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The tadpole of *Chiasmocleis carvalhoi* and the advertisement calls of three species of *Chiasmocleis* (Anura, Microhylidae) from the Atlantic rainforest of southeastern Brazil

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Abstract

The tadpole of *Chiasmocleis carvalhoi* and the advertisement calls of three species of *Chiasmocleis* (Anura, Microhylidae) from the Atlantic rainforest of southeastern Brazil. The tadpole of *Chiasmocleis carvalhoi* is figured and described for the first time from individuals collected in the State of Rio de Janeiro, southeastern Brazil. The advertisement calls of *C. atlantica*, *C. capixaba*, and *C. carvalhoi* also are described and compared with the advertisement calls of others *Chiasmocleis* species restricted to Atlantic Rainforest. The advertisement calls of *C. atlantica*, *C. capixaba*, and *C. carvalhoi* are similar, consisting of one pulsed note of harmonic structure emitted repetitively. Our study corroborates the monophyly of the genus *Chiasmocleis* based on similarities in advertisement calls. Calls of syntopic species (*C. atlantica* with *C. carvalhoi* and *C. capixaba* with *C. schubarti*) were less similar than those of closely related allopatric species.

Keywords: Anura, Microhylidae, *Chiasmocleis atlantica*, *Chiasmocleis capixaba*, *Chiasmocleis carvalhoi*, tadpole description, advertisement call, Atlantic rainforest, southeastern Brazil.

Introduction

As currently recognized, the Neotropical microhyline genus *Chiasmocleis* Méhelÿ, 1904 includes 18 species, distributed from Panama to southern South America, north and east of the Andes (Caramaschi and Cruz 2001, Caramaschi and Pimenta 2003, Frost 2003). Eight species are restricted to the Atlantic Rainforest: *C. alagoana* Cruz, Caramaschi, and Freire, 1999; *C. atlantica* Cruz, Caramaschi, and Izecksohn, 1997; *C. capixaba* Cruz, Caramaschi, and Izecksohn, 1997; *C. carvalhoi* Cruz, Caramaschi, and Izecksohn, 1997; *C. cordeiroi* Caramaschi and Pimenta, 2003; *C. crucis* Caramaschi and Pimenta, 2003; *C. leucosticta* (Boulenger, 1888), and *C. schubarti* Bokermann, 1952. Descriptions of male vocalizations are known for *C. carvalhoi* (Nelson 1973, Hartmann *et al.* 2002), *C. leucosticta*, and *C. schubarti* (Nelson 1973). Tadpoles have been described for only two species of *Chiasmocleis*

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(*Chiasmocleis* cf. *shudikarensis* and *C. ventrimaculata*, Hero 1990, Schlüter and Salas 1991). Furthermore, only the tadpole of *C. ventrimaculata* was described in detail (Schlüter and Salas 1991). Herein, we describe the tadpole of *C. carvalhoi* and the advertisement calls of *C. atlantica*, *C. capixaba*, and *C. carvalhoi*. In addition, we compare the advertisement calls among the *Chiasmocleis* species from the Atlantic Rainforest.

