

Tonal calls as a bioacoustic novelty in two Atlantic Forest species of *Physalaemus* (Anura: Leptodactylidae)

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Abstract. The frog genus *Physalaemus* has almost 50 species with vocalizations that are mostly composed of a single note. This note tends to have a broad harmonic structure or a pulsed structure. The sister species *P. lateristriga* and *P. olfersii* have pulsed advertisement calls that have been described as a noisy and long-lasting warbling sound. We provide the first account of inclusion of tonal sounds as part of the vocal repertoire of these species. Pure tones can (1) be long and form the entire call; (2) form prefixes of variable length separated by silence from the advertisement call; (3) be brief and form the onset or the offset of the regular advertisement call. Tonal calls may be an evolutionary novelty and they are not known from other populations of *P. olfersii* and *P. lateristriga*. Identification of the mechanism of sound production and of the behavioural roles of these unique calls may help elucidate the evolution of call complexity in frogs.

Keywords. Animal communication, bioacoustics, Leiuperinae, vocal repertoire.

INTRODUCTION

Most anurans communicate using stereotyped signals with relatively simple acoustic structure (Gerhardt and Huber, 2002; Köhler et al., 2017). Various call traits can be shared among the species of a clade and yet enough variation is present, in most cases, to make the advertisement call of each species unique. Advertisement call differences can form major prezygotic isolation barriers and can be used to separate morphologically cryptic species (Blair, 1958; Johnson, 1966).

The vocal repertoires of frogs in the Neotropical genus *Physalaemus* are almost always simple with calls composed of a single note (Tárano, 2001; Giaretta et al., 2009). The calls of this group are of particular interest because its sister genus *Engystomops*, which used to be included in *Physalaemus*, contains the túngara frog (*E.*

pustulosus; Lynch, 1970; Nascimento et al., 2005; Lourenço et al., 2015), a model organism in behavioural and acoustic communication studies. A variety of studies has explored the evolution of an optional secondary pulsed sound (chuck) that the male túngara frog can add to the end of his regular advertisement call (whine; Ryan, 1985). The laryngeal mechanism underlying the addition of the chuck has not been fully described, however, and further insight may be gained from comparisons with other species in the group.

In both *Physalaemus* and *Engystomops*, advertisement calls tend to be long and have a pronounced harmonic structure with a smooth amplitude envelope (Cannatella et al., 1998; Wilczynski et al., 2001; Provete et al., 2012). Descending frequency modulation throughout the call is very common with most of the change concentrated at the beginning. Some species, however, have pulsed calls

Table 1. Acoustic terminology adopted in this study.

Traits	Description
<i>Temporal traits</i>	
Call length (sec)	Time from initial 10% to final 10% of amplitude of one call
Rise time (%)	Point of maximum amplitude relative to call length
Call rate per minute	(Total number of calls - 1) / time from beginning of first call to beginning of last call
<i>Spectral traits</i>	
Dominant frequency (Hz)	Frequency containing the greatest energy in one call. It matches the fundamental frequency in tonal calls.
Frequency Modulation (Hz)	Dominant frequency at 10% final minus that at initial 10% of one call

and lack the descending frequency modulation (Bokermann, 1966; Padiál and Köhler, 2001; Weber et al., 2005b). *Physalaemus olfersii* and *P. lateristriga* are examples of this latter type of advertisement call (Drewery et al., 1982; Giaretta et al., 2009; Cassini et al., 2010). Based on acoustic structure and laryngeal morphology, their call was speculated to be possibly produced in the same manner as the chuck encountered in the call of the túngara frog (Drewery et al., 1982).

Here, we report on previously unknown call complexity in the genus *Physalaemus* generated through the incorporation of tonal sounds. We document its occurrence in *P. olfersii* and *P. lateristriga*. We also discuss the potential role and the mechanism underlying this striking acoustic novelty based on the vocal biomechanics of the group.

