

# Advertisement and combat calls of the glass frog *Centrolene lynchi* (Anura: Centrolenidae), with notes on combat and reproductive behaviors

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## Abstract

**Advertisement and combat calls of the glass frog *Centrolene lynchi* (Anura: Centrolenidae), with notes on combat and reproductive behaviors.** We describe the advertisement and combat calls, combat behavior, and reproductive behavior of the glass frog *Centrolene lynchi*, an endemic species of the cloud forests of Ecuador. The typical advertisement call consists of a tonal note and 1–3 peaked notes, each with 1–3 pulses. Variations on this advertisement call include the addition of a short note before the tonal note, as well as the absence of the tonal note. These modifications may be similar to the described call modifications of other anurans in response to the presence of conspecific females and males, potential predators and/or changes in the sound environment. Additionally, we describe the combat call emitted during previously undescribed venter-to-venter combat between males. The discovery of this combat behavior is consistent with the hypothesis that venter-to-venter combat behavior is a shared derived trait of the subfamily Centroleninae. Finally, we describe male reproductive habits and development of the species, including egg attendance by males and implied male parental care.

**Keywords:** Bioacoustics, call description, combat behavior, glass frog, parental care.

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## Resumen

**Canto de anuncio y de combate de la rana de cristal *Centrolene lynchi* (Anura: Centrolenidae), con notas sobre su comportamiento de combate y reproductivo.** Se describen los cantos de anuncio y de combate de la rana de cristal *Centrolene lynchi*, una especie endémica de los bosques nublados del Ecuador. El canto de anuncio típico consiste en una nota tonal seguida por 1–3 notas pulsadas, cada una de las cuales tiene 1–3 pulsos. Entre la variación observada en el canto de anuncio está la adición de una nota corta que precede a la tonal, así como también la ausencia de esta última. Estas modificaciones son similares a las observadas en otros anuros y pueden estar relacionadas a la presencia de hembras, otros machos, depredadores y/o cambios en el ruido ambiental. Adicionalmente, se describe el canto de combate emitido durante las peleas entre machos. El combate entre machos es similar al observado en otras especies de la subfamilia Centroleninae y ha sido hipotetizado como una sinapomorfía de la misma. Finalmente, se presenta información sobre los hábitos reproductivos de los machos, cuidado parental y desarrollo de las puestas de huevos.

**Palabras claves:** Bioacústica, comportamiento de combate, cuidado parental, descripción de cantos, rana de cristal.

## Resumo

**Cantos de anúncio e de combate de *Centrolene lynchi* (Anura: Centrolenidae), com notas sobre seu comportamento de combate e reprodutivo.** Descrevemos aqui os cantos de anúncio e de combate de *Centrolene lynchi*, uma espécie endêmica dos bosques nublados do Equador. O canto de anúncio típico consiste em uma nota tonal seguida por 1–3 notas pulsadas, cada uma das quais com 1–3 pulsos. Entre a variação observada no canto de anúncio está a adição de uma nota curta que precede a nota tonal e a ausência desta última. Essas modificações são similares às observadas em outros anuros e podem estar relacionadas com a presença de fêmeas, outros machos, predadores e/ou mudanças no ruído do ambiente. Adicionalmente, descrevemos o canto de combate emitido durante as lutas entre machos. O combate entre machos é similar ao observado em outras espécies da sub-família Centroleninae e já foi hipotetizado como uma sinapomorfia do grupo. Finalmente, apresentamos informações sobre os hábitos reprodutivos dos machos, cuidado parental e desenvolvimento dos ovos.

**Palavras-chave:** Bioacústica, comportamento de combate, cuidado parental, descrição de vocalizações.

## Introduction

Glass frogs (family Centrolenidae) comprise 147 currently recognized species that inhabit the Neotropics (Guayasamin *et al.* 2009). Frogs in this family have varied and adaptive reproductive behaviors, including egg attendance and male parental care (McDiarmid 1978, Hayes 1991, Cisneros-Heredia and McDiarmid 2007, Vockenhuber *et al.* 2009, Delia *et al.* 2010). Although the occurrence of male parental care originally was reported in as many as 50% of the species in this family (McDiarmid 1978), recent debate

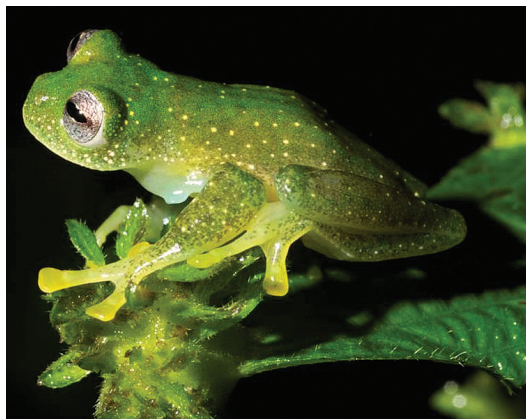
about what constitutes parental care has dramatically decreased these numbers. The most recent definition of parental care is post-laying behavior that increases hatching efficacy and survivorship/ survivorship of offspring (Delia *et al.* 2010). Based on this definition, only three species exhibit male parental care—*Hyalinobatrachium fleischmanni*, *H. valeroi*, and *H. colymbiphylum* (Delia *et al.* 2010). Male egg attendance has been reported in nine additional species, but these studies did not present unequivocal evidence of male parental care (Cisneros-Heredia and McDiarmid 2007, Vockenhuber *et al.* 2009).

