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Advertisement Call and Tadpole of *Chiasmocleis mantiqueira* Cruz, Feio and Cassini, 2007 (Anura, Microhylidae)

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Abstract.—*Chiasmocleis mantiqueira* is a small microhylid frog recently described from an Atlantic rain forest fragment found in the state of Minas Gerais, southeastern Brazil, whose biology is poorly know. Here, the tadpole and the vocalizations of this species are described from the type locality, along with some information about its natural history. Characteristics of larval external morphology of *C. mantiqueira* are similar to other described tadpoles of the genus: oral disc without keratinized mouthparts, spiracle single, posterior ventral, and medial vent tube. The body is depressed, rectangular in dorsal view and oval in lateral view, and the tail has a flagellum. The species exhibits an explosive breeding behavior, like other Neotropical microhylids. However, unlike other species in this genus, males of *C. mantiqueira* do not have a vocal sac, and its call is composed of a single harmonic, not a pulsed, note. Thus, the characteristics of the call of *C. mantiqueira* contradict the hypothesis that call structure can support the monophyly of *Chiasmocleis*, and further data are required to clarify the relationship of the species in this genus.

The genus *Chiasmocleis* Méhelÿ, 1904, currently contains 25 species distributed from Panamá to southern South America, 20 of these species occurring in Brazil (Funk and Cannatella, 2009; Frost, 2010; Sociedade Brasileira de Herpetologia, 2009). Species are morphologically similar, and consequently the taxonomy of the genus is considered problematic (Peloso and Sturaro, 2008). The use of additional characters may be of great value to clarify the taxonomic status of certain populations and to allow phylogenetic inference. For anurans, larval and bioacoustic characteristics are examples of valuable characters. Larval morphology can provide important contributions to the understanding of amphibian systematics (Cruz, 1982). Anuran advertisement calls are generally species specific (Gerhardt, 1988), and their specificity serves as an isolating mechanism and a useful taxonomic character (Duellman and Trueb, 1994).

The larvae and vocalizations of six species of *Chiasmocleis* have been described: *Chiasmocleis albopunctata* Boettger, 1885; *Chiasmocleis carvalhoi* Cruz, Caramaschi and Izecksohn, 1997; *Chiasmocleis hudsoni* Parker, 1940; *Chiasmocleis panamensis* Dunn, Trapido, and Evans, 1948; *Chiasmocleis shudikarensis* Dunn, 1949; and *Chiasmocleis ventrimaculata* (Andersson, 1945) (Zimmerman and Bogart, 1988; Hero, 1990; Schlüter and Salas, 1991; De La Riva et al., 1996; Lescure and Marty, 2000; Wogel et al., 2004; Schlüter, 2005; Oliveira-Filho and Giaretta, 2006; Vera Candioti, 2006; Rodrigues et al., 2008). For two species only the tadpoles were described: *Chiasmocleis alagoanus* Cruz, Caramaschi, and Freire, 1999, and *Chiasmocleis anatipes* Walker and Duellman, 1974 (Duellman, 1978; Nascimento and Skuk, 2006). For seven species only the advertisement call is known: *Chiasmocleis atlantica* Cruz, Caramaschi and Izecksohn, 1997; *Chiasmocleis avilapiresae* Peloso and Sturaro, 2009; *Chiasmocleis bassleri* Dunn, 1949; *Chiasmocleis capixaba* Cruz, Caramaschi, and Izecksohn, 1997; *Chiasmocleis leucosticta* (Boulenger, 1888); *Chiasmocleis mehelyi* Caramaschi and Cruz, 1997; *Chiasmocleis schubarti* Bokermann, 1952; and *Chiasmocleis supercilialbus* Morales and McDiarmid, 2009 (Nelson, 1973; Hartmann et al., 2002; Wogel et al., 2004; Morales and McDiarmid, 2009; Santana et al., 2009; Barros et al., 2010).

According to Caramaschi and Cruz (1997), the genus *Chiasmocleis* may not be monophyletic. The monophyly of *Chiasmocleis* has not been adequately tested even in recent molecular-based phylogenies of the Microhylidae (Van der Meijden, 2007; only two species were included). However, Harttman et al. (2002) suggest that call similarities among species of *Chiasmocleis* support the monophyly of the genus.