MATERIAL AND METHODS

Vocalizations were recorded in the field using analogue recorders (Marantz PMD 420 with Sony Metal-SR 100 Audio Cassette) and supercardioid microphones (Sennheiser ME-80) positioned at an approximate distance of 2 m from the calling males. The recordings were digitized at a 44.1-kHz sampling rate and 16-bit sample size. Calls were recorded from two localities in the Brazilian state of São Paulo (south-eastern Brazil): *Physalaemus olfersii*—Parque Estadual da Serra do Mar, Núcleo Santa Virgínia, São Luiz do Paraitinga (-23.332200°, -45.096828°; 979 m a.s.l.); *Physalaemus lateristriga*—Ribeirão Branco (-24.358600°, -48.743000°; 840 m a.s.l.). Voucher specimens and recordings are housed at the Célio F. B. Haddad collection (CFBH). Information on sound recordings is as follows: *Physalaemus lateristriga*—(1) CFBH 63, recorded on 4 February 1995, at 22:20 h, air temperature 19°C; (2) MGP 5011401, recorded on 14 January 2005, at 19:30 h, air temperature 19°C, water temperature 22°C; call voucher is one male of the series CFBH 16564-16567, 16569-16575, 16577-16582, 16584-16585. *Physalaemus olfersii*—(1) MGP 4111701-4111705, recorded on 17 November 2004.

The acoustic analysis was conducted in Soundruler (Gridi-Papp, 2007), a package of MATLAB scripts (Matlab, 2004) that

allows for unbiased quantification of acoustic traits using automated procedures. Call rate, however, was measured manually in Audacity (Audacity Team, 2017). The settings used in the frequency analysis included FFT size = 1024 samples, FFT overlap = 90%, window type = Hanning, contrast = 70%. Settings for automated recognition of pulses were (in samples): pulse detection (smoothing = 250, resolution = 25); pulse delineation (smooth factor = 1, smoothing = 25, resolution = 1). Settings for frequency tracking range: 1000 Hz each step. Critical amplitude ratio: CFBH 63 (-1; disabled); MGP 5011401 (5.0). A 500-Hz high-pass filter at 48 dB was applied to the sound file CFBH 63 in Audacity to reduce background noise. Temporal traits were measured from oscillograms and spectral traits from spectrograms. The acoustic terminology is summarized in Table 1. A call-centred approach (*sensu* Köhler et al., 2017) was used for the acoustic characterization of acoustic signals described in the present study.

RESULTS

Tonal calls were recorded from two males of *P. lateristriga* that only produced tonal calls during the recording. Three males of *P. olfersii* were also recorded making tonal calls but these were shorter and positioned as prefixes or suffixes of the regular (pulsed) advertisement calls.

The two males of *P. lateristriga* that only produced tonal calls were recorded at the same locality and temperature but with a separation of 10 years. Both exhibited an interruption within the call but it corresponded to a frequency shift in one individual and to a silent gap in the other. The tonal calls ($n = 8$; Fig. 1A) of the first male *P. lateristriga* (CFBH 63) lasted 0.83-1.40 sec (1.18 ± 0.09 ; min-max, $x \pm SD$) and were emitted at a rate of 17.8 calls/min. Rise time was 8.3-89.3% (62.4 ± 27.1) of the call's length. The dominant frequency of the call was 1572-1701 Hz (1641.9 ± 51.2). The call started at about 1500 Hz and gradually modulated up to approximately 1750 Hz. An abrupt shift in frequency separated the initial part of the call from the last 10-20% (see Fig. 1A). The final section of the call had a downward frequency modulation approximately from 1300 Hz to 1100 Hz.