Centrolenids also exhibit territorial combat behavior that ranges from primitive amplexus-like grasping to derived venter-to-venter grappling (Bolívar *et al.* 1999, Guayasamin *et al.* 2009), and have advertisement calls that have significant interspecific variation (Marquéz *et al.* 1996, Catenazzi *et al.* 2009). The variety of these calls, reproductive behaviors, and combat behaviors make these traits useful for systematic analysis (Marquéz *et al.* 1996, Bolívar *et al.* 1999, Guayasamin *et al.* 2009). However, these aspects of centrolenid ecology and behavior have been described for only a few species. Of 147 total, the calls of fewer than 20% of species have been described quantitatively (e.g., Starrett and Savage 1973, Señaris and Ayarzagüena 1993, Grant *et al.* 1998, Lescure and Martin 2000, Myers and Donnelly 2001, Catenazzi *et al.* 2009), and combat behavior has been described for only 11 species, or 7.4% of the family (McDiarmid and Alder 1974, Duellman and Savitzky 1976, Greer and Wells 1980, Jacobson 1985, Bolívar *et al.* 1999, Guayasamin and Barrio-Amorós 2005, Kubicki 2007, Delia *et al.* 2010, Rojas-Runjaic and Cabello 2011).

One species with undescribed call and combat behavior is *Centrolene lynchi* (Duellman 1980) (Figure 1), endemic to the cloud forest of the Pichincha Province of Ecuador. Herein, we describe the advertisement and combat calls of this taxon and provide information on natural history, reproductive ecology, and combat behavior for the species.

## Materials and Methods

We observed 21 males of *Centrolene lynchi* from January 09–18 April 2010 at Reserva Las Galarías, a cloud forest reserve at 1800 m in the Pichincha Province of Ecuador (00°00'33" S, 78°44'15" W). The record of *C. lynchi* at Reserva Las Galarías is an altitudinal and range extension for the species. The study plot was an 800-m transect that included both banks of Lucy's Creek, a fast-flowing stream. We located the 21 males by their vocalizations and flagged the



**Figure 1** - Adult male *Centrolene lynchi* at Reserva Las Galarías, Pichincha, Ecuador. Photo by Luis A. Coloma.

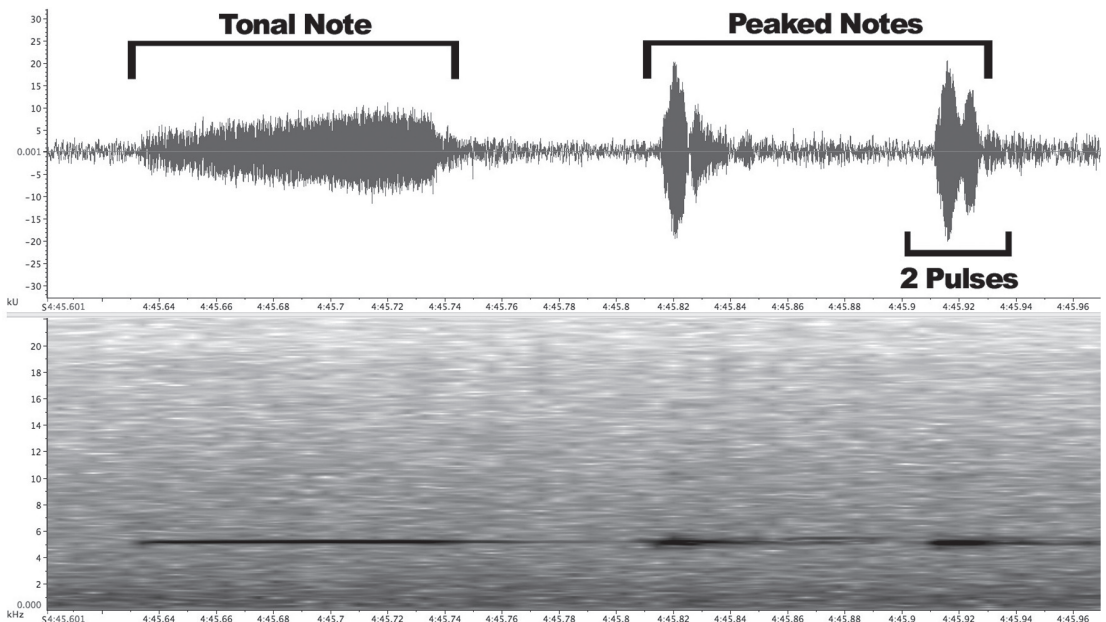
locations of the frogs, along with the locations of the egg clutches of each individual male found within the transect, and gave an identification number to each. Voucher specimens are housed at Museo de Zoología of the Pontificia Universidad Católica del Ecuador (QCAZ 40191–2, 40194). We walked the entire transect each night, noting the presence or absence of each male at the flagged site, the health of the egg clutch, whether the male was calling or quiet, the position of the male with respect to the eggs, and the temperature and humidity.

