Frogs in the family Craugastoridae exhibit direct development and eggs are deposited in moist locations, rather than in water. Free from dependence on standing water, suitable egg deposition sites presumably are common for many species including those dwelling primarily in the leaf litter, such as Stejneger’s Flesh-bellied Frog (*Craugastor stejnegerianus*). The lack of communal breeding/calling sites for *C. stejnegerianus* results in males calling from dispersed locations. Calling from the surface of low vegetation may help attract nearby females, as the squeak-like calls are relatively faint. This adult female frog was photographed on 13 June 2016 near Tinamastes, Provincia de Puntarenas, Costa Rica.
Temporal and spectral analysis of the advertisement call of *Craugastor stejnegerianus* (Anura: Craugastoridae) in Costa Rica

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**Abstract:** The vocalizations of many of the anuran species in the mega-diverse family Craugastoridae remain unknown. One such species is *Craugastor stejnegerianus*, a frog found in tropical forests of Costa Rica and western Panama. We recorded the vocalizations of this species at Refugio Nacional de Vida Silvestre Hacienda Barú, Provincia de Puntarenas, Costa Rica. The typical advertisement calls consisted of a single, short note (\(\bar{x} = 0.051\) s) with a mean call interval of 30.7 s and a mean dominant frequency of 3,335.5 Hz. A second type of call was noted, which consisted of a short trill (\(\bar{x} = 0.12\) s) with a mean dominant frequency of 4,392.8 (± 121.8) Hz. While the advertisement call lengths were similar to those described previously for another frog in the *Craugastor podiciferus* species group (*C. podiciferus*), the calls differed with regard to the number of harmonics in the call and the mean dominant frequency. Our results will facilitate identification of closely related species during call surveys. Further, cryptic species likely remain within this group, and our findings will contribute to future taxonomic efforts.

**Key Words:** advertisement call, bioacoustics, frog, *Craugastor podiciferus* species group

**Resumen:** Las vocalizaciones de muchas de las especies de anuros de la megadiversa familia Craugastoridae siguen siendo desconocidas. Una de esas especies es *Craugastor stejnegerianus*, una rana que se encuentran en las selvas tropicales de Costa Rica y el oeste de Panamá. Grabamos las vocalizaciones de esta especie en el Refugio Nacional de Vida Silvestre Hacienda Barú, Provincia de Puntarenas, Costa Rica. Las llamadas típicas de advertencia constaban de una única nota corta (\(\bar{x} = 0.051\) s), con un promedio de llamada de intervalo de 30.7 s y una frecuencia dominante media de 3,335.5 Hz. Un segundo tipo de llamada fue detectada, y constaba de un trino corto (\(\bar{x} = 0.12\) s) con una frecuencia dominante media de 4,392.8 (± 121.8) Hz. Mientras que las longitudes de las llamadas de advertencia fueron similares a las descritas anteriormente para otra rana del complejo de especies de *Craugastor podiciferus* (*C. podiciferus*), las llamadas diferían con respecto al número de armónicos en la llamada y la frecuencia dominante media. Nuestros resultados facilitarán la identificación de especies estrechamente relacionadas durante muestreos acústicos. Además, es probable que especies cripticas permanezcan dentro de este grupo, y nuestras conclusiones contribuirán a futuros esfuerzos taxonómicos.

**Palabras Claves:** Bioacústicas, llamadas de aviso, rana, serie de especies *Craugastor podiciferus*
INTRODUCTION

The family Craugastoridae includes an abundant and diverse group of frogs containing 19 genera and 783 species (Frost, 2016). Many of these species are difficult to distinguish based on morphology alone, which has led to a confusing taxonomic history for this group, and this situation only recently has been clarified by using molecular data (Crawford and Smith, 2005; Frost et al., 2006; Heinicke et al., 2007). Because of the cost and time required for molecular analyses, however, these methods not always are available to field researchers. Temporal and spectral properties of frog vocalizations are unique to each species and can be used as an aid in identification during field surveys, and also might be useful for distinguishing between cryptic species.

