Reproductive biology of the Brazilian sibilator frog
Leptodactylus troglodytes

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This study reports on previously unknown characteristics of the reproductive biology of *Leptodactylus troglodytes*, including reproductive site, courtship and territorial behaviour, advertisement, territorial, courtship and reciprocation calls, fecundity and architecture of underground chambers. The ability of tadpoles to produce foam was also tested in the laboratory. Individuals of *L. troglodytes* bred near temporary ponds in shaded areas and eggs were laid in underground chambers. Three courtship behaviour sequences were recorded. Courtship and reciprocation calls are important during courtship to maintain association between the bonding pair. Reciprocation calls emitted by females may indicate gender avoiding attacks by males. The underground chambers examined had a complex architecture consisting of multiple internal spherical rooms. Foam nests with eggs/larvae in different stages of development were found inside the chambers. Tadpoles of *Leptodactylus troglodytes* were able to regenerate foam nests within three hours after the beginning of the experiment. This behaviour was also found in other species of the *L. fuscus*, *L. pentadactylus* and *L. marmoratus* species groups.

Key words: architecture of underground chambers, behaviour, fecundity, reproductive site, tadpoles, vocalization

INTRODUCTION

Amphibians, especially the anurans, exhibit greater reproductive diversity than any other group of tetrapod vertebrates (Duellman & Trueb, 1986). Salthe & Duellman (1973) defined the reproductive modes as a combination of traits that includes oviposition site, ovum and clutch characteristics, rate and duration of development, stage and size of hatchling and type of parental care, if any. The greatest diversity of species is found in the neotropics, where anurans have evolved a remarkable number of reproductive modes (Duellman & Trueb, 1986; Haddad & Prado, 2005).

The neotropical genus *Leptodactylus* comprises 87 currently recognized species (Frost, 2009) that are clustered into five phenetically defined groups: *Leptodactylus ocellatus*, *melanonotus*, *pentadactylus*, *fuscus* and *marmoratus* species groups (*sensu* Heyer, 1969). Reproductive features among species and groups are remarkably diverse and show a tendency towards a terrestrial mode of life (e.g. Heyer, 1969, 1978). Members of the *L. fuscus* group lay their eggs in underground chambers, constructed by the male, and tadpoles subsequently complete their development and metamorphosis in water as free swimming and feeding larvae (Heyer, 1978). The sibilator frog *Leptodactylus troglodytes* A. Lutz (1926) (hole-dwelling thin-toed frog of Caramaschi et al., 2005) is a small frog of the *L. fuscus* species group distributed throughout northeastern, southeastern and central Brazil, being restricted to cerrado and caatinga biomes (Frost, 2009; IUCN et al., 2008). There is limited information on the reproductive biology of this species (Cascon & Peixoto, 1985; Crump, 1995; Arzabe & Almeida, 1997; Reading & Jofré, 2003).

Here we describe characteristics of the reproductive biology of *Leptodactylus troglodytes*, including reproductive site, courtship and territorial behaviour, vocalizations, fecundity and the architecture of underground chambers. We also test the ability of tadpoles to produce/regenerate foam. We compare these features to those previously reported for *L. troglodytes*, other members of the *L. fuscus* group and other *Leptodactylus* species.

MATERIALS AND METHODS

Fieldwork was carried out at Cocos municipality, Bahia state (Santa Luzia Farm, 14º49'S, 45º58'W; three nights of observations) and Buritizeiro municipality, Minas Gerais state (Jatobá Farm, 17º07'S, 44º52'W; two nights of observations) in October 2004 and November 2005, respectively. The climate of both cerrado areas has two well-defined seasons characterized by wet/warm (approximately September to March) and dry/mild (approximately April to August) cycles. Savanna and grassland (part of the complex cerrado landscape physiognomy) characterize the study areas (Ribeiro & Walter, 2001). We inspected reproductive sites for calling males,
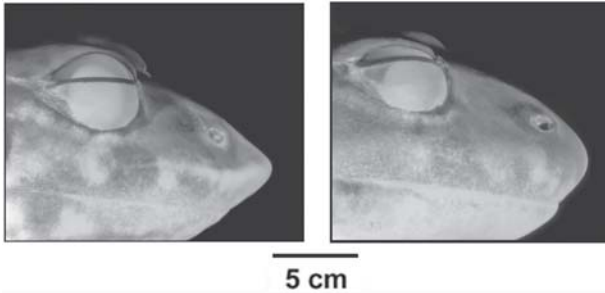
Fig. 1. Lateral view of a *Leptodactylus troglodytes* male (left) (spatula-like shape) and female (right) snout of *Leptodactylus troglodytes* (photo from Santa Luzia Farm, Cocos municipality, Bahia state, Brazil).

bonding pairs and foam nests in underground chambers, where continuous observations of the most relevant behavioural events (Martin & Bateson, 1986) were carried out. The daily calling pattern was observed for two 24-h periods. We also marked calling sites ($n=6$) to tentatively establish distances among calling males.