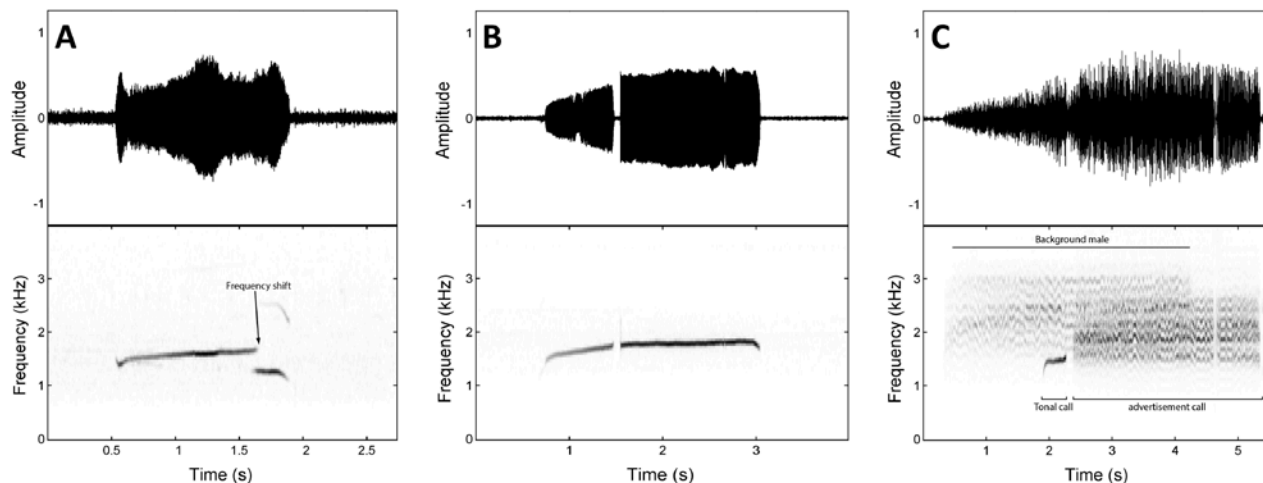


Fig. 1. (A-B) Two males of *Physalaemus lateristriga* recorded from Ribeirão Branco (São Paulo, Brazil): (A) Tonal call from the recording CFBH 63; (B) Tonal call from the recording MGP 5011401. (C) Male *Physalaemus olfersii* recorded from Santa Virgínia (São Paulo, Brazil). A vocal bout from the recording MGP 4111701 depicting a short tonal call followed by a typical advertisement call (overlapped by another male calling in the background).