We observed combat behavior on 05 March 2010, at a temperature of 20.7°C and 100% humidity. The interaction was recorded with a Sony HDR-XR520V video recorder for 6 min, and analyzed in the lab. To extract the sound from the video, we employed iMovie 8.0.5. This file was extracted in a reduced quality AIFF format. We analyzed the sound clip using Raven 1.2.1 for Mac OSX (Cornell Lab of Ornithology).

We recorded 48 calls of 7 males during the nights of 27 February, and 13–14 April 2010. The temperature and humidity values for these days were 19.5°C and 100%, 19.9°C and 100%, and 20.6°C and 100%, respectively. Calls were

recorded using an Olympus Linear PCM Recorder LS-10 and microphone. Individuals were recorded for 5 min at a distance of 60–400 cm, and the audiospectrogram and oscillogram for each 5-min segment was produced and analyzed using the program Raven 1.2.1 for MacOSX (Cornell Lab of Ornithology). Because these frogs were part of a larger behavioral study and human manipulation likely would influence their behavior, we did not measure the SVL of recorded males; however, SVL in male *Centrolene lynchi* is 23.3–26.0 mm (mean = 24.8 mm; Duellman, 1980). We measured six aspects of the call for this study: (1) call duration (ms); (2) note duration (divided into tonal and peaked notes; ms); (3) interval between notes (ms); (4) type and number of notes per call; (5) number of pulses per pulsed note; and (6) tonal note and pulsed note dominant frequency (Hz). For acoustic variables, we follow the terminology of Duellman and Trueb (1994). Notes were divided

into two categories, tonal notes and peaked notes, based on distinct wave-form and placement in the call. Tonal notes usually are the first note of the call and are defined by flat, non-peaked amplitude sustained throughout the duration of the note (Figure 2). Peaked notes usually are the second through last notes in each call. Peaked notes are characterized by a clear amplitude peak and often are pulsed (Figure 2). Pulses are defined as a visible increase and decrease of amplitude peaks on the oscillogram within a single note. Notes are defined by a single and complete amplitude rise from and return to the base frequency of the white noise produced by the river (Figure 2). Taxonomy for glassfrogs follows the proposal by Guayasamin *et al.* (2009). Digitalized calls are housed at the Centro de Investigación de la Biodiversidad y Cambio Climático, Universidad Tecnológica Indoamérica, Quito, Ecuador, and are available upon request.



**Figure 2** - Oscillogram and audiospectrogram of a typical advertisement call, with a labeled tonal note, peaked note, and pulses.

## Results

### Call Description

Males call 2–5 m above the stream from large, broadleaf plants, branches, and ferns. The ambient sound environment included the noisy whitewater stream and many calls from *Centrolene peristictum*. The minimal distance between male *Centrolene lynchi* was 5 m, although most were separated by much greater distances. Calls emitted from different males rarely overlapped

each other. The recordings were taken during overcast nights that included at least some precipitation. The typical advertisement calls produced by each male are summarized in Table 1.

The typical advertisement call is relatively short and consists of a tonal note followed by 1–3 peaked notes (Figure 2). The first note of the call usually is longer than the following notes and is tonal, showing no clear frequency peak. The following notes are shorter and pulsed, with

**Table 1** - Measurements ( $\pm$  SD) of the typical advertisement calls as produced by seven males, including call duration (ms), tonal note duration (ms), peaked note duration (ms), the interval between notes (ms), the range and most frequent number of notes per call and pulses per peaked note, the dominant frequency of the tonal note (Hz), the dominant frequency of the peaked notes (Hz). The final column represents the average of all males measured.