We recorded vocalizations with a digital recorder in linear PCM mode (Sony MZ 700 Hi-MD) with a unidirectional Audio-Technica ATR55 Camcorder Condenser Telemike microphone; sampling rate was set at 22,050 Hz, with 16 bit resolution. Audiospectrograms were produced using SoundRuler (Gridi-Papp, 2003–2007), Audiospectrogram (Horne, 2001) software (territorial call), and Adobe® Audition® 3. For analysis we used an FFT (Fast Fourier Transformation) set at 2048 points (necessary to analyse the recordings made), frequency resolution at 21.5 Hz, and low and high band limit at 1500 and 7000 Hz, respectively. We measured the following parameters: call length (time from beginning to end of one call), call rate (total number of calls – 1/time from beginning of first call to beginning of last call), call rise time (time from beginning of call to point of maximum amplitude) and frequency modulation (measured as the difference between final and initial call frequency). The dominant frequency was measured at the peak amplitude of each call (Cocroft & Ryan, 1995; Márquez et al., 1995). Inter-call interval is defined as the time elapsed between two calls (Tárano, 2001). Although we did not conduct playback experiments, the determination of the possible function of a particular anuran vocalization (often classified as advertisement, courtship, territorial and reciprocation calls) was based on the context in which calls were emitted and the response elicited in neighbouring individuals. We collected most of the data at Santa Luzia Farm, Cocos municipality; data from Jatobá Farm, Buritizeiro municipality are identified in the results. The temperatures registered for each call are given in the figure legends.

We used behaviour, vocalization and secondary sexual characters, such as snout shape, to determine an individual's sex. The male of *L. troglodytes* has a spatula-like snout that is used to dig and prepare the reproductive chambers. This trait is also found in species of the *L. fuscus* and *L. marmoratus* groups (Heyer, 1978).

We determined the ovarian complement based on a female collected in the field (Santa Luzia Farm, at Cocos municipality) and three museum specimens (São Domingos and Chapada Gaúcha municipalities, both in Goiás State, housed in Coleção Herpetológica da Universidade de Brasília–CHUNB) (numbers below). We considered eggs with a diameter equal to or larger than 2 mm as mature (Giaretta & Kokubum, 2004). We prepared plaster moulds to determine the underground chamber architecture (see details in Giaretta & Kokubum, 2004).

We collected foam nests ($n=3$), together with some of the underground chambers, in the field and transported them to the lab, where they were maintained at room temperature (±25 °C). To confirm species identification, we collected tadpoles ($n=2$) from foam nests located in underground chambers and kept them in the laboratory until they completed metamorphosis. Developmental staging followed Gosner (1960). We tested if tadpoles could generate foam similar to that originally produced by the parents when constructing the foam nest. Tested tadpoles ranged from 8.4 to 13.4 mm total length (Gosner stages 26–27). We freed tadpoles from three clutches (two clutches containing 30 each and one with 40 tadpoles) from the original foam by gently washing them in dechlorinated tap water. We placed each group of tadpoles in a plastic tube without water. We maintained the plastic tubes in a moist and shaded area under natural photoperiod (Kokubum & Giaretta, 2005); temperature ranged between 23 and 29 °C and tubes were checked at intervals of 2–4 h over a 96-h period.

