



Larval morphology and advertisement call of *Phyllodytes acuminatus* Bokermann, 1966 (Anura: Hylidae) from Northeastern Brazil

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Abstract

This paper describes the tadpole and advertisement call of *Phyllodytes acuminatus*, based on specimens from the Parque Nacional do Catimbau, in the municipality of Buíque, State of Pernambuco, Northeastern Brazil. The overall morphology of *P. acuminatus* tadpole is similar to that of most species of the genus. The presence of a double row of marginal papillae surrounding all the oral apparatus (except on most of the upper labium which has a dorsal gap) was a characteristic that differentiate *P. acuminatus* from the other species of the genus. Furthermore, the call structure of the species (unpulsed notes with harmonic structure) fits it in the group composed of *P. kautskyi* and *P. melanomystax*.

Key words: Lophiohylini, tadpole, vocalization, Parque Nacional do Catimbau, Caatinga

Resumo

O girino e o canto de anúncio de *Phyllodytes acuminatus* são descritos baseados em espécimes provenientes do Parque Nacional do Catimbau, no município de Buíque, estado de Pernambuco, nordeste do Brasil. A morfologia geral de *P. acuminatus* é similar a das demais espécies do gênero. A presença de uma fileira dupla de papilas marginais circundando o aparato oral (exceto em parte do lábio superior) foi uma característica que diferenciou *P. acuminatus* das demais espécies do gênero. Além disso, a estrutura do canto (nota sem pulsos com estrutura harmônica) alocou a espécie no grupo composto por *P. kautskyi* e *P. melanomystax*.

Palavras-chave: Lophiohylini, girino, vocalização, Parque Nacional do Catimbau, Caatinga

Introduction

One of the major obstacles in amphibian surveys during faunal inventories is identifying the larval forms (Rossa-Feres & Nomura 2006). Tadpoles are more easily located and captured than adults given that they remain longer at the reproduction site (Altig & McDiarmid 1999a). As such, knowledge of larval morphology can be an important source of information for taxonomic, natural history, and ecological studies of anuran species (Heyer *et al.* 1990; Duellman & Trueb 1994; Altig & McDiarmid 1999b). Additionally, amphibian advertisement call (*sensu* Wells 1977) plays a number of different roles, including announcing their location within the reproduction site to other individuals, attracting reproductive females, defending the courtship site and signaling aggressive behavior (Fouquette 1960; Littlejohn & Lofthus-Hills 1968; Haddad & Cardoso 1992); it can also be used in the taxonomic elucidation of closely related species (*e.g.* Pombal *et al.* 1995; Napoli & Cruz 2005; Nunes *et al.* 2007).

The genus *Phyllodytes* Wagler, 1830 is currently composed of 11 species with distribution restricted to Eastern

Brazil (Frost 2013). All of them are characterized by having odontoids on the mandible and use bromeliads for refuge, mating, egg laying, and larval development (Bokermann 1966; Caramaschi *et al.* 1992; Faivovich *et al.* 2005). *Phyllodytes acuminatus* Bokermann, 1966 is distributed only in the states of Alagoas and Pernambuco, Northeastern Brazil, in the Atlantic Rain Forest and Caatinga biomes (Santos & Silva 2010; Frost 2013). It is a moderate-sized species for the genus (SVL 21.1–24.9 mm) with a pointed snout in dorsal view and subacute in lateral view, no vestige of a membrane and poorly developed adhesive discs, abdomen with few large tubercles, and males with two pairs of highly developed odontoids (Cardoso 2009).

With the aim of finding new useful characters to help understand the systematic relationships of *Phyllodytes acuminatus*, we herein describe its larval external morphology and advertisement call structure. We compare these features with known tadpoles and advertisement calls of other species in the genus.