Materials and Methods

During a community study from February 2001 to January 2002, at Reserva Biológica de Duas Bocas (20°16' S, 40°28' W; 200 m above sea level), Municipality of Cariacica, State of Espírito Santo, southeastern Brazil, we recorded advertisement calls of one male of *Chiasmocleis capixaba* in a temporary pond within the forest. Observations of breeding activities of *C. atlantica* and *C. carvalhoi* in a temporary pond of approximately 750 m^2 also were made at the edge of a forest at Reserva Biológica União (22°31' S, 41°56' W), Municipality of Rio das Ostras, State of Rio de Janeiro, southeastern Brazil. Fieldwork was conducted from September 2002 to May 2003 for a total of 48 nights. An amplectic pair of *C. carvalhoi* was kept in laboratory until oviposition. The tadpoles were reared and sequentially preserved in 5% formalin to provide a representative sampling of the developmental sequence. We also collected tadpoles in the natural habitat and compared them with those reared in laboratory. The description of the tadpoles was based on larval Stages 28, 30, 31, 33, 35, and 39 (Gosner 1960). Several stages were used to assess ontogenetic changes during development. Measurements were made on larvae at Gosner´s Stage 35. The dental formula and measurements follow the guidelines provided by Altig (1970) and Altig and McDiarmid (1999), with exception of the interorbital and internostril distances, which was taken between the inner edges of the eyes and nostrils. We used an ocular micrometer with a Zeiss stereomicroscope to take all measurements to the nearest 0.1 mm. The terminology describing the spiracle, anal tube, tail tip and oral apparatus follows that of Altig and McDiarmid (1999). Illustrations were prepared using a Zeiss stereomicroscope with a camera lucida. Voucher tadpoles of *Chiasmocleis carvalhoi* are deposited in Museu Nacional / UFRJ, State of Rio de Janeiro, Brazil (MNRJ 33772–33777).

Vocalizations of *Chiasmocleis capixaba* were recorded with an Aiwa TP-VS 480 recorder and a Le Son MK-60 microphone, and those of *C. atlantica* and *C. carvalhoi* were recorded with a Panasonic recorder, Model Nº RQ - L30. All vocalizations were digitized and edited at a sampling frequency of 22 kHz, FFT with 256 points, and 16-bit resolution, and analyzed with Avisoft-Sonagraph Light 1 (Version 2.7) and Cool Edit 2000. Vocalization terminology follows Duellman and Trueb (1986). A cluster analysis for the five rainforest species of *Chiasmocleis* (*C. atlantica*, *C. capixaba*, *C. carvalhoi*, *C. leucosticta*, and *C. schubarti*) was performed based on call duration and dominant frequency. The Average Linkage Method (Euclidean distance) was employed to verify the similarities among advertisement calls of the individual species (Mainly 2000). Call data for *C. leucosticta* and *C. schubarti* were taken from Nelson (1973).

Results

Description of the Tadpole of Chiasmocleis carvalhoi

The following description is based on an individual in Stage 35 (Figure 1). Overall body oval in lateral view and rectangular in dorsal and ventral views; body representing about 35% of total length; greatest body height in its posterior third; greatest body width immediately behind eyes; snout protruding in lateral view and truncate in dorsal and ventral views; eyes lateral, located on the anterior third of body; nostrils

absent; spiracle single, median, posterior ventral; spiracle long, reaching or passing the opening of the anal tube, spiracle height at least half of the height of the anal tube (spiracle broad); anal tube medial, with opening directed downward, and attached directly to ventral fin; dorsal fin originating at the end of the body; fins approximately straight and parallel for first two-thirds tail; caudal tip flagellate; greatest caudal height found in anterior half of tail; oral disc terminal with paired, semicircular labial flaps suspended in front of the mouth and separated by an inverted, U-shaped medial notch (Figure 1D); jaw sheaths, papillae, and tooth rows absent. Morphometric data are in Table 1.

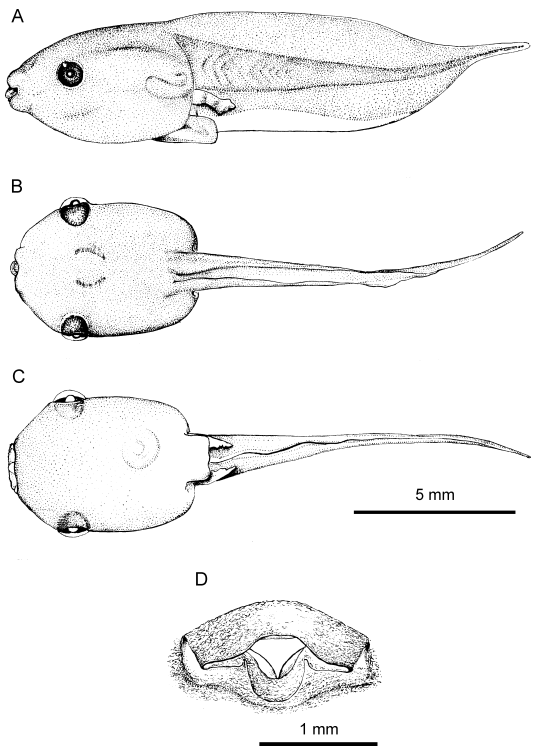

Figure 1. Tadpole of *Chiasmocleis carvalhoi*, Stage 35 (MNRJ 33772). (A) Lateral, (B) dorsal, (C) ventral views; (D) oral disc.