We measured the snout–vent length of tadpoles, two newly metamorphosed juveniles, two males, a female and moulds of underground chambers with a Mitutoyo digital
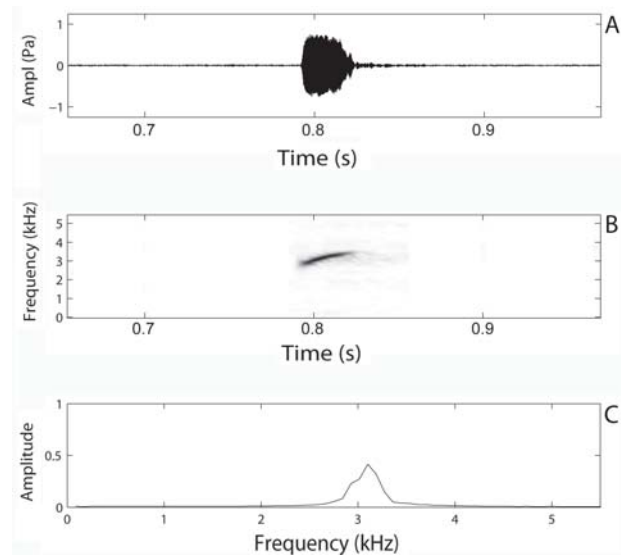
Fig. 2. Waveform of the call (A), audiospectrogram (B) and power spectrum (C) of one advertisement call of *Leptodactylus troglodytes*. Jatobá Farm, Buritizeiro municipality, Minas Gerais state, Brazil (23 November 2005; 2312, air temperature 24 °C, specimen not collected). Recording file: *Leptodactylus troglodytes* canto 063 Fazenda Jatobá Buritizeiro MG.
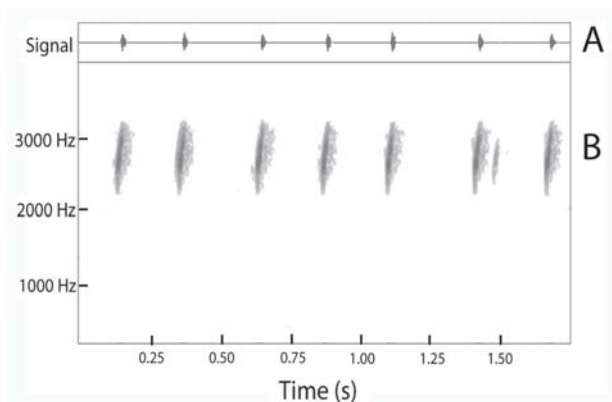
Fig. 3. Waveform (A) and audiospectrogram (B) of seven territorial calls of *Leptodactylus troglodytes*. Santa Luzia Farm, Cocos municipality, Bahia state, Brazil (1 November 2004, 2300, air temperature 18 °C). Recording file: *Leptodactylus troglodytes* territorial2 Fazenda Santa Luzia Cocos BA.

caliper (0.01 mm). Adult (AAG-UFU 3575, 3576, 3577 (call vouchers), 3578; CHUNB 35417, 35418, 35419) and tadpole (AAG-UFU 4208, 4209) voucher specimens are deposited in the Museu de Biodiversidade do Cerrado, in Universidade Federal de Uberlândia (AAG-UFU) and Coleção Herpetológica da Universidade de Brasília, in Universidade de Brasília (CHUNB).

RESULTS

Calling site and behaviour

We found *Leptodactylus troglodytes* individuals breeding in artificial, temporary, small (approximately 2.5 m wide × 1.5 m deep) rivulets (Santa Luzia and Jatobá Farms) along with *Leptodactylus labyrinthicus* (*n*=1). Males started calling in the late afternoon (approximately 1700) and continued to call until 0800–0900 the next day, although calling was not continuous. Males called from the margins (≥1.5 m) of the rivulets, hidden amidst sparse vegetation or in holes in bare soil. The distances among calling males were always greater than 2 m (mean = 3.45±1.26 SD; 2.0–4.8 m). During the first night of observations, we found an intruding male calling closer than 2 m from the resident male, who responded with a territorial call. Territorial calls were also emitted in response to silent approaching females (*n*=1). At approximately 2000, we detected 11 males calling along a 30-m transect.

We observed three partial sequences of courtship behaviour at Santa Luzia Farm. The courtship behaviour sequences included: 1) male emitting advertisement calls, 2) a receptive female approaching the male, 3) male emitting courtship calls or not (changing and modulating the calls), 4) female signalling receptivity by touching male's flanks or tympanum with her snout, 5) male guiding the female to a previously excavated reproductive chamber, 6) male entering the chamber first, followed by female, 7) immediately after pair entering the chamber, male returning to seal the entrance with his head or not, 8) pair

remaining in the chamber up to four hours, 9) female leaving the chamber first, followed by the male. In one instance, we saw the male reconstruct the chamber using both his snout and dorsum.