Material and methods

Tadpole collection and call recordings of *Phyllodytes acuminatus* were conducted in Parque Nacional do Catimbau (08°29'24.4" S, 37°16'59.3" W, 863 m a.s.l), Buíque Municipality, Pernambuco State, Northeastern Brazil, in June 2010. Tadpole description was based on an individual at stage 38 (Gosner 1960). *Phyllodytes acuminatus* is the only species of the genus recorded at the study site. Morphometry, ontogenetic changes, and individual variations were based on three lots gathering 10 tadpoles between stages 25 and 42. Terminology and morphometric characteristics analyzed follow Altig and McDiarmid (1999b), except for interorbital and internasal distance (measured from the inner edges of the eyes and nostrils), snout-eye distance (measured from the anterior edge of the eyes to the midpoint of the snout), and nostril-eye distance (measured between the posterior edge of the nostril and anterior edge of the eye). All values are in millimeters and were measured with a Zeiss stereomicroscope ocular micrometer, with the exception of total length and body length, height and width, which were measured with 0.1 mm calipers. Methylene blue staining (2%) was used to enhance the visualization of the oral disc papillae.

Advertisement calls of two males were recorded with a Marantz PMD 660 portable digital recorder and a Sennheiser ME65/K6 directional microphone on 3 June 2010, between 19:00h and 21:00h, at an air temperature of 18 °C. Calls were digitalized with a sampling frequency of 44.1 kHz and a resolution of 16 bits, and then analyzed using the Cool Edit Pro 2.1 software. The oscillogram, spectrogram, and power spectrum were produced with the SoundRuler software (version 0.9.6.0) using the following parameters: FFT=512; Overlap=75%; Window=Hanning and Contrast=0.6. The terminology used to describe the call is in accordance with Duellman and Trueb (1994) and Gerhardt (1998).

Voucher specimens are deposited in the herpetological collection of Museu de História Natural da Universidade Federal de Alagoas (lots MUFAL 8383–85, tadpoles; MUFAL 8386–87, adults). Calls are deposited in the herpetological collection of the Universidade Federal Rural de Pernambuco (CHUFRPE V0065, digital archive).

Results

Tadpole description (Fig.1). Body is depressed (wider than high), ovoid in lateral and ventral views, with maximum height at the middle third of the body, just behind the spiracle and with maximum width in the middle third of the body; body length corresponding to approximately 41% of total length. Snout rounded in dorsal and ventral views, slightly pointed in lateral view. Eyes dorsally positioned and dorsolaterally directed; located at the end of the upper third of the body, eye diameter representing 60% of interorbital distance and 5.6% of body length. Nostrils, circular, positioned dorsolaterally and antero-laterally directed, situated in two small cavities at nostril level, closer to the snout than the eyes; internasal distance representing 41% of interorbital distance. Spiracle sinistral, at the middle third of the body length and below the midline of the body height, directed posterodorsally at a 45° angle to the midline of the body and with a circular opening, inner wall fused to body, with its extremity being free and longer than the external wall. Vent tube central, attached to the ventral fin, with opening facing the right side. Oral apparatus (Fig. 1D) in anteroventral position, non-emarginated, surrounded by a double row of marginal papillae, with a dorsal gap. Papillae are small and conical. Several submarginal papillae are concentrated

laterally and adjacent to the marginal papillae. Labial tooth row formula (LTRF) is 2(2)/4; A1 and A2 of equal size. P1, P2 and P3 of equal size. P4 is slightly smaller than the others. Jaw sheaths are pigmented, with finely serrated edges. Upper jaw arch-shaped and lower jaw is slightly concave.

Maximum tail musculature height represents 44% of body height and tapers towards the tip of the tail; myomeres and myosepta are visible along the entire length of the tail. Dorsal and ventral fins are approximately equal in size. Dorsal fin begins at the body-tail junction, initially parallel to the body, extending upward to the end of the middle third and continuing downward to the tip of the tail. Ventral fin begins at the end of the body, proceeding in a straight line through the middle third where it converges towards the tip of the tail, which is slightly rounded. The lateral line system is not visible.

Measurements (in mm; N=1; Stage 38). Total length 26.0; body length 10.6; body height 5.4; body width 6.9; tail length 15.3; tail height 3.9; dorsal fin height 0.8; ventral fin height: 0.8; tail muscle height: 2.4; snout-eye distance: 1.3; internasal distance: 0.4; nostril-eye distance: 0.6; eye diameter: 0.6; interorbital distance: 1.0; oral apparatus width: 1.4; spiracle length: 1.1, and vent tube length: 1.3. Table 1 summarizes the measurements of the remaining tadpoles described.