Coloration

In life, dorsum brown extending onto the lateral surface of body, somewhat below the median line of body; a pair of curved, bracket-shaped, and light-cream lines between the eyes; tail musculature and fins light-brown; ventrally, belly translucent and without pigmentation, particularly on its anterior two thirds. In preservative, brown color pattern faint.

Variation

The spiracle morphology and the presence of bracket-shaped marks vary among developmental stages. We observed a total of 21 tadpoles, in addition to the one described above. In four (at Stages 28, 29, 30, and 31), the spiracle is short and narrow. Six (N = 1, Stage 30; N = 3, Stage 31; N = 1, Stage 32; N = 1, Stage 35) have long, narrow spiracles, and 11 tadpoles (N = 1, Stage 32; N = 1, Stage 33; N = 8, Stage 35; N = 1, Stage 39) have long, broad spiracles.

Bracket-shaped marks are not present between the eyes in five tadpoles (N = 1, Stage 28; N = 3, Stage 30; N = 1, Stage 39); however, these marks are evident in 16 other individuals (N = 4, Stage 31; N = 3, Stage 33; N = 9, Stage 35).

Vocalizations and Notes on Reproduction

The advertisement calls of *Chiasmocleis atlantica*, *C. capixaba*, and *C. carvalhoi* are similar (Figures 2-4). They consist of one pulsed note that is emitted repetitively, and according to spectrogram (Figure 4), the calls possess harmonic structure. The call duration, number of pulses, pulse duration, and intervals between pulses of *C. atlantica* are greater than in *C. capixaba* and *C. carvalhoi*; the interval between notes in *C. capixaba* is longer than in *C. carvalhoi* and *C. atlantica* (Table 2). The dominant frequency corresponds to the fundamental frequency in all three species (Figures 2 and 4).

Table 1 – Measurements (mm) of the 11 tadpoles of *Chiasmocleis carvalhoi* at Stage 35 of Gosner (1960).

CHARACTER	MEAN ± SD	RANGE
Total length	16.60 ± 0.50	15.50–17.20
Body length	5.80 ± 0.10	5.60–6.00
Body height	3.30 ± 0.10	3.10–3.50
Body width	4.00 ± 0.10	3.80–4.20
Tail length	10.80 ± 0.50	9.70–11.30
Tail height	3.70 ± 0.10	3.40–3.90
Tail musculature height	1.40 ± 0.10	1.30–1.50
Dorsal fin height	1.32 ± 0.07	1.20–1.44
Ventral fin height	1.48 ± 0.05	1.44–1.60
Eye diameter	0.84 ± 0.04	0.80–0.88
Interorbital distance	2.90 ± 0.10	2.70–3.00
Snout-eye distance	1.60 ± 0.10	1.40–1.80
Oral disc	1.51 ± 0.04	1.45–1.55

Table 2 – Summary of acoustic parameters of the vocalizations of *Chiasmocleis atlantica*, *Chiasmocleis capixaba*, and *Chiasmocleis carvalhoi*. Values are presented as mean ± SD, [range], (N).