Within the family Craugastoridae is a group of nine closely related frog species regarded as the *Craugastor podiciferus* species group by Hedges et al. (2008) and Padial et al. (2014). The frogs in this group are similar in terms of morphology and habitat, and even have similar advertisement calls to the human ear (Savage and Emerson, 1970; Savage, 2002). Among the nine species in this group is *C. stejnegerianus* (Stejneger’s Flesh-bellied Frog), a common and locally abundant litter frog occurring in the lowland and premontane forests on the Pacific versant of Costa Rica and western Panama, the Meseta Central region, and on the periphery of the Atlantic lowlands in proximity to Laguna Arenal (Savage, 2002). This small (12 to 18 mm snout–vent length [SVL]) frog is reported to be diurnal (Savage, 2002), but also is active at night, especially during and after rain and during bouts of breeding activity (Gómez-Hoyos et al., 2016; JET, JOC, pers. observ.).

Despite the fact that *C. stejnegerianus* is common and locally abundant, little has been reported regarding its microhabitat and behavior. Additionally, no formal analysis of the advertisement call of *C. stejnegerianus* exists in the literature. Savage (2002) reported that the call of *C. stejnegerianus* consists of a “single low squeak, repeated after a pause.” Nonetheless, details of the temporal and spectral properties of the call have not been reported. To further our knowledge of this species’ natural history and to allow for bioacoustical comparisons with the advertisement calls of related species, our objective was to fully describe the temporal and spectral properties of the advertisement call of *C. stejnegerianus*.

MATERIALS AND METHODS

We made observations and recordings of *Craugastor stejnegerianus* on 18 May and 28 May 2015. On both occasions, we made in situ recordings of *C. stejnegerianus* in a tropical lowland forest on the Pacific versant of Puntarenas, Costa Rica, within Refugio Nacional de Vida Silvestre Hacienda Barú (09.270268°N, -83.880851°W; WGS 84; elev. 15 m). We made audio recordings using a Tascam® DR-100 portable digital recorder coupled with a Sennheiser® MKE-600 shotgun microphone. Sample recordings are archived at the Fonoteca Zoológica Animal Sound Library at the Museo Nacional de Ciencias Naturales of Madrid, Spain (www.fonozoo.com; accession #s 9965–9968).

We located the frogs by traversing the trail and listening for vocalizations. When an audible call was heard, we conducted a search to visually locate the frog, and once found, we held a shotgun microphone at a distance of 1 m or less from the frog. We made most recordings under ambient light conditions; however, we used a red LED light to visually confirm that the calls originated from *C. stejnegerianus*.

We attempted to capture vocalizing frogs to obtain morphometric data and vouchers. We inspected the size of the tympanic membrane in captured frogs to determine their sex (tympanic membrane approximately equal in
diameter to the size of the eye in males, smaller in females); further, we assumed that sampled individuals were males based on their calling behavior. We obtained the SVL and body mass by using a dial caliper and a Pesola® 10g spring scale. In addition, we measured the air temperature, relative humidity, and wind speed with a digital Merlin® 3000.

We recorded the vocalizations of eight frogs, totaling 106 calls. The number of calls per frog ranged from two to 20. We determined the vocalization type based on definitions by Wells (2007), and defined the call variables as in Heyer et al. (1990). We analyzed and graphically constructed calls using Raven Pro sound analysis software version 1.5 (Bioacoustics Research Program, 2014). We constructed time waveforms and spectrograms to determine the temporal parameters of all calls. The temporal properties measured for each series of frog calls included note duration and call interval.

We scored the call quality, and used only those considered good to excellent \( n = 42 \) for spectral analysis. We constructed audio spectrogram slices with the use of fast Fourier transform, and time grids with a hop size of 128 samples, with 50% overlap, and frequency grid DFT size of 256 samples with grid spacing of 172 Hz, Hann’s sampling window (256 samples), and a 3 dB filter bandwidth of 248 Hz. The spectral properties measured included low and high frequencies, dominant frequency, fundamental frequency, the number of harmonics, and the emphasized harmonic. We determined the dominant frequency by using the slice spectrogram feature in Raven Pro to evaluate the emphasized harmonic at the beginning (~5%) of the call, at the peak frequency, and at the end (~95%) of the call for the distribution of maximum energy. We then calculated the mean dominant frequency for the entire note using these values. Similarly, we calculated the mean of the fundamental frequency.