Herein, we describe the tadpole and the advertisement call of *Chiasmocleis mantiqueira* from Parque Estadual da Serra do Brigadeiro, State of Minas Gerais, Brazil. We also present some aspects of the natural history of this species.

Materials and Methods

Study Site.—The Parque Estadual da Serra do Brigadeiro is a conservation unit that covers about 15,000 ha in the Atlantic Rain Forest biome, most of this area consisting of rain forest remnants (Lacerda et al., 2009). Its area ranges over parts of the municipalities of Ervália, Fervedouro, Sericita, Araponga, Miradouro, Pedra Bonita, Muriaé and Divino, in the Zona da Mata region of the state of Minas Gerais, Brazil. The highest portions of a set of mountains integrating the Mantiqueira Mountain Range Complex are inserted in the park, at a maximum height of 1,985 m above sea level (Cruz et al., 2007).

Two aquatic environments were surveyed. The Lagoa Seca (20°42′S and 42°29′W), located in the municipality of Araponga, central region of the park, is a temporary pond at 1,380 m, covering an area of approximately 150 m^2. The water surface is covered by bulrush (Juncaceae), with a rock outcrop covered by herbaceous vegetation and bromeliads. The Lagoa das Bromélias (20°53′S and 42°31′W), located in the southern portion of the park, in municipality of Ervália, is a temporary pond at 1,227 m, with about 200 m^2 of water surface in the wet season. This pond is inserted in a forest patch with rich epiphytic flora mainly represented by Bromeliaceae and Orchidaceae (Caramaschi et al., 2008).

Data Collection.—Tadpoles at stages 26–42 were collected from Lagoa Seca and Lagoa das Bromélias in January, November, and December 2009. The tadpoles were preserved immediately after collection in 10% formalin, and some of them were kept alive until their complete metamorphosis to confirm the identity of the species. Measurements and terminology follow Altig and McDiarmid (1999), and tadpole stages were defined according to Gosner (1960). The morphometric characterization was based on a series of six tadpoles in Stage 36. The measurements in millimeters were obtained using a stereomicroscope with a millimetric ocular.

Males were observed calling at Lagoa das Bromélias on 21 October 2009. Calls of one male were recorded at 2130 h, air temperature 23°C, using a Panasonic RR-US450® digital recorder with an internal microphone with a sampling

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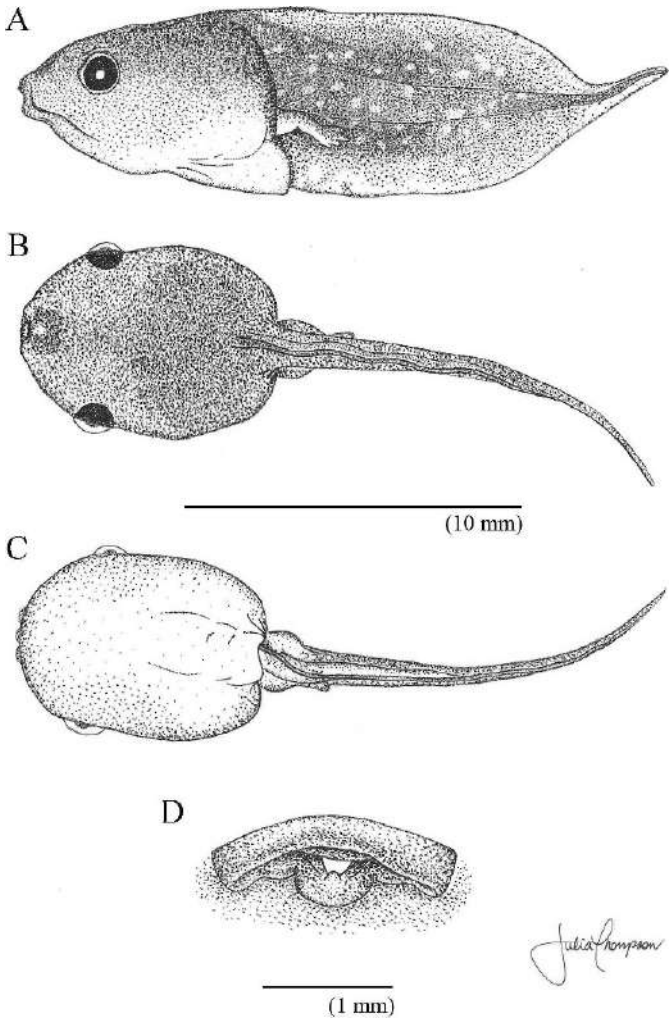