In response to the male courtship call the female emitted reciprocation calls (described below). The two males measured 48.4 and 51.7 mm (SVL) and were also characterized by a more prominent and acuminate snout when compared to the female (SVL = 53.6 mm; Fig. 1).

Call repertoire

Each advertisement call (*n*=5 frogs; two individuals from Santa Luzia Farm and three from Jatobá Farm; one call analysed for each individual; Fig. 2) lasts 364–576 ms
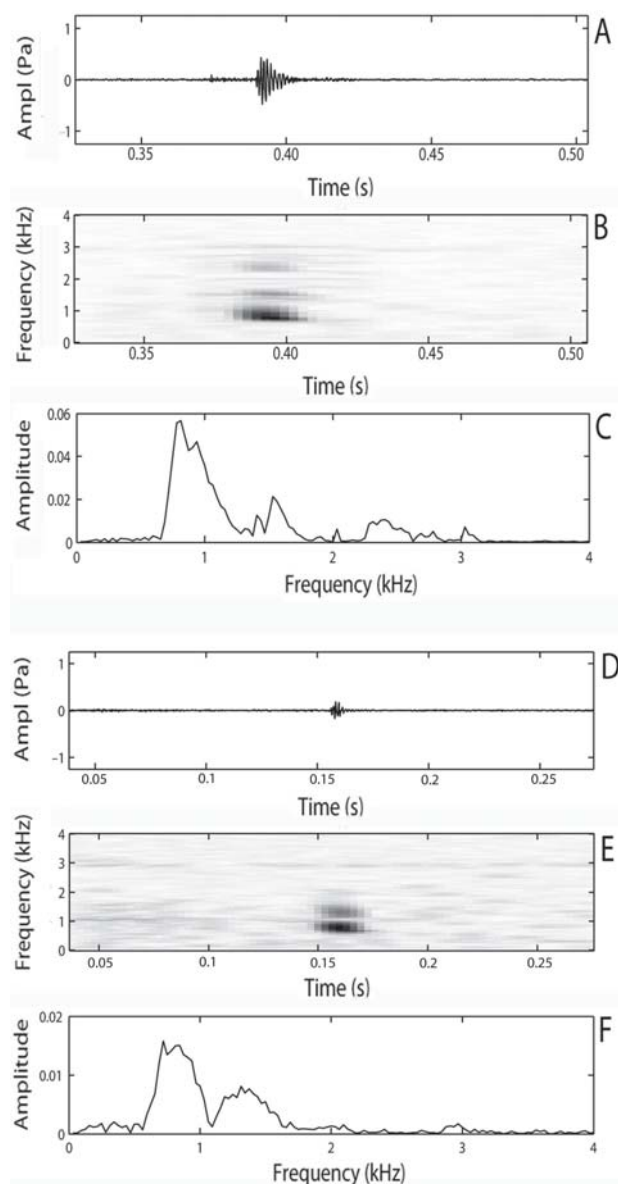
Fig. 4. Courtship (A, B, C) and reciprocation (D, E, F) calls of *Leptodactylus troglodytes*. A,D) Waveform of calls; B,E) audiospectrogram of calls; C,F) power spectrum. Santa Luzia Farm, Cocos municipality, Bahia state, Brazil (1 November 2004; air temperature 18 °C). Recording file: *Leptodactylus troglodytes* corte – recíproco Fazenda Santa Luzia Cocos BA.
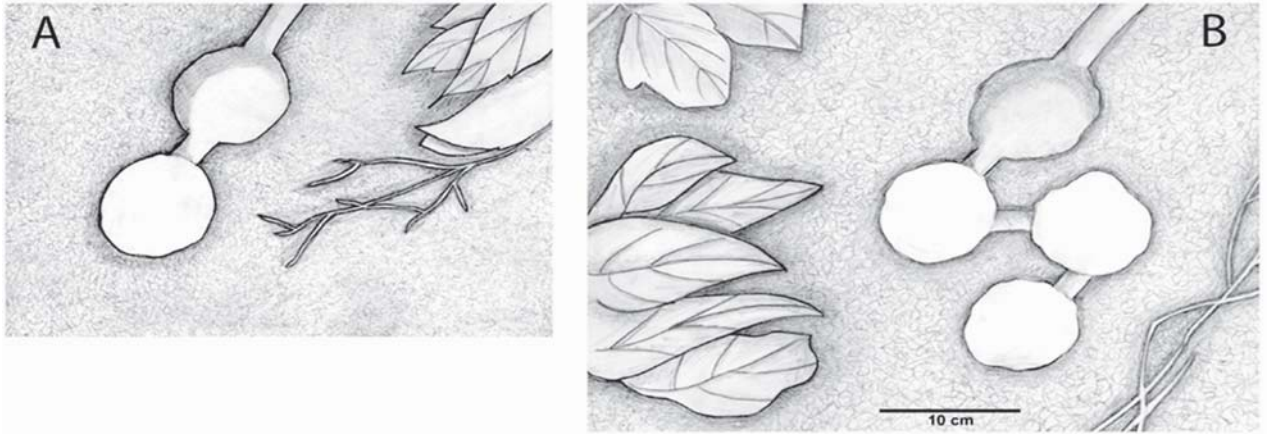
Fig. 5. Diagrams representing the architecture of the underground chamber of *Leptodactylus troglodytes* (side view from rivulet). A) Underground chamber with two rooms (internal chambers); the second room (below) contained the foam nest. B) Underground chamber with four rooms (internal chambers); the three rooms in the lower portion of the figure contained the foam nests.