Statistic	Male number							Average
	1	2	3	4	5	6	7	
Number of calls ( <i>n</i> )	10	5	9	5	6	9	4	48
Date	13 Apr	13 Apr	13 Apr	14 Apr	14 Apr	14 Apr	27 Feb	
Temperature (°C)	19.9	19.9	19.9	20.6	20.6	20.6	19.5	
Humidity (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
Call duration (ms)	241.8	305.6	263.6	319.2	268.6	358.0	297.7	293.5
SD	32.4	35.0	11.2	36.8	40.0	24.1	12.0	27.5
Tonal duration (ms)	102.5	107.8	92.4	97.5	97.2	97.3	108.0	100.4
SD	26.2	7.9	23.7	15.0	37.6	20.6	10.5	20.2
Peaked duration (ms)	18.1	15.3	19.9	16.4	14.1	18.0	27.3	18.5
SD	3.4	2.6	6.2	3.4	6.2	4.4	6.3	4.7
Note interval (ms)	80.1	74.5	60.3	75.7	91.7	78.0	68.8	75.6
SD	26.4	6.6	10.3	3.7	4.8	8.2	6.5	9.5
Notes/ call	1-2	2-3	2-3	2-3	1-2	2-3	2-2	1-3
Most frequent	1	2	2	2	1	3	2	2
Pulses/ Peaked note	1-3	2-2	2-3	2-2	1-2	1-2	2-3	1-3
Most frequent	2	2	2	2	1	2	2	2
Tonal frequency (Hz)	5261.3	5047.4	5531.6	5139.3	5443.6	5340.2	5311.5	5296.4
SD	112.9	77.1	84.1	44.5	38.5	0.0	49.7	58.1
Peaked frequency (Hz)	5269.3	5074.0	5467.2	5055.3	5364.9	5395.0	5225.4	5264.4
SD	54.8	71.6	78.0	41.4	128.9	88.5	44.5	72.5

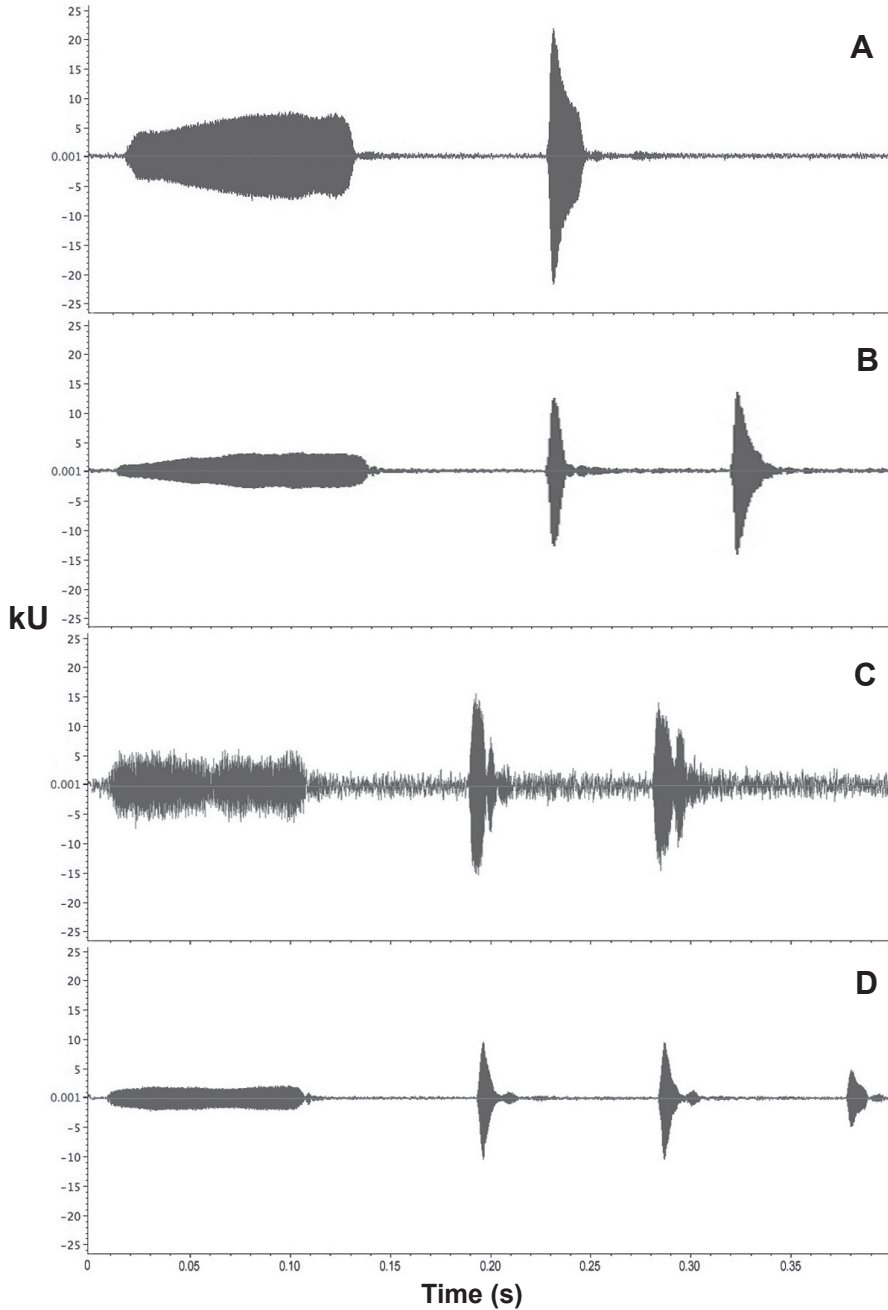
clear frequency peaks. These peaked notes are emitted in groups of 1–3 and contain 1–3 pulses each (Figure 3). Notes are separated by 9.0–138.0 ms. The dominant frequencies of tonal and peaked notes are similar and high (Table 1), and show no frequency modulation (Figure 2).