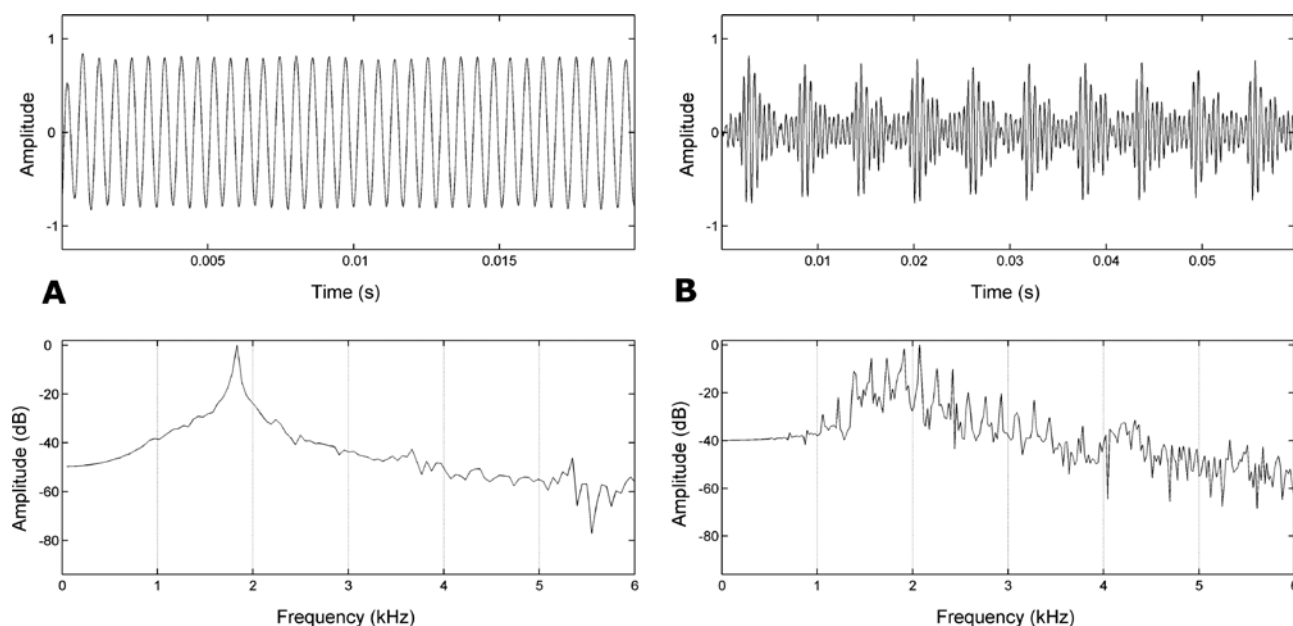


Fig. 2. Waveform and 4096-FFT amplitude spectrum of (A) a 20-msec section from a tonal call (recording MGP 5011401; *P. lateristriga*), and a 60-msec section from (B) a typical advertisement call (MGP 4111704; *P. olfersii*), respectively.

The tonal calls ($n = 14$; Fig. 1B) of the second male *P. lateristriga* (MGP 5011401) lasted 0.90–2.57 sec (2.12 ± 0.39) and were emitted at a rate of 8.84 calls/min. Rise time was 33.5–95.2% (66.1 ± 20.4) of the call's length. The calls had a silent gap of 0.0689–0.1076 sec (0.0817 ± 0.0105) near the first third or half of the call's length (see Fig. 1B). The duration of the first part of the call varied from 0.33 to 1.05 sec (0.78 ± 0.19), while

that of the second part varied from 0.22 to 1.63 sec (1.09 ± 0.46). The dominant frequency was 1787–1830 Hz (1811.9 ± 22.1). Ascending frequency modulation occurred throughout the call, stabilizing in the last 25%, with a drop in frequency of only 50–100 Hz in the last 10%. The dominant frequency started at 1450–1600 Hz (1470.4 ± 55.0) and ended at 1700–1750 Hz (1728.8 ± 21.4).

Unlike *P. lateristriga*, males of *P. olfersii* did not produce call bouts containing only tonal calls in our recordings. Three male *P. olfersii* (MGP 4111701–3) emitted (Fig. 1C) a tonal call shortly followed by the typical pulsed advertisement call. However, the tonal portion was consistently shorter than those described earlier for *P. lateristriga*. All three males of *P. olfersii* were recorded while calling in antiphony with neighbouring conspecific males (Fig. 1C). Two other recorded males of *P. olfersii* (MGP 4111704–5) did not emit tonal calls, but changed to a tonal-like structure at the very final portion of a few calls: 50–70 msec in duration and dominant frequency of 1250–1750 Hz.

In both species, the tonal calls were very narrowly tuned whereas the pulsed calls had energy distributed through a wide frequency range (Fig. 2). The dominant frequency was always centred near 1700 Hz but in the tonal calls the amplitude of the fundamental frequency was 40 dB higher than that of any other harmonic, whereas in the pulsed calls the amplitude of the dominant frequency was less than 5 dB higher than that of the closest side-band.

DISCUSSION

The acoustic structure of the tonal calls diverged sharply from that of the pulsed advertisement calls in *Physalaemus lateristriga* and *P. olfersii*. The striking contrast between these structures made *P. olfersii* males stand out acoustically from the chorus when they added tonal sounds to their calls. Tonal calls also made *P. lateristriga* stand out in their diverse acoustic assemblage of tropical anurans and insects due to the rarity of tonal sounds in nature (Rossing, 2007).

A single other species of *Physalaemus* (*P. fernandezae*) has been reported to produce tonal sounds (Barrio, 1965). This species, different from *P. lateristriga* and *P. olfersii*, appears to always produce tonal-only advertisement calls and no other call types have been described. The brief acoustic description provided by Barrio (1965) indicates other similarities with the calls recorded in our study. In addition to being tonal, the calls of *P. fernandezae* have high fundamental frequency and ascending frequency modulation. These shared characteristics are unlikely to reflect common descent because *P. fernandezae* is not closely related to the *P. olfersii* group, within which *P. olfersii* and *P. lateristriga* are sister taxa (Lourenço et al., 2015). Tonal calls are therefore likely to have evolved independently in these two groups and the accompanying acoustic similarities may reflect a common laryngeal mechanism for the production of tones rather than homology.