(mean = 451.0±8.92 SD), emitted in a sequence of regular calls repeated 56–80 times per minute (mean = 68.7±12.05 SD). The mean call rise time is 585.33±36.23 SD, ranging from 518 to 622 ms. The call is tonal and lacks harmonic structure (Fig. 2). The dominant frequency measures 3143.85 Hz and mean frequency modulation was 559.86±49.73 SD, range 517–603 Hz. Higher frequencies were not found when the calls were re-analysed.

The territorial, courtship and reciprocation calls observed in *Leptodactylus troglodytes* have not been previously reported for this species. Two territorial call recordings were obtained from two males. They were produced when males were less than 60 cm apart (Fig. 3) and lasted a mean of 454.33±18.83 SD (Fig. 3A), with inter-call intervals averaging 530±32.51 SD. Calls were emitted as a sequence of regular calls (but irregular when compared to the advertisement call) repeated 145–240 times per minute. The dominant frequency averaged 3121.70 Hz ± 28.94 SD) and mean frequency modulation was 1001.82 HZ ± 60.33 SD. Table 1 summarizes both advertisement and territorial call parameters for *L. troglodytes*.

The courtship and reciprocation calls are partially described here due to the limited number of available recordings. These two calls are very similar as can be seen by the shape of the oscillogram and dominant frequency, as described below. Courtship calls were recorded for two males. Courtship calls (Fig. 4A–C) are produced when the male perceives the presence of a female. The calls lasted 93–117 ms and had an ascending modulation at the beginning of the call (Fig. 4A). The dominant frequency ranged from 796.87 to 859.37 (Fig. 4B). On one occasion a reciprocation call was heard as a response to a male courtship call, emitted while a male was leading a female to the reproductive chamber (Fig. 4D–F). All calls analysed lasted 31 ms (Fig. 4D) and the dominant frequency was 828.12 Hz.

Architecture of underground chambers

Two underground chambers were constructed in muddy soil, amid grass roots about three metres from water. They consisted of two and four compartments, respectively. We analysed the structure of one of the chambers and found that it had a tunnel 75 mm long and 38 mm wide, and two sequential, spherical (70–75 mm) compartments linked by shorter tunnels (about 40 mm long) (Fig. 5A). The other chamber was similar, but consisted of four compartments, three of which contained foam nests with eggs and tadpoles (Fig. 5B). Clutches in each compartment contained tadpoles in various early stages of development. We used these clutches to examine fecundity and used some tadpoles from these clutches to implement the foam-producing behaviour test.

Fecundity and behaviour of tadpoles

The eggs were cream-coloured and egg diameter was 2.5±0.2 mm (range 2.3–2.7). Three foam nests had both eggs and tadpoles within the foam; the numbers of eggs and tadpoles in each nest were 1) 150 eggs and 10 tadpoles, 2) 143 eggs and 12 tadpoles and 3) 85 eggs and 79 tadpoles; all tadpoles were in stages 26 or 27 and had a mean size of 11.8 mm ± 1.1 SD (range 10.2–13.4). We examined four preserved females with a mean of 274 eggs ± 121.6 SD (range 130–427); individual eggs had a mean size of 2.1±0.1 mm (range 2.0–2.1; *n*=30). Eggs hatched within

Table 1. Comparative summary of advertisement and territorial call parameters of *Leptodactylus troglodytes*.