TABLE 1. Measurements (in mm) of 17 morphometric characters of seven different stages of the *Phyllodytes acuminatus* tadpoles (lots MUFAL 8383–85) from Parque Nacional do Catimbau, Municipality of Buíque, Pernambuco State, Brazil. (mean±standard deviation). Legend: stage (St), total length (TL), body length (BL), body height (BH), body width (BW), tail length (TL), tail height (TH), dorsal fin height (DFH), ventral fin height (VFH), tail muscle height (TMH), snout-eye distance (SED); internasal distance (ID), nostril-eye distance (NED); eye diameter (ED), interorbital distance (ID), oral apparatus width (OAW), spiracle length (SL) and vent tube length (VTL).

Characters	St 28 (N=1)	St 30 (N=1)	St 33 (N=1)	St 36 (N=1)	St 38 (N=3)	St 39 (N=1)	St 42 (N=2)
TL	20.5	22.9	21.3	24.4	25.7±0.5	27.1	27.7±0.0
BL	8.2	9.9	9.4	9.5	10.8±0.3	11.6	18.8±1.6
BH	4.6	3.6	5.2	4.4	5.3±0.4	5.6	3.2±0.1
BW	5.2	6.1	6.4	6.7	7.0±0.3	7.9	6.7±0.3
TL	12.3	13.1	11.9	14.8	14.9±0.4	15.5	14.9±1.6
TH	2.6	3.8	4.0	3.1	3.6±0.9	2.8	3.2±2.1
DFH	0.8	1.5	1.2	0.4	0.9±0.3	1.1	1.4±0.4
VFH	1.2	1.1	1.1	0.5	1.0±0.2	0.8	1.2±0.4
TMH	2.0	2.1	1.7	3.1	2.2±0.3	2.5	1.8±0.2
SED	1.5	1.9	2.2	1.6	1.8±0.5	1.2	2.2±0.3
ID	0.3	0.4	1.2	0.3	0.7±0.5	0.4	1.0±0.7
NED	0.5	1.5	1.1	0.7	0.7±0.3	0.6	1.0±0.0
ED	0.5	1.0	1.0	0.6	0.8±0.3	0.7	1.0±0.2
ID	0.9	1.2	2.2	0.9	1.4±0.6	0.9	1.7±0.4
OAW	1.8	2.0	2.5	1.3	1.7±0.6	1.4	1.4±0.1
SL	0.9	–	0.9	1.0	0.8±0.3	0.9	–
VTL	0.7	–	1.0	1.1	1.0±0.4	0.6	–

Coloration. In formalin, the body is translucent, with grayish-brown and black punctuations distributed uniformly on dorsum and lateral regions. The intestinal spiral is partially visible on ventral surface. The tail is also translucent with star-shaped black spots distributed over the entire surface. Oral apparatus has the same star-shaped black spots on the anterior lip and on each side of the jaw sheath.

Ontogenetic variation (in comparison with stage 38). In stage 26, the typical LTRF is still not developed with only three posterior rows; the marginal papillae are smaller and less numerous. In stage 42 there is an increase in the number of black punctuations on the body, giving it a much darker appearance; the vent tube is no longer visible and the caudal fins are much lower. In stage 43, internasal distances decrease considerably. The dark

coloring extends into limbs and tail. A black line emerges from the tympanum to the nostrils, a distinguishing feature of the adults. Rows of ventral granules, also characteristic of the adults, begin to appear on the abdomen; denticles and jaw sheath disappear.

Natural history notes. Tadpoles were found in bromeliads (*Encholirium spectabile* and *Aechmea leptantha*), usually a single individual per plant. However, some bromeliads contained two individuals, normally at different stages of development. Eggs were laid on the inner surface of bromeliad leaves, near the central tank, similar to that described by Bokermann (1966).