The addition of a tonal prefix or suffix adds complexity to the advertisement call of *P. olfersii*. A complex vocal repertoire has been described for *P. spiniger* but it did not include tonal sounds (Haddad and Pombal, 1998; Costa and Toledo, 2013). In *Engystomops*, some species (*E. pustulosus*, *E. petersi*) are known to optionally add sounds with a distinct acoustic structure to their advertisement call as ornaments that make the call more attractive to females (Ryan and Rand, 1993).

The behavioural significance of the tonal calls in the *P. olfersii* group is unclear. All recorded individuals of *P. olfersii* and *P. lateristriga* vocalized from the ground within a few meters from the water. Neither females nor other males were observed to interact with the callers during shifts between calls including or not tonal sounds. The callers themselves did not change position, orientation, or indicate any change of behavioural context when switching between emitting or not tonal sounds. In other anurans, the second most commonly reported call type is emitted during aggressive interactions (Wells, 2007), but in *Engystomops pustulosus* the second most common sound (the chuck) increases the attractiveness of the call to females (Ryan, 1985). The tonal call could also be an accidental acoustic consequence of variation in the structure or mechanics of the larynx and may lack an exclusive role.

The mechanistic origin of such distinctive acoustic signals is intriguing. The dominant frequency in both the pulsed advertisement calls and the tonal calls is about 1700 Hz and the main difference between them is the presence or absence of pulsing. This aspect is also present in the advertisement call of *E. pustulosus* in which the chuck is pulsed while the whine is not. Large laryngeal fibrous masses have been described in some species of leiuperine frogs including *E. pustulosus* and *P. olfersii* (Ryan and Drewes, 1990). Such structures attached to vocal cords have proven to be necessary for the production of the chuck but not of the whine (Gridi-Papp et al., 2006). The onset of the chuck in *E. pustulosus* has been suggested to be controlled by muscles that deform the larynx (Drewery et al., 1982; Ryan and Drewes, 1990). Alternatively, a mathematical model indicates that the mechanical interactions between the fibrous mass and the vocal cords may determine the onset of the chuck with base on laryngeal airflow (Kime et al., 2018). One way or the other, the lack of pulsing in the tonal calls of *P. lateristriga* and *P. olfersii* could result from halting the vibration of the laryngeal fibrous masses.

Without pulses, the calls of *P. olfersii* and *P. lateristriga* could be expected to exhibit a pronounced harmonic structure like those of most leiuperine frogs, but instead, they are tonal. This raises the question of whether the ton-

al calls are being produced by vocal cord vibration or as whistles, by oscillations of airflow as it passes between the arytenoid cartilages. Some evidence in favour of a reduced role of vocal cords can be observed in the frequency modulation of the call. The *P. olfersii* group can present subtle ascending frequency modulation at the attack phase and descending frequency modulation during the decay phase of the call as best seen in *P. feioi*, *P. orophilus*, and *P. soaresi* (Weber et al., 2005a; Cassini et al., 2010). This matched modulation of amplitude and frequency is expected whenever the vocal cords do not block the airflow immediately before the onset and after the offset of the call (Gridi-Papp, 2014). The tonal calls could be produced by the vocal cords, however. While not the highest among leiuperines (see comparison in Provete et al., 2012: Table 5), the advertisement calls of most species in the *P. olfersii* species group have a high dominant frequency (Weber et al., 2005a; Cassini et al., 2010). Extensive filtering of the harmonics could concentrate all the energy in the fundamental frequency and result in tonal calls.

This study shows that males of *P. lateristriga* produce pulsed or tonal calls and males of *P. olfersii* can switch between these two structures within a call. These species offer a unique opportunity for study of the acoustic mechanisms that underlie two call modes that are fixed in other species of *Physalaemus*. Furthermore, tonal calls are not known from other populations of either species, indicating that this might be an opportunity to document the evolution of a novel acoustic signal. Finally, this case seems analogous to that of *E. pustulosus*, in which males optionally incorporate a categorically different signal into their calls. The study of this system may provide new insight into the evolution of diversity in acoustic communication.

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