CHARACTERISTICS	*Chiasmocleis atlantica*	*Chiasmocleis capixaba*	*Chiasmocleis carvalhoi*
Call duration (ms)	137 ± 22 [64–186] (128)	52 ± 5 [47–61] (39)	48 ± 5 [42–54] (40)
Interval between notes (ms)	28 ± 5 [23–47] (126)	59 ± 7 [54–86] (34)	37 ± 1 [34–43] (36)
Number of pulses	16.7 ± 2.1 [7–22] (316)	8.7 ± 0.8 [8–10] (39)	9.3 ± 0.9 [8–10] (69)
Pulse duration (ms)	5 ± 0.5 [4–6] (69)	4 ± 0.3 [3–4] (50)	3 [3] (11)
Interval between pulses (ms)	4 ± 0.6 [3–6] (60)	3 ± 0.5 [2–3] (50)	2 ± 0.4 [2–3] (11)
Dominant frequency (kHz)	3.54 ± 0.11 [3.32–3.75] (125)	4.75 ± 0.06 [4.61–4.87] (38)	4.84 ± 0.07 [4.69–4.96] (21)

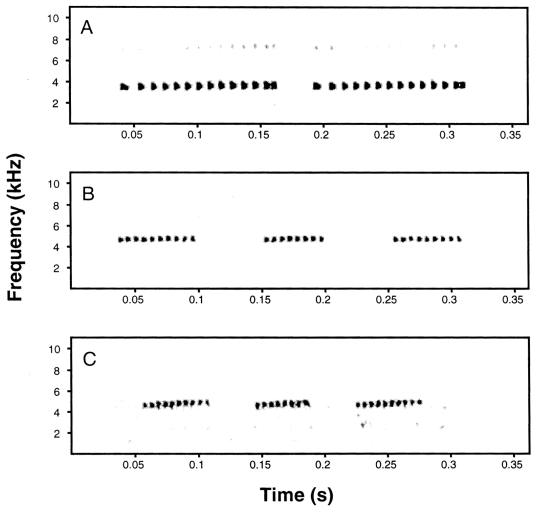

Figure 2 - Sonograms of advertisement calls of (A) *Chiasmocleis atlantica* (with two pulsed notes), air temperature of 23ºC, (B) *Chiasmocleis capixaba* (with three pulsed notes), air temperature of 20ºC, and (C) *Chiasmocleis carvalhoi* (with three pulsed notes), air temperature of 23ºC.

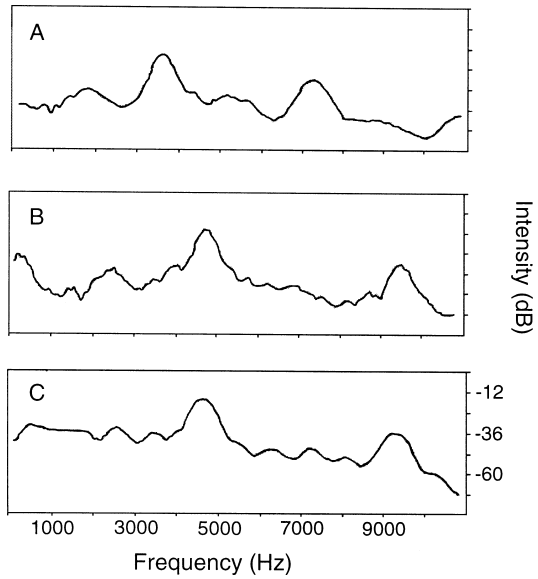

Figure 4 - Frequency spectrum, along the X-axis, and amplitude, in dB, along the Y-axis, of advertisement calls of (A) *Chiasmocleis atlantica*, (B) *Chiasmocleis capixaba*, and (C) *Chiasmocleis carvalhoi*.

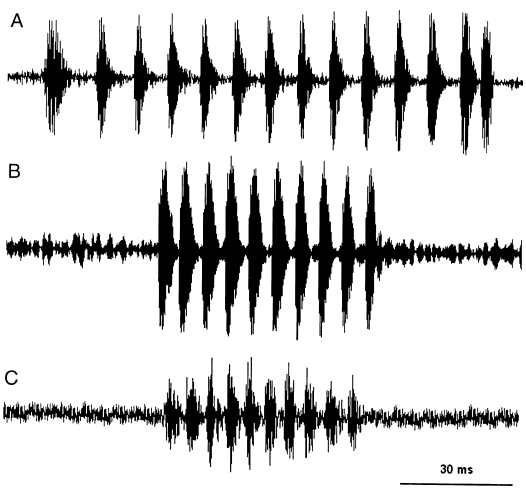

Figure 3 - Waveforms showing one pulsed note of advertisement call of (A) *Chiasmocleis atlantica*, (B) *Chiasmocleis capixaba*, and (C) *Chiasmocleis carvalhoi*.