This typical advertisement call was produced by all seven males recorded. Additionally, two

of the seven males emitted two distinct, modified call types. The first call variation was the addition of a brief, low amplitude note before the tonal note, termed here a pre-tonal note (Figure 4A). Calls with an additional pre-tonal note were produced two times by Male 1, and three times by Male 5. They are longer than the typical advertisement call (Tables 1, 2). The call consists

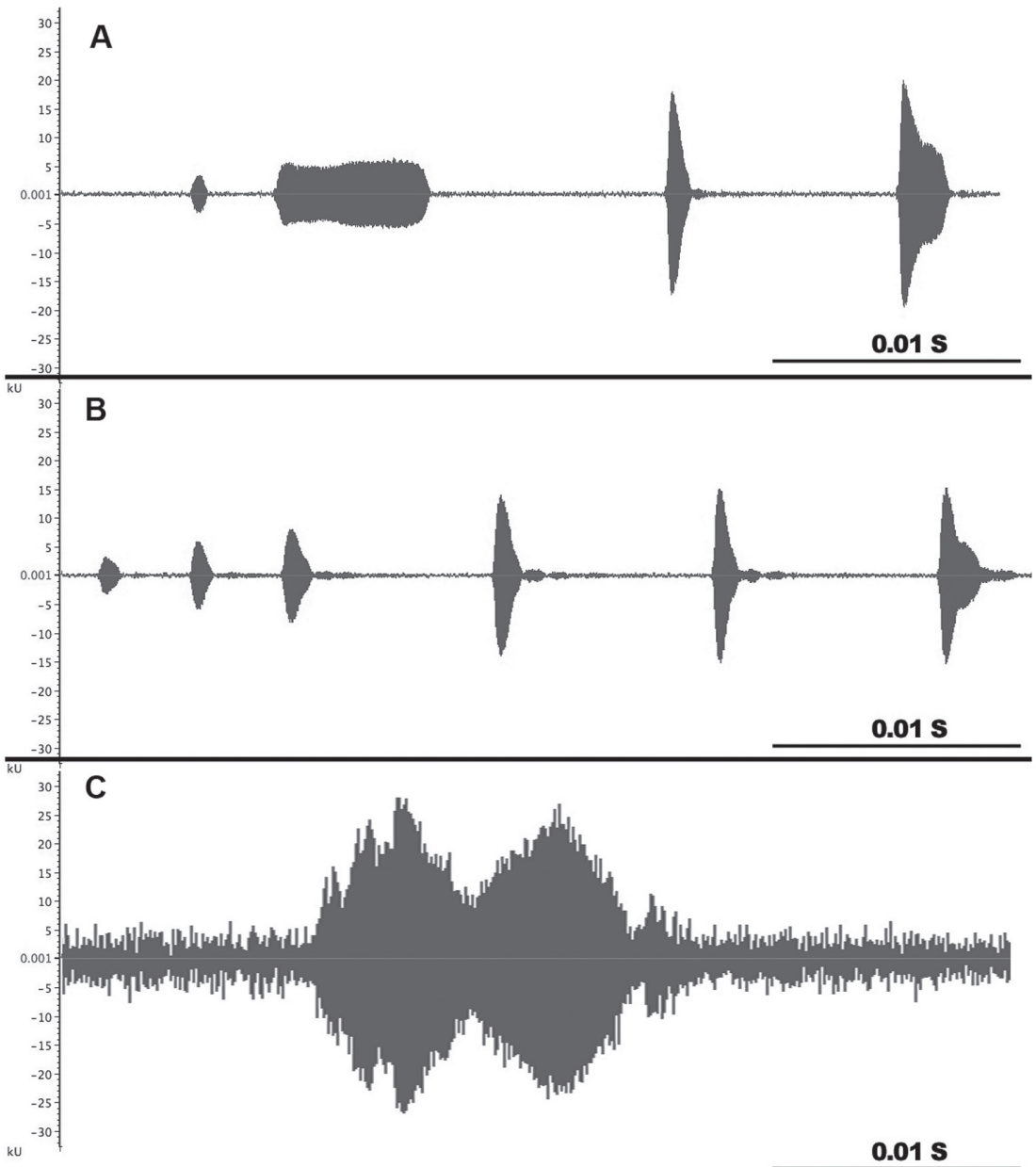
**Table 2** - Measurements of additional calls produced by *Centrolene lynchi*, including call duration (ms), pre-tonal note duration (ms), tonal note duration (ms), peaked note duration (ms), the interval between notes (ms), the range and most frequent number of notes per call and pulses per peaked note, the dominant frequency of the pre-tonal note (Hz), the dominant frequency of the tonal note (Hz), and the dominant frequency of the peaked notes (Hz).