RESULTS

Field Observations

Although typically found in the leaf litter on the forest floor, we found male *Craugastor stejnegerianus* calling from the upper surface of green leaves at heights of approximately 10–20 cm. The frogs sat with their head elevated while calling, and sometimes adjusted their orientation on the leaf between calls. One frog was observed to sit with the digits on the leaf, but with the tarsi raised, in a posture similar to that observed in vocalizing *C. underwoodi* (JOC, pers. obsrv.). We estimated the density of calling males to be approximately 1–2 males m\(^{-2}\). When disturbed by motion or excessive use of white light, the frogs ceased vocalizing for 10–15 min after the disturbance. We noted that the call of *C. stejnegerianus* is not as audible as those of some sympatric species, such as the Common Tink Frog, *Diasporus diastema*. From our anecdotal observations, we estimated the audible distance to be less than 3–5 m.

On 18 May from 0110 to 0200 h, we made observations and recordings approximately 30 m from the beginning of the Pizote trail at Refugio Nacional de Vida Silvestre Hacienda Barú. The weather conditions consisted of overcast skies, but no rain. The relative humidity was 85.4% and air temperature was 28.3°C with little to no wind. Initially, we obtained five calls from an individual at a distance of ≤ 1 m. After locating a second male, we recorded 12 high quality vocalizations with the microphone positioned at a distance of ~20–30 cm. We captured, measured, and weighed this male, and recorded its SVL as 16.4 mm and its body mass as 0.6 g; we then preserved the specimen as a voucher and deposited it in the Museo de Zoología at the Universidad de Costa Rica (UCR22454; Fig. 1).

On 28 May from 0009 and 0130 h, we made observations and recordings approximately 100 m from the start of the Pizote trail. The weather conditions consisted of partly cloudy skies under a waxing gibbous moon, with rain having fallen approximately 1.5 h earlier. The relative humidity was 99.1% and air temperature was 25.5°C with no wind. We recorded the vocalizations of six frogs, with the microphone positioned approximately 20–30 cm from each frog. We collected, measured, and weighed four of the six frogs, and their SVL ranged from 16.9 to 19.6 mm and their body mass from 0.4 to 1.0 g (Table 1).
Table 1. Advertisement calls of *Craugastor stejnegerianus* (Anura: Craugastoridae) recorded 18 May (0110 to 0200 h) and 28 May 2015 (0009 to 0130 h) at Refugio Nacional de Vida Silvestre Hacienda Barú, Provincia de Puntarenas, Costa Rica (values reported as \( \bar{x} \pm \text{standard deviation} \)).

<table>
<thead>
<tr>
<th>Individual</th>
<th>Temporal Parameters</th>
<th>Spectral Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frog ID</td>
<td>SVL (mm)</td>
<td>Mass (g)</td>
</tr>
<tr>
<td>A</td>
<td>17.0</td>
<td>1.0</td>
</tr>
<tr>
<td>B</td>
<td>19.6</td>
<td>0.6</td>
</tr>
<tr>
<td>C</td>
<td>16.9</td>
<td>0.9</td>
</tr>
<tr>
<td>D</td>
<td>—</td>
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</tr>
<tr>
<td>E</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>F</td>
<td>17.3</td>
<td>0.4</td>
</tr>
<tr>
<td>G</td>
<td>16.4</td>
<td>0.6</td>
</tr>
<tr>
<td>H</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>17.4 (1.3)</strong></td>
<td><strong>0.7 (0.24)</strong></td>
<td><strong>106</strong></td>
</tr>
</tbody>
</table>

Fig. 1. Dorsolateral and ventral views of a male *Craugastor stejnegerianus* (Stejneger’s Flesh-bellied Frog) collected to obtain photos and morphometric data (SVL = 16.4 mm, body mass = 0.6 g, pictured units are cm), then deposited as a voucher specimen in the Museo de Zoología at the Universidad de Costa Rica (UCR22454). Advertisement calls were recorded *in situ* at Refugio Nacional de Vida Silvestre Hacienda Barú, Provincia de Puntarenas, Costa Rica.