The numbers of pulses in these three species are positively correlated with time (Figure 5). *Chiasmocleis atlantica* has the most accentuated increase in the number of pulses as the male calls (Pearson coefficient correlation; r = 0.81, 0.55, and 0.64; p = 0.001, *C. atlantica*, *C. capixaba*, and *C. carvalhoi*, respectively).

Males were observed calling above or within leaf litter around temporary ponds after heavy rains, mainly in October 2001 (*C. capixaba*) and in November 2002 (*C. atlantica* and *C. carvalhoi*). The latter two species are syntopic and their reproductive activities overlap. Breeding aggregations of *C. atlantica* and *C. carvalhoi* were observed on only six nights (11, 12, and 13 November 2002, 10 December 2002, 14 January 2003, and 19 March 2003). A bout of calling activity on 12 November 2002 of these two species lasted eight hours, starting at 18:00 h and finishing at

02:00 h. We observed reproductive aggregations of *Chiasmocleis capixaba* only in temporary ponds, but never around the lake (a permanent body of water in which several other anuran species were observed) located about 50 m from the temporary pools.

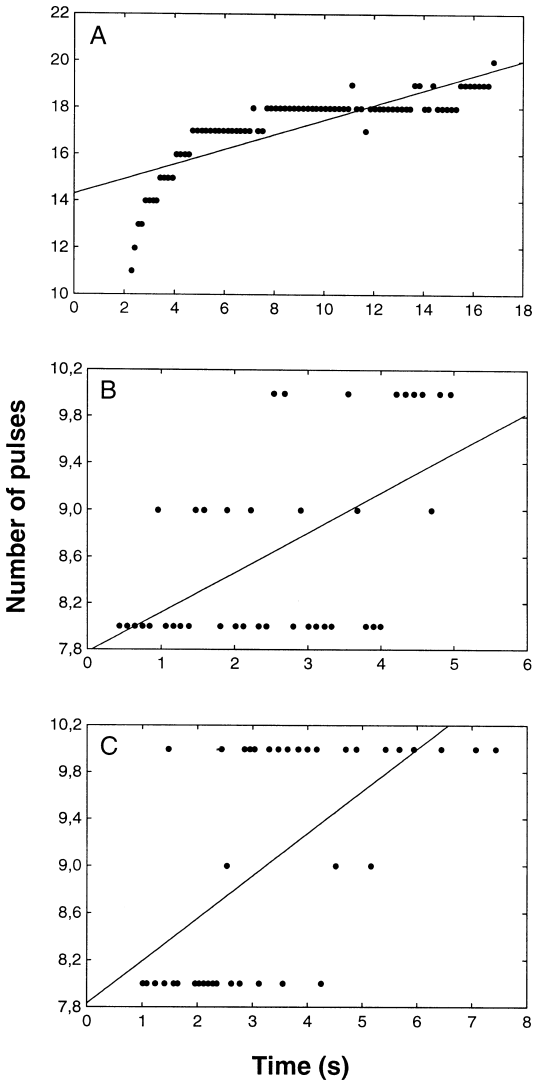

Figure 5 - Correlations between the number of pulses and time of vocalizations of (A) *Chiasmocleis atlantica*, (B) *Chiasmocleis capixaba*, and (C) *Chiasmocleis carvalhoi* (for all Pearson correlations $P = 0.001$).