	Call type				
	No tonal	No tonal	Pre-tonal	Pre-tonal	Combat
Male	1	5	1	5	8
Number of calls (n)	1	1	2	3	5
Date	14 Apr	14 Apr	14 Apr	14 Apr	05 Mar
Temperature (°C)	20.6	20.6	20.6	20.6	20.7
Humidity (%)	100.0	100.0	100.0	100.0	100.0
Call duration (ms)	632.0	364.0	396.0, 506.0	311.0, 346.0, 340.0	150.4 ± 6.7
Pre-tonal duration (ms)	—	—	20.0, 22.0	6.0, 7.0, 8.0	—
Tonal duration (ms)	—	—	66.0, 100.0	31.0, 65.0, 130.0	—
Peaked duration (ms)	17.0 ± 2.9	11.0 ± 1.7	25.0 ± 4.3	17.0 ± 5.6	—
Range	14.0–21.0	9.0–12.0	19.0–29.0	11.0–23.0	
Note interval (ms)	72.0 ± 31.7	51.8 ± 26.9	75.3 ± 35.3	62.9 ± 23.9	—
Range	63.0–143.0	29.0–77.0	18.0–114.0	25.0–83.0	
Notes/ Call	5	6	2, 2	2, 3, 6	1
Pulses/ Peaked note	1-2	1-2	1-2	1-2	2
Most frequent	2	1	1/2	2	2
Pre-tonal frequency (Hz)	—	—	5426.4, 5340.2	5340.2, 5340.2, 5340.2	—
Tonal frequency (Hz)	—	—	5426.4, 5168.0	5512.5, 5426.4, 5426.4	—
Peaked frequency (Hz)	5357.4 ± 94.4	5368.9 ± 49.8	5189.5 ± 43.1	5387.2 ± 45.0	4892.3 ± 38.5
Range	5254.1–5426.4	5340.2–5426.4	5168.0–5254.1	5340.2–5426.4	4823.4–4909.6



**Figure 3** - Oscillograms depict normal variation in the typical advertisement call. Calls contain a tonal note and 1–3 peaked notes, each consisting of 1–3 pulses. (A) A call consisting of a tonal note followed by a single, one-pulse note. (B) A call with a tonal note followed by two notes with one pulse each. (C) A call with a tonal note and two notes composed of two pulses each. (D) A call with a tonal note followed by three notes composed of one pulse each.





**Figure 4** - Additional calls produced by *Centrolene lynchi* include a call with a pre-tonal note, a call lacking the pre-tonal note, and one-note combat call. **(A)** A call containing the pre-tonal (left most) note. **(B)** A call that lacks a tonal note and consists of only six peaked notes. **(C)** Combat call produced during male-male, upside-down hanging grappling combat.



of a short, peaked pre-tonal note, a tonal note, and 2–6 peaked notes of 1–2 pulses each. Distances between notes are short and variable, and dominant frequencies of the tonal and peaked notes does not vary greatly from the typical advertisement call (Table 2). One possible explanation for the addition of an extra note in related species is the attraction of females during phonotaxis (Greer and Wells 1980), but we were unable to make any contextual observations that would support this hypothesis in *Centrolene lynchi*.

The second of these modified calls lacks the tonal note and consists of only 5 or 6 peaked notes of 1–2 pulses each (Figure 4B). Calls without the tonal note are longer than the typical call and were produced one time each by Males 1 and 5 (Table 2). They did not differ in dominant frequency or note interval from the typical advertisement call.

#### *Combat Behavior and Call*

We observed combat behavior on the night of 05 March 2010, with a temperature of 20.7°C and 100% humidity. The observation started mid-conflict, with Male A (above), hanging by his feet from the stem of a large leaf and Male B (below) grasping Male A around the body above the arms, dangling with his legs free (Figure 5). The males faced each other and were positioned head-to-venter. As typical grappling behavior for members of the subfamily Centroleninae is venter-to-venter (Guayasamin *et al.* 2009); it is likely that combat began in the more typical condition and progressed to the head-to-venter position as Male B began to lose grip of the stem, clinging to Male A to avoid falling. Throughout the observation, Male A sporadically shook his body, rocked back and forth, and pushed with his arms against Male B. During this combat, Male A produced eight single-note calls, possibly to loosen his opponent's grip (Bolívar *et al.* 1999). Consistent with this hypothesis, Male B did not call, whereas Male A called and maintained an inflated posture

throughout the observation. After several (> 5) minutes of combat, Male B lost his grip on Male A and fell to the vegetation below. For a short video clip of the combat, including the combat call, please visit: <http://www.youtube.com/watch?v=dae0bi8-Vcs>.