FIG. 1. Tadpole of *Chiasmocleis mantiqueira* (MZUFV 147) at Stage 36: (A) lateral view; (B) dorsal view; (C) ventral view; and (D) oral disc.

frequency of 16,000 Hz and 16-bit resolution. Calls were analyzed with AVISOFT-SASLab Light for Windows (v. 3.74) and SoundRuler (V. 0.9.6.0). Audiospectrograms were produced according to the following parameters: FFT = 256, Frame = 100, Overlap = 75, and flat top filter. The sonogram, oscillogram, and power spectrum were performed in SoundRuler (v. 0.9.6.0). Terminology follows Duellman and Trueb (1994) and Tárano (2001). For comparison among the calls described for the genus, data summarized in Santana et al. (2009), Morales and McDiarmid (2009), and Barros et al. (2010) were used.

Adult voucher specimens and tadpoles of *C. mantiqueira* are housed in the Museu de Zoologia João Moojen, Universidade Federal de Viçosa, Viçosa, state of Minas Gerais, Brazil (adults: MZUFV 10023–10027; 10140–10143; tadpoles: MZUFV Lots 81, 120, 131, 147).

RESULTS

External Morphology of Tadpoles.—The description is based on six tadpoles at Stage 36. The body is depressed, rectangular in dorsal view, oval in lateral view, and about 40% of total length (Figs. 1A, B). The greatest body height is in its posterior third, and greatest width is in its middle third. The snout is rounded in dorsal and ventral view and acuminated in lateral view. Eyes are laterally positioned and directed above the midline of body. Interorbital distance is 4.4–5.2× of eye diameter. Nostrils are absent. Oral disc is terminal, with paired, semicircular labial flaps suspended in front of the mouth and separated by an inverted U-shaped medial notch, without keratinized mouthparts, and a fleshy salience in the medial position of lower jaw (Fig. 1D).

Spiracle single, long, posterior ventral; distal border wide projecting over the vent tube (Fig. 1C). Vent tube medial, with both walls attached directly to the ventral fin and the opening directed ventrally.

Tail length is about 60% of total length; the tail musculature is moderately developed, gradually tapering to the end, and has a flagellum. Dorsal fin emerging on the posterior third of the body originating anteriorly to the body-tail junction. Dorsal and ventral fins similar in height. Measurements of the tadpoles are given in Table 1.

Coloration in preservative: Dorsum is dark brown with a median white spot on the snout region, behind the oral disc. Belly is whitish with a few melanophores that become scarcer in the final third of the body. The tail is transparent with melanophores concentrated on the anterior half of the musculature and dorsal fin. Some individuals may have small white spots (without pigments) in the tail.

Tadpole Variation.—The nostrils are absent until Stage 40. From Stage 41, tadpoles present small nares, circular, dorsally positioned with the opening directed dorsally, without projections on marginal rim.

Advertisement Call.—The advertisement call is composed of a single, not pulsed note (Fig. 2) with a mean duration of 0.04 ± 0.02 sec (0.012–0.072 sec) (mean ± standard deviation [range]). The notes showed marked harmonic structure identifiable in the spectrogram. In the oscillogram, the call is bell-shaped, slightly descending. Mean dominant frequency (taken in the

TABLE 1. Measurements (in millimeters) of 18 tadpoles of *Chiasmocleis mantiqueira* at Gosner Stages 28, 36, and 41. Values are means ± standard deviation; range is given in brackets.