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Call Analyses

We confirmed that the advertisement call is a single, short squeak as described by Savage (2002), with a mean call duration of 0.051 (± 0.01) s. The call interval varied, with a range of 2.0 to 93.0 s, and a mean call interval of 30.7 (± 22.4) s (Table 1). Although we lacked sufficient data to quantify bout parameters, anecdotal observations suggest that calling occurs in bouts with neighboring frogs chorusing together. Calling bouts were separated by periods of silence ranging from approximately five to 15 min.

The advertisement calls were well modulated with harmonics clearly defined, ranging in number from eight to 12 with a mean of 10.3 (± 1.5), and the emphasis on the second harmonic (Fig. 2). The inverted chevron pattern in the spectrogram shows frequency modulation across the call. For example, the mean starting frequency of the emphasized harmonic was 3,526.7 (± 285.9) Hz, which rose slightly to a peak of 3,579.8 (± 213.3) Hz, and then dropped to a mean frequency of 2,899.5 (± 463) Hz at the end of the call. Other harmonics followed a similar pattern of modulation (Fig. 2). Among the 42 high quality calls, the mean low frequency was 1,360 (± 316.7) Hz and the mean high frequency 20,158.3(± 1,693.3) Hz. The mean dominant frequency for all calls was 3,335.5 (± 239.9) Hz. The mean frequency of the fundamental harmonic for high quality calls was 2,069.6 (± 520.4) Hz (Table 1). Although audibly similar, several of the presumed advertisement calls (n = 6) displayed a slightly different spectrogram that exhibited flat or down-sweeping harmonics, and fewer total harmonics (Fig. 2).

![Fig. 2. Time waveform (top) and frequency spectrogram (bottom) of: (A) a typical advertisement call, (B) an infrequently produced, down-sweeping call, and (C) a trill (buzz-like) call of a male Craugastor stejnegerianus (Stejneger’s Flesh-bellied Frog) recorded in situ at Refugio Nacional de Vida Silvestre Hacienda Barú, Provincia de Puntarenas. The frog producing call (A) was collected, preserved, and deposited in the Museo de Zoología at the Universidad de Costa Rica (UCR22454). Recordings of all three calls were archived at the Fonoteca Zoologica Animal Sound Library at the Museo Nacional de Ciencias Naturales of Madrid, Spain (www.fonozoo.com; accession #s 9968 [A], 9967 [B], and 9966 [C]).]
In addition to the advertisement calls, a second call type was noted in one male that produced three separate whirring or buzz-like sounds (trills), with a mean call length of 0.12 (± 0.02) s. Two of these three calls were of sufficient quality for spectral analysis, and consisted of 11 and 14 pulses, respectively, with a mean pulse length of 0.006 (± 0.001) s. The two calls had a mean dominant frequency of 4,392.8 (± 121.8) Hz, a mean low frequency of 1,337.2 (± 38.6) Hz, and a mean high frequency of 19,649.0 (± 578.9) Hz (Fig. 2).

**DISCUSSION**

Our work corroborates earlier informal descriptions of *Craugastor stejnegerianus* vocalizations (Savage 2002). Males call at night in chorus-like bouts of activity that seem associated with rain events during the wet season. The males we observed were calling from positions on leaf surfaces of low-growing vegetation and produced a faint, squeak-like call. The absence of vocal slits and a vocal sac in *C. stejnegerianus* likely contribute to the relatively low energy of their vocalizations. The dispersed nature of calling males, the short call duration, and the low energy of the call may explain, in part, why the call of *C. stejnegerianus* has not been formally described.