The combat call produced during this encounter is markedly different in structure from the advertisement call of the species (Figure 4C). It consists of a short single note containing two pulses at a dominant frequency lower than that of the advertisement call (Table 2). Like the advertisement and modified calls, the combat call is not frequency modulated.

#### *Courtship and Egg Deposition*

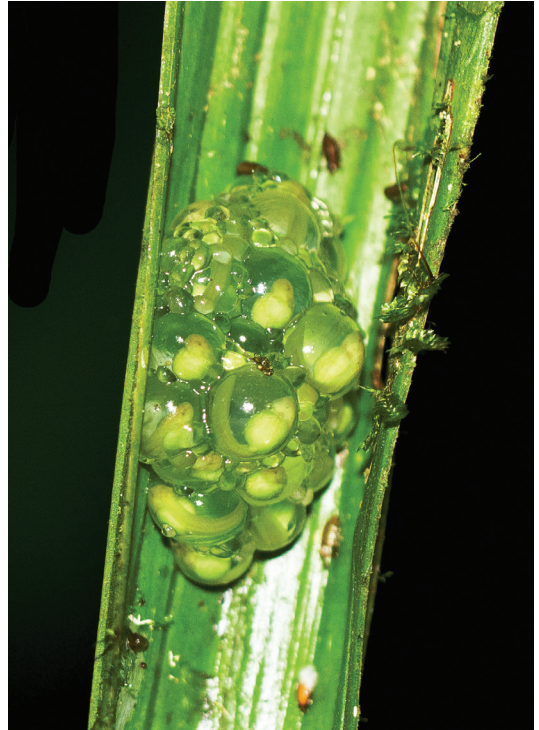
Male *Centrolene lynchi* repeat a typical advertisement call to attract females to their territory. Females are attracted by this call to join the male in amplexus, after which, the female places the brilliant green eggs in clutches of 20–24 eggs (Figure 6) on vegetation in the male's territory ( $n = 7$ , mean = 21.7, SD = 1.6). Most eggs are placed on top of large leaves or ferns above a fast-flowing stream at a heights of 165–600 cm ( $n = 14$ , mean = 313.3 cm, SD = 78.7). There were two exceptions—one egg clutch was observed under a leaf, and another was hanging on a vine. After placing the egg clutch, the female departs.

#### *Male Egg Attendance*

Twenty-one males were located within consistent territories throughout the season for a total of 271 observations. Of these, 111 are of males with eggs and 160 are of males without eggs. The males' calling rates vary depending on whether or not they were attending an egg clutch. Males calling from plants without a clutch called in 97.5% of the observations ( $n = 156$ ), whereas males sitting on or near one or more clutches called in only 65.8% of the observations ( $n = 73$ ). Of the males with one or more clutches ( $n = 111$ ), males were found on top of the eggs in



**Figure 5** - Combat behavior of *Centrolene lynchi* is carried out in an upside-down, venter-to-venter grappling position. Captured mid-conflict. Combat call produced by Male A (above), while Male B (below) was silent throughout the observation. See a clip of this video at: <http://www.youtube.com/watch?v=dae0bi8-Vcs>



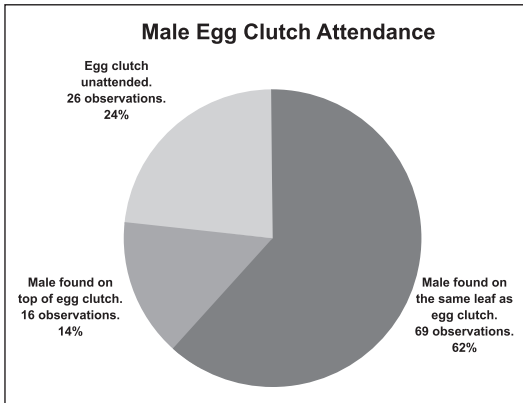
**Figure 6** - An egg mass of *Centrolene lynchi* on a palm, photographed in the field. Photo provided by Carl Hutter.

14.4% of the observations ( $n = 16$ ) and on the same leaf as the eggs in 62.2% of the observations ( $n = 69$ ). The egg clutch was unattended in 23.4% of the observations ( $n = 26$ ; Figure 7). Each male that we observed was located on the same leaf as the eggs for multiple (up to 10) sequential observations. In contrast, males without eggs often positioned themselves on various leaves and were more likely to be absent or not locatable in their territory. The consistent proximity of males to their clutch is indicative of male egg attendance (Hayes 1991, Delia *et al.* 2010). However, because we cannot show that this behavior increased offspring survival, we

cannot report explicit evidence for parental care as defined by Delia *et al.* (2010).