Characters	Stage 28 (N = 6)	Stage 36 (N = 6)	Stage 41 (N = 6)
Total length	14.17 ± 0.85 (13.66–15.84)	19.98 ± 0.70 (19.11–21.19)	22.57 ± 1.06 (21.48–23.96)
Body length	5.38 ± 0.23 (5.25–5.84)	8.00 ± 0.22 (7.72–8.32)	8.42 ± 0.37 (8.02–8.91)
Body height	3.60 ± 0.18 (3.37–3.86)	5.05 ± 0.20 (4.85–5.35)	4.83 ± 0.24 (4.55–5.15)
Body width	4.37 ± 0.17 (4.16–4.65)	6.06 ± 0.19 (5.84–6.34)	6.29 ± 0.30 (5.74–6.63)
Tail length	8.79 ± 0.63 (8.32–10.00)	11.98 ± 0.59 (11.19–12.97)	14.16 ± 1.22 (12.67–15.54)
Maximum tail height	4.60 ± 0.19 (4.46–4.95)	5.97 ± 0.48 (5.25–6.53)	6.19 ± 0.31 (5.64–6.53)
Tail muscle height	1.72 ± 0.15 (1.49–1.88)	2.84 ± 0.68 (2.08–3.76)	2.48 ± 0.29 (2.18–2.97)
Dorsal fin height	1.37 ± 0.12 (1.29–1.58)	1.90 ± 0.18 (1.58–2.08)	2.08 ± 0.11 (1.98–2.18)
Ventral fin height	1.72 ± 0.17 (1.49–1.98)	1.95 ± 0.15 (1.78–2.08)	2.23 ± 0.17 (1.98–2.48)
Eye diameter	0.86 ± 0.05 (0.79–0.89)	1.16 ± 0.08 (1.09–1.29)	1.17 ± 0.07 (1.09–1.29)
Interorbital distance	4.24 ± 0.19 (4.06–4.55)	5.61 ± 0.18 (5.25–5.74)	5.59 ± 0.38 (4.95–5.94)
Internarial distance	–	–	0.91 ± 0.07 (0.79–0.99)

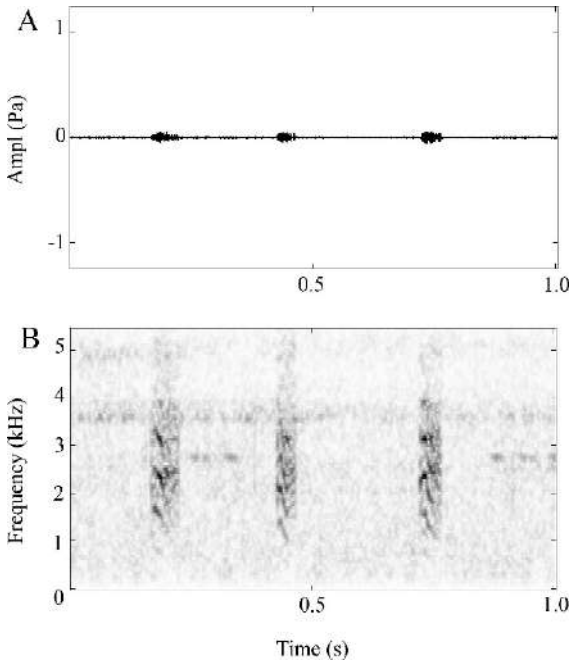

FIG. 2. Advertisement call of *Chiasmocleis mantiqueira*: (A) oscillogram and (B) audiospectrogram of three notes (air temperature = 23°C).

point of maximum energy of the oscillogram) is 3,146.23 ± 829 Hz (1,884.06–4,944.23 Hz).

Notes on Reproduction and Natural History.—In Lagoa Seca, tadpoles were found in November, and represents the first record from the type locality, increasing the upper altitudinal range for this species from 1,227 m to 1,380 m. In Lagoa das Bromélias, tadpoles were found in January, November, and December. In November, tadpoles were abundant and distributed throughout the pond, not forming aggregations or schools (Fig. 3A). Most of the larvae were at early developmental stages (28–33). In December, most of the larvae were at later stages (36 to 39) and were less abundant. No tadpole was observed in January 2010, although they were abundant in January 2009 when the pond was completely full. Predation events by water bugs (Hemiptera: Belostomatidae) and Odonata larvae were also observed during this month.

Adult individuals of both sexes were found at Lagoa das Bromélias on 20 October 2009 at 1820 h, hidden on the forest leaf litter around 5 m from the pond, without any evidence of reproductive activity. However, on the night of 21 October at about 1900 h, several individuals were observed aggregated around the pond margins, the majority of them males, located both in the leaf litter and on a rock near to the pond edge (Fig. 3B). Males were calling simultaneously, and some specimens of both sexes were found hidden under rocks, where the males were also emitting the call. By around 2100 h, many individuals were observed floating on the water surface. Mating pairs were not observed, although some collected females exhibited mature eggs.