In the *Craugastor podiciferus* species group, formal descriptions of vocalizations only exist for *C. podiciferus* (Schlaepfer and Figeroa-Sandi, 1998). A comparison of the spectrograms for the advertisement calls of *C. podiciferus* to those of *C. stejnegerianus* shows a similar inverted chevron pattern, and similar note lengths ranging from 0.043 to 0.057 s. The squeak call for *C. podiciferus*, however, has fewer harmonics (three vs. 10 for *C. stejnegerianus*). Our observations indicate that the quality of the recording influences the number of detectable harmonics. Consequently, the difference in the number of harmonics between *C. podiciferus* and *C. stejnegerianus* possibly is a function of the call recording quality rather than true spectral differences. The squeak call of *C. podiciferus* also differed in having a lower mean dominant frequency (2,700 Hz) as compared to *C. stejnegerianus* (3,355.5 Hz).

We also compared the trill (buzz-like call) of a male *C. stejnegerianus* to the trill of a male *C. podiciferus* described by Schlaepfer and Figeroa-Sandi (1998). Although the spectrograms had a similar appearance (poor frequency modulation and no visible harmonics), the trill of *C. podiciferus* differed in having a fewer number of pulses (8–9 vs. 11–14 for *C. stejnegerianus*) and a higher mean dominant frequency (5,500 Hz vs. 4,392.9 Hz for *C. stejnegerianus*).

A comparison of the advertisement call of male *C. stejnegerianus* to the reciprocal call of female *C. podiciferus* (Schlaepfer and Figeroa-Sandi, 1998) showed both to have a similar inverted chevron pattern and similar note duration (0.051 vs. 0.057 s). They differed, however, in the number of harmonics (8–12 vs. 5 for *C. podiciferus*), and the mean dominant frequency (3,355.5 vs. 3,100 Hz for *C. podiciferus*). At locales where *C. stejnegerianus* and *C. podiciferus* are syntopic, advertisement calls and reciprocal calls of the respective species may be similar enough to necessitate spectrographic analysis to differentiate the species.

We presume that the majority of the vocalizations described herein were advertisement calls. Additional work is necessary to determine whether other vocalizations, such as the trill (buzz-like call), and the down-sweeping calls cited herein, are used for courtship, and/or aggressive calls (Wells and Schwartz, 2007). Further work also is necessary to describe bout duration and bout interval, as well as to determine if *C. stejnegerianus* utilize female reciprocal calling similar to that described for *C. podiciferus*.

Calling activity in frogs can vary based on fluctuations in abiotic conditions, such as temperature, rainfall, seasons, elevation, and possibly lunar phases (Gayou, 1984; Brooke et al., 2000; Saenz et al., 2006; Grant et al., 2012). Saenz et al. (2006) suggested that variations in these abiotic conditions might lead to a diversity of breeding strategies due to changing environmental conditions. We noted anecdotaly that calling activity of *C. stejnegerianus* increased during or after rain events and when the humidity was high. We do not know, however, whether specific abiotic conditions might trigger or affect *C. stejnegerianus* breeding activity, and whether breeding is opportunistic or predictable based on environmental conditions. Further work will help identify environmental variables that influence reproductive behavior.

*Craugastor stejnegerianus* is considered to be a common species in Costa Rica and western Panama by the IUCN SSC Amphibian Specialist Group (2015), with a large, stable population. One limitation of our study is that it was limited to a single study site within the geographical range of this species. Additional work from other sites
may provide evidence of variation in advertisement calls within the species, and provide additional comparisons to the calls of similar cryptic species, such as the recently identified *C. gabbi* and a likely unidentified species in the region of Cordillera de Tilarán (Crawford, 2003; Arias et al., 2016).

*Craugastor stejnegerianus* has been assigned to a species group in which morphological similarities and taxonomic uncertainties make it difficult to distinguish between species in the field. In such cases, acoustic signals can be a useful tool, along with genetic analysis, for species identification (Kelley et al., 2001; Angulo and Reichle, 2008). Acoustic signals are diagnostic because anuran advertisement calls are species-specific, and are used for mate recognition. As such, these calls function as prezygotic barriers, leading to reproductive isolation between cryptic species (Kelley et al., 2001), and ultimately to divergence in both morphology and call characteristics. Our results provide comparative data for future efforts in clarifying phylogenetic relationships within this confusing group of small, drab litter frogs.

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**Literature Cited**


Twining and Cossel, Jr.


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