino* from the Floresta Nacional de Silvânia is similar to that previously described for this species from the type locality by Giaretta et al. (2007). Furthermore, we observed that pulse number per call is the most important acoustic parameters to distinguish the advertisement call of individuals from Silvânia Municipality referred to *I. juipoca* (Sazima & Cardoso, 1978; Haddad et al., 1988; Giaretta et

al., 2007). Our results allowed us to properly identify species belonging to the *Ischnocema* genus recorded in the Flona de Silvânia, State of Goiás, and also extend, for approximately 260 km, the distribution of *I. penaxavantinho*, which was previously considered restricted to the State of Minas Gerais (Giarretta et al., 2007; Frost, 2014). Finally, this kind of study demonstrates the importance of including vocalizations to differentiate species that are both taxonomically cryptic or similar.

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