DISCUSSION

Characteristics of larval external morphology of *C. mantiqueira*, such as oral disc, eye position, spiracle and vent tube, are similar to the described tadpoles of the genus. However, the tadpole of *C. mantiqueira* differs from that of *C. alagoanus* by the absence of a pair of dark spots in an arc shape around the interocular region (Nascimento and Skuk, 2006), and from *C. carvalhoi* and *C. ventrimaculata* by the absence of a pair of curved, bracket-shaped and light-cream lines between the eyes (Schlüter and Salas, 1991; Wogel et al., 2004). The coloration of *C. mantiqueira* is also distinct from that of *C. anatipes* (body olive-tan dorsally and yellowish-white ventrally; Duellman, 1978) and *C. panamensis* (reddish-brown dorsum and caudal musculature, with a longitudinal white line at the base of the tail; Vera-Candioti, 2006). It differs from *C. albopunctata* by the absence of wart-ornamented flaps at the border (Oliveira-Filho and Giaretta, 2006) and from *C. hudsoni* by the presence of a flagellum at the end of the tail (Rodrigues et al., 2008). The tadpole of *C. shudikarensis* was not described with sufficient detail for comparison (Hero, 1990).

Although based on calling from a single male, and therefore limited in inference, the nonpulsed call presents a harmonic structure differentiating the vocalization of *C. mantiqueira* from all the other species of the genus (see Santana et al., 2009; Morales and McDiarmid, 2009). Hartmann et al. (2002) considered the pulsed call structure (a qualitative parameter) to support the monophyly of the genus *Chiasmocleis*, the hypothesis followed and reinforced later by Wogel et al. (2004) and Santana et al. (2009). The present finding does not support the hypothesis of Hartmann et al. (2002). We verified the male specimens collected and some of the paratopotypes (MZUFV 7312–7316) in search of vocal sacs and vocal slits, because Cruz et al. (2007) did not mention this character in the diagnosis of the species. Although for several species of *Chiasmocleis* these are

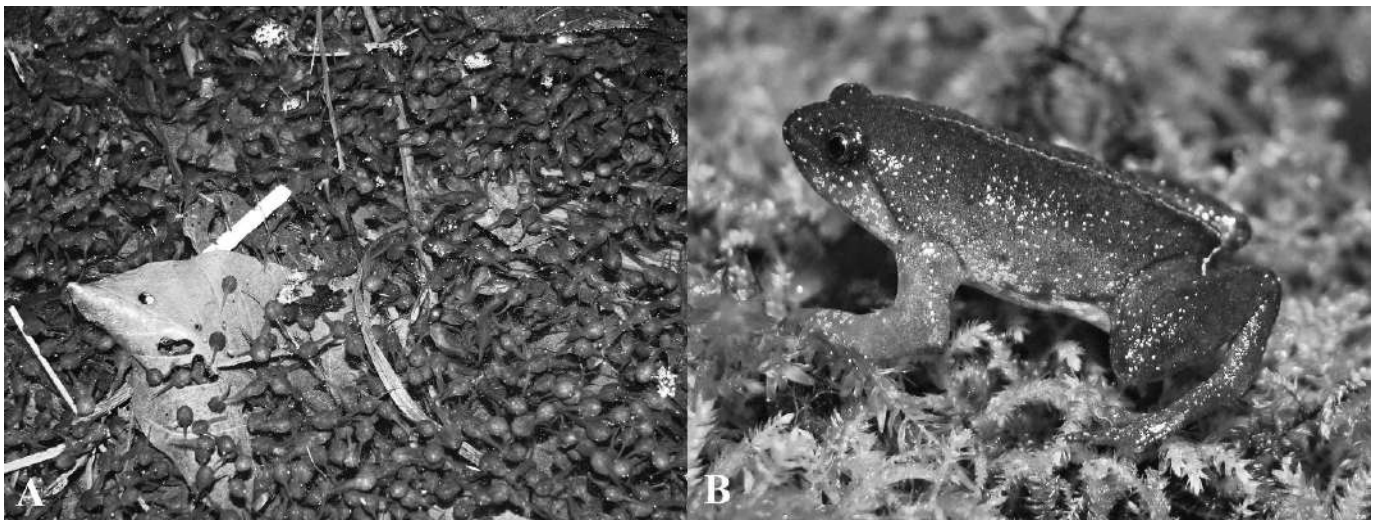

FIG. 3. Individuals of *Chiasmocleis mantiqueira* from type locality: (A) tadpoles and (B) adult male. Photos by J. S. Dayrell and E. T. Silva.