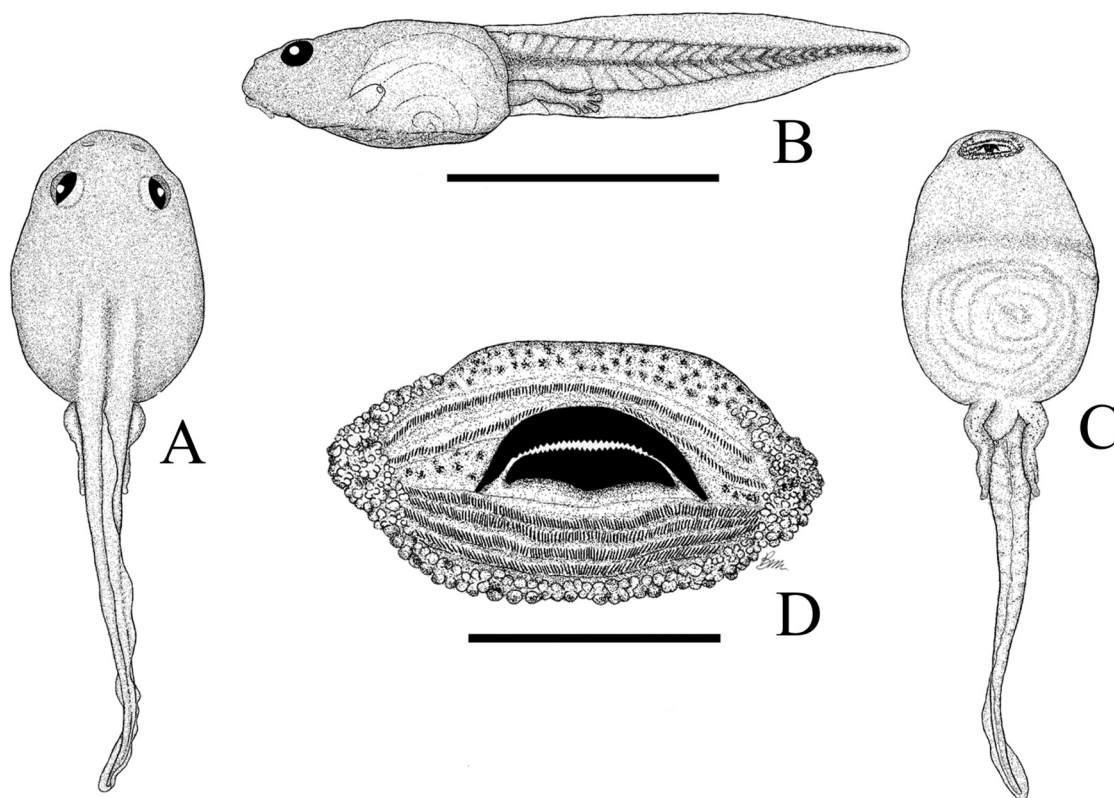


FIGURE 1. Tadpole of *Phyllodytes acuminatus* at Gosner stage 38 (lot MUFAL 8385) from Parque Nacional do Catimbau, Municipality of Buíque, Pernambuco state, Brazil. (A) dorsal, (B) lateral, and (C) ventral views, scale bar = 10 mm; (D) oral apparatus, scale bar = 1 mm.

Advertisement call (Fig. 2). The advertisement call of *Phyllodytes acuminatus* (N=90 calls; two males) consists of a single note, emitted in sequences of one to four (a three-note bout is the most frequent), presenting three to seven detectable harmonics. The dominant frequency oscillated between the fundamental frequency [\bar{x} = 2.14 ± 0.09 kHz (2.07–2.33), N=25] and the second harmonic [\bar{x} = 4.28 ± 0.15 kHz (4.05–4.57), N=65]. A total of 7% of calls analyzed exhibited a third harmonic [\bar{x} = 6.70 ± 0.22 kHz (6.29–6.89), N=6]; 19% exhibited a fourth harmonic [\bar{x} = 8.45 ± 0.32 kHz (8.10–9.22), N=17]; 32% exhibited a fifth harmonic [\bar{x} = 10.76 ± 0.49 kHz (8.96–11.36), N=29]; 41% exhibited a sixth harmonic [\bar{x} = 12.66 ± 0.40 kHz (12.05–13.52), N=37], and 1% exhibited a seventh harmonic [\bar{x} = 14.55 kHz, N=1]. Call duration ranges from 0.03 to 0.17s (\bar{x} = 0.10 ± 0.03, N=90). The interval between calls was 0.27–0.47s (\bar{x} = 0.35 ± 0.05, N=66) and between sequences of calls was 6.03–53.4s (\bar{x} = 18.99 ± 11.18, N=34).