Call parameters	Advertisement call	Territorial call
Call length (ms)	364–576	226–678
Inter-call interval (ms)	599–1010	497–572
Call rate (per min)	56–80	145–240
Dominant frequency (Hz)	3144	3058–3144
Frequency modulation (Hz)	517–603	909–1060

Fig. 6. *Leptodactylus troglodytes* tadpoles (one of the three tested groups removed from their original clutches). The foam was produced within 3 hours of the beginning of the experiment.

83–93 h after collection. One of the recently metamorphosed specimens had a snout–vent length of 15.9 mm.

The three groups of tadpoles removed from their original clutches all produced new and copious foam within 3 hours of the beginning of the experiment (Fig. 6). The foam consisted of small bubbles released from the mouth of the tadpoles and appeared different from the original foam nest generated by parents.

DISCUSSION

Call repertoire

Advertisement calls have been previously reported for *Leptodactylus troglodytes* in the state of Bahia, Andaraí municipality (Heyer, 1978), and Mangue Seco (Nunes & Juncá, 2006). The tonal advertisement calls of *L. troglodytes* reported here are similar in spectral features, such as dominant frequency and ascending frequency modulation, when compared to previously reported calls. However, the duration of notes and inter-note intervals in the populations in our study (364–576 ms and 599–1010 ms, respectively) are different from those reported for Mangue Seco (50–70 and 18–159 ms, respectively). This incongruence could be explained by the difference in air temperature at the time that recordings were made in Mangue Seco (20ºC; Nunes & Juncá, 2006) and Jatobá Farm (24ºC; this study).

The production of courtship and reciprocation calls and physical contact during courtship behaviour are probably employed to maintain communication between the male and female during courtship, as has also been reported in *L. fuscus* (Freitas et al., 2001). Reciprocation calls provide information to the male that the approaching individual is a receptive female, rather than an intruding competitive male, preventing male attacks (Wells, 1977; Silva et al., 2008, and review by Schlaepfer & Figeroa-Sandí, 1998). Reciprocation calls have been reported for a few anurans, including some *Leptodactylus* species (Schlaepfer & Figeroa-Sandí, 1998; Davis et al., 2000; Bernal & Ron, 2004; Silva et al., 2008). Most commonly, females do not initiate calling, but rather respond vocally to the calls of males (Schlaepfer & Figeroa-Sandí, 1998). These calls have thus been termed reciprocation or reciprocal calls (Duellman & Trueb, 1986; Roy et al., 1995). Although rarely reported, female vocalizations are more frequent than originally assumed, and may have evolved by co-opting the pre-existing advertisement calling pathway common in both sexes. This process may be an adaptation for mate location that could be present in most, if not all species (Emerson & Boyd, 1999).

Nest architecture

As reported for other species of the *Leptodactylus fuscus* species group, *L. troglodytes* builds its underground chambers in open areas that are regularly flooded by nearby water sources after heavy rains (Martins, 1988; Lima et al., 2006). Underground chambers have been suggested as mechanisms to protect the eggs and larvae (e.g. Haddad & Sawaya, 2000), whereas thermal differences and different soil types may be factors affecting nest architecture (Arzabe & Prado, 2006). Other environmental selective pressures possibly moulding nest architecture in *Leptodactylus* may be related to habitat type (e.g. open or forested areas) and behavioural breeding strategies (e.g. exotrophic and endotrophic eggs).

Despite the great number of *Leptodactylus* species (*L. fuscus* and *L. marmoratus* groups) that build underground nests, the architecture of the underground chambers has been described for only a few species (Sazima, 1975; Sazima & Bokermann, 1978; Martins, 1988; Crump, 1995; Giaretta & Kokubum, 2004; Arzabe & Prado, 2006; Ponssa & Barrionuevo, 2008; Kokubum & Souza, 2008; Oliveira Filho & Giaretta, 2009). Underground chambers can differ among species in shape, size, presence or absence of an entrance tunnel, number of openings to the chamber and number of internal compartments. The underground chamber of *L. bufonius* is jar-shaped (Cei, 1949), while those of *L. mystacinus* are approximately spherical (Sazima, 1975; Giaretta & Oliveira Filho, 2006; Oliveira Filho & Giaretta, 2009). In *L. furnarius* the chambers have no entrance tunnel (Giaretta & Kokubum, 2004), while in *L. fuscus* (Arzabe & Prado, 2006) and *L. troglodytes* they possess an access tunnel. Among species of the *L. fuscus* group, multiple internal compartments have been reported only for *L. mystaceus* (Arzabe & Prado, 2006). The presence of multiple compartments may allow the accommodation of additional nests (Toledo et al., 2005; Arzabe & Prado, 2006) and may be related to territorial behaviour.