#### *Egg Development*

Nine clutches were observed throughout their development from deposition until hatching. Clutches developed 23–29 days before hatching ( $n = 9$ , mean = 25.66, SD = 2.0), and males attended up to three clutches at a time. At least three males attended multiple clutches in similar developmental stages, which suggests polygyny. During this time, the egg capsule shifted in coloration from bright green to slightly brownish



**Figure 7** - Males *Centrolene lynchi* attended eggs in 76% of observations. Males were not visible or the eggs were unattended for the other 24%. Total observations of males with eggs = 111.

yellow as the tadpoles developed. Seven males abandoned the eggs and their territory 1–3 days before the eggs hatched, and in all such cases, the eggs hatched successfully. In two cases, males attended their clutches until the eggs hatched.

## Discussion

We describe four markedly different calls produced by eight males of the glass frog *Centrolene lynchi*. The most frequently produced call represents a typical advertisement call, followed by two modified advertisement calls and one combat call. The four call types differ from one another in temporal as well as spectral traits. The basic advertisement call has two components—a tonal note, followed by 1–3 peaked notes. The comparison of this call to those of other anurans provides insight and guidance for call analysis. For example, in another Neotropical anuran, *Engystomops pustulosus*, similar tonal notes have been hypothesized to contain species information, whereas non-tonal notes have been shown to enhance attractiveness to females in other groups (Ryan

and Drewes 1990). Modified advertisement calls in other species have been produced in response to acute changes in a male's environment. Previously described scenarios include the presence of a nearby female (Greer and Wells 1980, Rand and Ryan 1981, Ryan and Drewes 1990), presence of a nearby male (Rand and Ryan 1981, Martínez-Rivera and Gerhardt 2008), changes in the sound environment (Parris *et al.* 2009), and the threat of predation (Tuttle and Ryan 1982, Tuttle *et al.* 1982). For example, in the case of female presence, *Hyalinobatrachium fleischmanni* and *Engystomops pustulosus* have been shown to add mews and chucks respectively to their call in order to attract approaching females (Greer and Wells 1980, Ryan and Drewes 1990). Females of other Neotropical frog species have been shown to prefer an increased number of notes per call (Rand and Ryan 1981, Martínez-Rivera and Gerhardt 2008). This implies that the pre-tonal note added by *C. lynchi* (this publication) similarly may serve to increase female attraction. Such temporal modifications also have been shown in the laboratory to increase in the information content of an advertisement call (Ryan 2001). An alternate hypothesis is that some anurans produce a short pseudo-note in front of their typical call as a means of warming up their vocal chords, which also may be the case in *C. lynchi*.

Another possible impetus for call modification was demonstrated by Parris *et al.* (2009), in which *Litoria ewingii* and *Crinia signifera* have been shown to increase their call frequency in response to changes in sound environment produced by traffic noise. Although the *Centrolene lynchi* in this remote region of cloud forest is far removed from traffic and other man-made noise disturbances, the rushing river above which these frogs live produces a similarly harsh acoustic environment. In this environment, call modifications could be made in response to rainfall, the presence of conspecific and/or heterospecific calls, or changes in the water level of the stream, all of which would dramatically affect ambient noise and occur frequently in the cloud forest.

Changes in these factors, temperature, and humidity may account for some of the variation in call dominant frequency and represent a possible future direction of study.


Last, calls of other Neotropical species have been demonstrated to be used by bats to locate frogs for predation (Tuttle and Ryan 1981), and longer calls have been shown to increase predation risk (Ryan *et al.* 1982); frogs have been shown to call less and shorten their calls in the presence of a predator (Tuttle and Ryan 1982, Tuttle *et al.* 1982). In the case of *Centrolene lynchi*, the range of which overlaps that of many potential predators including bats, perhaps the removal of the tonal note and production of shorter calls could be an anti-predator response.

Many male *Centrolene lynchi* were located on the same leaf as the eggs or on top of eggs for many nights in a row. Although this is an impressive demonstration of male egg attendance that may imply parental care, and studies in related taxa report similar sustained male proximity to the eggs (Cisneros-Heredia and McDiarmid 2007, Vockenhuber *et al.* 2009), more recent definitions of parental care have discredited the assumption that repeated male presence constitutes true parental care unless it can be demonstrated that this male attendance confers a survival advantage to the offspring (Delia *et al.* 2010). Thus, future analyses of offspring survival in the conditions of male presence and male removal will be necessary to test this hypothesis and confirm male parental care in this species.

Finally, with respect to combat behavior, we observed head-to-venter and hanging grappling position in *Centrolene lynchi* which probably began from an initial venter-to-venter combat position. This fits with the predictions by Guayasamin *et al.* (2009) and is congruent with the hypothesis that complex venter-to-venter combat behavior is a derived behavioral trait shared by members of the subfamily Centroleninae. Recent findings by Delia *et al.* (2010) reported the same venter-to-venter behavior for *Hyalinobatrachium fleischmanni*, introducing the possibility that this derived combat behavior

either (1) has evolved more than once within Centrolenidae or (2) represents a synapomorphy of the family. More observations are needed to support or reject these hypotheses.

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