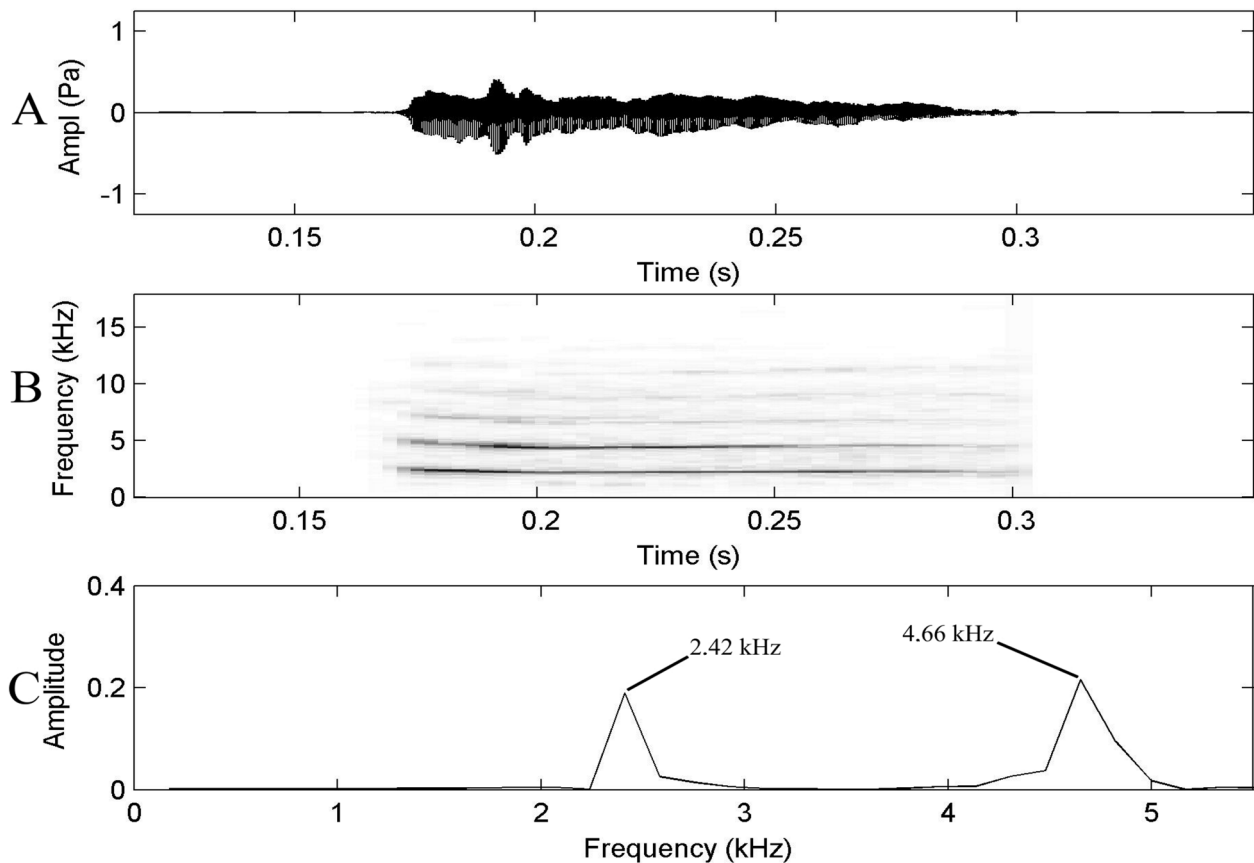


FIGURE 2. Advertisement call of *Phyllodytes acuminatus* (MUFAL 8386) recorded in the Parque Nacional do Catimbau, Municipality of Buíque, Pernambuco State, Brazil, on June 3, 2010, 19:50h. Air temperature 18 °C. (A) oscillogram, (B) spectrogram and (C) power spectrum.