The dendrogram obtained from the cluster analysis of the acoustic parameters reveals that the species of *Chiasmocleis* that occur in the Atlantic Rainforest comprise two assemblies (Figure 6). One group, composed of *C. atlantica*, *C. leucosticta*, and *C. schubarti*, includes species of medium size (body size range from 19.0–26.3 mm in males; Cruz *et al.* 1997) that have longer calls with lower dominant frequencies. *Chiasmocleis capixaba* and *C. carvalhoi* constitute the other group; these species are smaller (body size range from 14.7–18.3 mm in males; Cruz *et al.* 1997) and have shorter calls with higher dominant frequencies.

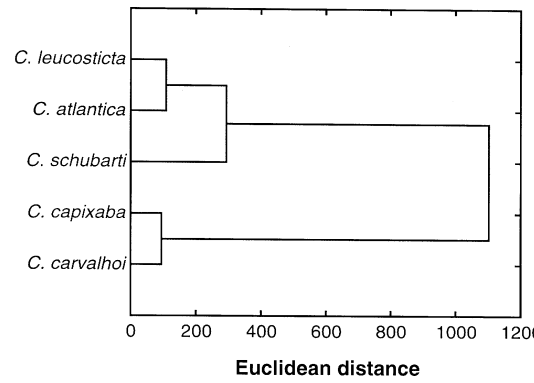

Figure 6 - Cluster analysis (Euclidean distance) among acoustic parameters (call duration and dominant frequency) of advertisement calls of species of the genus *Chiasmocleis* from Atlantic Rainforest.

Discussion

The tadpole of *Chiasmocleis carvalhoi* is similar in coloration and general aspect to the tadpole of *C. ventrimaculata* described and illustrated in Schlüter and Salas (1991). However, the dorsal fin of *C. carvalhoi* originate at the end of the body and is approximately straight for the firsts two thirds of its length, whereas in *C. ventrimaculata* the dorsal fin originates close to tail musculature

and rise in its two thirds of tail. Furthermore, the height of the tail musculature of *C. carvalhoi* is less than that of *C. ventrimaculata* (Schlüter and Salas 1991).

The advertisement call of *Chiasmocleis carvalhoi* described by Hartmann *et al.* (2002) is structurally similar to the one described here, with the exception of the harmonic structure and distinctness of pulses. Differences in some acoustic parameters (e.g., call duration and interval between calls) measured here and in Hartmann *et al*. (2002) resulted from differentiated analysis: in our study each pulsed note was considered a call, while in their study a call corresponded to a series of pulsed notes. Thus, as analyzed herein, call duration did not vary and interval between calls was conservative.

Our study corroborates the monophyly of the genus *Chiasmocleis* proposed by Hartmann *et al*. (2002) based on similarities in advertisement calls. Pulsed calls could represent a synapomorphy for this group, even if polarity of this character and vocalizations of the closely related genera (as proposed by Wild 1995, Hartmann *et al.* 2002) are unknown.

Reproductive aggregations of *Chiasmocleis capixaba* were observed only in temporary ponds, never in the nearby permanent body of water. A similar behavior was also observed in *Chiasmocleis shudikarensis* (Zimmerman and Bogart 1988). The "*Chiasmocleis* rain pools" (as defined by these authors) were exclusively pools on the forest floor and never semi-permanent forest pools or puddles besides streams, which were less ephemeral and more frequently filled. A bout of calling activity on 4 November 2001 of *C. capixaba* lasted eight hours, starting at 19:00 h and finishing at 03:00 h.

The species groups emerging from the cluster analysis seem to reflect the correlation between body size and the acoustic properties of the calls, in which call duration and dominant frequency are positively and negatively correlated with body size, respectively (Zweifel 1968, Zimmerman 1983, Sullivan and Wagner 1988, Giacoma *et al.* 1997). Moreover, in the syntopic species — *C. atlantica* and *C. capixaba* in Reserva Biológica União, RJ, and *C. capixaba* and *C. schubarti* in Reserva Biológica de Duas Bocas, ES — the members of species pairs possess distinct advertisement calls. This observation agrees with Duellman and Pyles' (1983) findings, in which calls of closely related, sympatric species tend to be less similar than those of closely related allopatric species.

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