Leptodactylus troglodytes is morphologically similar to *L. bufonius*, and they inhabit similar habitats. *L. troglodytes* seems to be the sister species of *L. bufonius*, as hypothesized by the cladistic analysis of the *L. fuscus* group by Ponssa (2008). The two species are parapatric, with *L. troglodytes* occurring in cerrado and caatinga for-

mations and *L. bufonius* occurring in the chaco/Pantanal region. It has been reported that in *L. bufonius* females close the chambers before leaving (Crump, 1995; Reading & Jofré, 2003), and the same behaviour was observed in *L. fuscus* from areas of cerrado (Lucas et al., 2008). This behaviour seems to be an adaptation to extremely seasonal regions. However, we did not observe this behaviour in either sex in *L. troglodytes*.

Arzabe & Almeida (1997) reported that *L. troglodytes* constructed the reproductive chamber in the presence of a receptive female. In our study, underground chambers were already constructed before the male began his advertisement calling. Also, we could not corroborate the existence of more than one opening to access the reproductive chamber in *L. troglodytes* (Arzabe & Almeida, 1997). However, these population-level differences probably reflect plasticity in the chamber construction behaviour of *L. troglodytes* males, and may also reflect the limitations of sample sizes in the present work. We also found that males of *L. troglodytes* may or may not block the tunnel entrance after the female has entered the chamber, as observed in *L. fuscus* (Martins, 1988) and *L. furnarius* (Giaretta & Kokubum, 2004).

Fecundity and behaviour of tadpoles

Given that the number of eggs/tadpoles found in the foam nests was lower than the number of eggs found in preserved females, it is possible that females may split their reproductive output over more than one mating event, thus increasing their own fitness. Another possibility is that a couple could split their eggs between more than one internal compartment in the same chamber.

Foam-making behaviour has been observed in tadpoles of other species of the *Leptodactylus fuscus* (*L. fuscus*, *L. mystaceus*, *L. furnarius* and *L. latinasus*), *L. pentadactylus* (*L. labyrinthicus*) and *L. marmoratus* (*Leptodactylus* sp. and *Leptodactylus thomei*) groups (Downie, 1984, 1989; Caldwell & Lopez, 1989; Almeida & Angulo, 2002, 2006; Kokubum & Giaretta, 2005; Kokubum & Souza, 2008; Ponssa & Barrionuevo, 2008). For most other species, foam-making behaviour by tadpoles remains speculative, and more studies are still needed. However, this behaviour may avoid contact with the bottom of the nest (Kokubum & Giaretta, 2005), facilitate respiratory/excretory processes (Downie & Smith, 2003) and/or avoid desiccation of tapoles, at least those inside chambers (Prado, pers. comm.). Downie & Smith (2003) studied the function of the foam generated by *L. fuscus* tadpoles. They emphasized that the foam may help the tadpoles to obtain moisture from the substratum, but also considered alternative hypotheses, such as the foam assisting with respiratory and excretory exchange. Data obtained on *L. labyrinthicus* suggest that water absorption is not the main function of the foam released by the tadpoles (Kokubum & Giaretta, 2005). The foam may, instead, act to avoid compaction of the tadpoles at the bottom of the basin or underground chamber, avoiding overcrowding and increasing respiratory and excretory efficiency (Kokubum & Giaretta, 2005). The ability of *L. troglodytes* tadpoles to regenerate foam supports a recently proposed hypothesis of a close relationship between the *L. fuscus* and *L. pentadactylus* groups (Ponssa, 2008; Ponssa & Barrionuevo, 2008). Species of the *L. marmoratus* group were considered to be more related to *L. lineatus* (not assigned to any *Leptodactylus* group) than to other *Leptodactylus* species by Ponssa (2008). However, Almeida & Angulo (2002) and Kokubum & Giaretta (2005) reported on foam-producing tadpoles in the *L. marmoratus* group. Thus, any phylogenetic inference on the nature of this kind of behavioural feature (homology or homoplasy) would be best examined when additional species are sampled and studied under a phylogenetic framework.

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