Discussion

Six of the 11 species of *Phyllodytes* have described tadpoles: *P. brevirostris* Peixoto and Cruz, 1988 (Vieira *et al.* 2009), *P. edelmoi* Peixoto, Caramashi, and Freire, 2003 (Peixoto *et al.* 2003), *P. luteolus* (Wied-Neuwied, 1824) (Bokermann 1966), *P. melanomystax* Caramaschi, Silva and, Britto-Pereira, 1992 (Caramaschi *et al.* 1992), *P. tuberculosis* Bokermann, 1966 (Bokermann 1966) and *P. gyrinaethes* Peixoto, Caramashi, and Freire, 2003 (Peixoto *et al.* 2003). The overall morphology of the tadpoles of *P. acuminatus* is similar to those of *P. brevirostris*, *P. edelmoi*, *P. luteolus*, and *P. tuberculosis*, in contrast to *P. melanomystax*, which shows the ventral fin originating at the end of anterior third of the tail (at the end of the body in *P. acuminatus* and in the other species), and *P. gyrinaethes*, which has an unusual general morphology. The presence of a double row of marginal papillae surrounding all the oral apparatus (except on most of the upper labium which has a dorsal gap) is a unique characteristic of *P. acuminatus* (a single row is present in *P. luteolus*, *P. tuberculosis* and *P. gyrinaethes*; one dorsally and two laterally and ventrally in *P. brevirostris*; one or two laterally and two or three ventrally in *P. edelmoi*; and two dorsolaterally and one ventrally in *P. melanomystax*) (Table 2). With the exception of *P. gyrinaethes*, the larvae of the genus now described exhibit the following characters: (1) body depressed and ovoid in dorsal and ventral views; (2) snout rounded in dorsal view; (3) eyes dorsal; (4) oral apparatus with one-three rows of marginal papillae and a large dorsal gap; (5) LTRF 2(2)/3; 2(2)/4 or 2(2)/5[6]; and (6) caudal tip broadly rounded (except in *P. luteolus*).

TABLE 2. Comparisons of external larval characters among *Phyllodytes* species. Characters followed by an asterisk (*) were inferred from the illustrations, but not stated in the text by the authors.

Species	Maximum total length in mm (stage)	Presence of a lateral constriction on the body	Snout shape in dorsal view	Number of rows of marginal papillae	Labial tooth row formula	Origin of the dorsal fin	Caudal tip	Source
<i>P. acuminatus</i>	24.4 (36)	No	Rounded	Two	2(2)/4	Body-tail junction	Slightly rounded	This study
<i>P. brevirostris</i>	25.3 (35)	No	Rounded	One dorsally and two laterally and ventrally	2(2)/5	Body-tail junction*	Broadly rounded*	Vieira <i>et al.</i> (2009)
<i>P. edelmoi</i>	27.1 (28)	No	Rounded	One or two laterally and two or three ventrally	2(2)/5[6]	Body	Slightly rounded	Peixoto <i>et al.</i> (2003)
<i>P. luteolus</i>	29.0 (36)	Yes	Rounded*	One	2(2)/4	Tail	Acute*	Bokermann (1966)
<i>P. melanomystax</i>	34.6* (36)	Yes	Rounded	Two dorsolaterally and one ventrally	2(2)/3	Body	Slightly rounded*	Caramaschi <i>et al.</i> (1992)
<i>P. tuberculosis</i>	31.0 (35)	No	Rounded	One	2(2)/4	Tail	Slightly rounded*	Bokermann (1966)
<i>P. gyrinaethes</i>	30.0 (34)	Yes	Flat, with a deep indentation on anterior margin	One	1(1)/5	Body-tail junction	Slightly rounded	Peixoto <i>et al.</i> (2003)