conspicuous traits (e.g., *Chiasmocleis gnoma*, *C. ventrimaculata*, *C. albopunctata*, *C. avilapiresae*; Canedo et al., 2004; Schlüter, 2005; Oliveira-Filho and Giaretta, 2006; Peloso and Sturaro, 2008), no evidence of vocal sac or vocal slits were found in *C. mantiqueira*.

Therefore, we associated the call structure of *C. mantiqueira* with two hypotheses. First, the absence of vocal sac and vocal slits may influence the call structure. Walkoviak (2007) cited that the acoustical characteristics of anuran calls are determined by the mechanical properties of the larynx and the sound-emitting apparatus. Various anuran species lack vocal sacs, and some further-derived frogs have lost vocal sacs secondarily (Wells, 2007). In some genera, some species have vocal sacs and long-distance vocal signals, but close relatives breeding in similar habitats do not (Duellman, 1970; Penna and Veloso, 1990), which could be the case here, considering the monophyly of *Chiasmocleis*. Thus, the call of *C. mantiqueira* could be used as a close-range communication, as stated by Wells (2007) for some anurans that lack vocal sacs.

Chiasmocleis mantiqueira is an explosive breeder, according to the classification of Wells (1977). This corroborates what has been observed for several other microhylid species (Rodríguez and Duellman, 1994; Kwet and Di-Bernardo, 1998; Rodrigues et al., 2003). The lack of both vocal sacs and long-distance vocal signals seems to be associated with explosive breeding behavior in permanent water or in sites that do not change in location from year to year (Wells, 1977, 2007). This last kind of aquatic site is the case of the temporary ponds where *C. mantiqueira* was found.

Another hypothesis is related to the possible paraphyly of the genus, as previously suggested by Caramaschi and Cruz (1997), because within the monophyletic cluster that contains *Chiasmocleis* according to Wild (1995), the call structure of its sister taxa (*Synapturanus*, *Myersiella*, and *Arcovomer*) is harmonic, and these genera also do not have vocal sacs (Hartmann et al., 2002). Nevertheless, the call of *C. mantiqueira* sounds like a wheezing, and for *Synapturanus*, *Myersiella*, and *Arcovomer*, it sounds like a whistle (Nelson, 1973; Hartmann et al., 2002; Giaretta and Martins, 2009).

Recently, two phylogenetic proposals based on molecular data were presented for Microhylidae (Frost et al., 2006; Van der Meijden et al., 2007). However, Frost et al. (2006) did not include *Chiasmocleis* in their analysis. On the other hand, in the proposal of Van der Meijden et al. (2007), *Chiasmocleis* is considered as the sister group of the other South American Microhylidae (except *Otophryne*). Nevertheless, these authors did not include *Synapturanus*, *Myersiella*, or *Arcovomer* in their phylogenetic proposal, these being genera close to *Chiasmocleis* according to Wild (1995). Thus, it is not possible to compare the call structure between *Chiasmocleis* and these genera based on these recent phylogenies, as Hartmann et al. (2002) did using the phylogeny presented by Wild (1995).

Heyer (1971) suggested that the prototype for the microhylid call would be composed of several pulses per note over a rather broad frequency range. However, Frost et al. (2006) suggested *Kalophrynus* and *Synapturanus* as Microhylidae basal genera, and neither possesses a pulsed call (Matsui et al., 1996; Menin et al., 2007). Thus, it is possible that the basal call for the family may be, in fact, not pulsed, and the call of *C. mantiqueira* could be a character reversion. Unfortunately, the analysis of Frost et al. (2006) was not detailed enough to reinforce this hypothesis (several genera were not included). Further phylogenetic studies that include all South American microhylid genera are necessary to better analyze the evolutionary history of the group, aiding the understanding of the distribution pattern of the call structure within the family. Likewise, additional information on other characters is necessary to clarify the phylogenetic relationship of *C. mantiqueira* in relation to the other *Chiasmocleis* species or even of the genus *Chiasmocleis* with its sister genera.

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