The advertisement call of six species of *Phyllodytes* has been previously described: *P. edelmoi* (Lima *et al.* 2008), *P. gyrinaethes* (Roberto & Ávila 2013), *P. kautskyi* Peixoto and Cruz, 1988 (Simon & Gasparini 2003), *P. luteolus* (Weygoldt 1981), *P. melanomystax* (Nunes *et al.* 2007) and, *P. tuberculosis* (Juncá *et al.* 2012). The main differences between the advertisement call of the *Phyllodytes acuminatus* and the remaining species of the genus are: (1) call duration (*P. acuminatus* shorter than *P. tuberculosis*, *P. edelmoi*, *P. luteolus*, *P. kautskyi*, *P. gyrinaethes*, and slightly higher than *P. melanomystax*); (2) number of notes (fewer in *P. acuminatus* than *P. edelmoi*, *P. tuberculosis*, *P. gyrinaethes*, and similar to *P. melanomystax* and *P. kautskyi*); and (3) call structure (which is pulsed in *P. edelmoi*, *P. gyrinaethes*, *P. luteolus*, and *P. tuberculosis*, and unpulsed with harmonic structure in *P. acuminatus*, *P. kautskyi*, and *P. melanomystax* (Table 3).

TABLE 3. Comparisons of some call parameters among *Phyllodytes* species.

Species	Call duration (s) (mean±SD)	Number of notes per call	Call structure	Dominant frequency range (kHz)	Source
<i>P. acuminatus</i> (2 males)	0.10±0.03	1	Unpulsed, with harmonics	2.07–4.57	This study
<i>P. edelmoi</i> (4 males)	5.20±0.44	22–29	Pulsed	1.49–3.32	Lima <i>et al.</i> (2008)
<i>P. gyrinaethes</i> (2 males)	1.70±0.30	4–6	Pulsed	2.53–3.09	Roberto & Ávila (2013)
<i>P. kautskyi</i> (1 male)	3.55±0.19	1	Unpulsed, with harmonics	0.87–1.81	Simon & Gasparini 2003
<i>P. luteolus</i> (N=not available)	≅5	8–15	Pulsed	2–6	Weygoldt 1981
<i>P. melanomystax</i> (3 males)	0.07±0.04	1	Unpulsed, with harmonics	1.39–3.11	Nunes <i>et al.</i> 2007
<i>P. tuberculosis</i> (3 males)	6.72±1.73	14–23	Pulsed	2.15–3.27	Juncá <i>et al.</i> 2012

Phyllodytes acuminatus presented the dominant frequency oscillating between values of fundamental frequency and dominant frequency, a feature also recorded by Nunes *et al.* (2007) for *P. melanomystax*. Furthermore, the call of *P. acuminatus* agrees with that of *P. luteolus* and *P. melanomystax*, none showing frequency modulation. This is in contrast with *P. kautskyi* that exhibits ascending modulation in the first half of the call and descending in the second half.

Caramaschi *et al.* (2004) proposed three species groups for *Phyllodytes* based on adult color pattern. *Phyllodytes acuminatus* is allocated to the *P. luteolus* species group along with *P. brevirostris*, *P. edelmoi*, *P. kautskyi*, *P. wuchereri* (Peters, 1873 “1872”) and *P. melanomystax*; although there is no evidence about the monophyly of these groups (Faivovich *et al.* 2005). Recently, Roberto and Ávila (2013), based on advertisement call data of *Phyllodytes* species, propose two different species groups: one with unpulsed notes with harmonic structure composed by *P. kautskyi* and *P. melanomystax* (*P. kautskyi* species group); and another with pulsed notes, composed by *P. edelmoi*, *P. luteolus* and *P. gyrinaethes* (*P. luteolus* species group). The call structure of *P. acuminatus* fits it in the group of *P. kautskyi* (unpulsed notes with harmonic structure). On the other hand, there is no clear concordance between the tadpole morphology and these two proposed groups, being necessary to properly test them to shed light on the phylogenetic relationships of the genus.

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