
VOCALIZATIONS OF FIVE SPECIES OF FROGS IN THE *CRAUGASTOR PODICIFERUS* SPECIES GROUP (ANURA: CRAUGASTORIDAE) FROM COSTA RICA

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Abstract.—Certain taxonomic groups appear superficially very similar to each other, which may disguise hidden diversity. In such cases, vocalizations can be important distinguishing characteristics, particularly for anurans. One such group of anurans is the *Craugastor podiciferus* species group, for which vocalizations of many species have not been previously published. Consequently, our aim was to quantitatively describe for the first time, the vocalizations of three species from this group: *Craugastor gabbi* (Gabb's Flesh-bellied Frog), *C. aff. persimilis* (Similar Flesh-bellied Frog), and *C. underwoodi* (Underwood's Flesh-bellied Frog), and to describe calls of two other species, *C. podiciferus* (Piglet Flesh-bellied Frog), and *C. stejnegerianus* (Stejneger's Flesh-bellied Frog), from additional localities. We recorded 510 calls from 29 individuals at five localities in Costa Rica. We confirmed that all five species have a tonal call consisting of a short (0.10–0.79 s), single, squeak-like note with a dominant frequency ranging from 2,483.5–6,175.7 Hz. We further confirmed that *C. podiciferus* produces a pulsed call, and document them for *C. gabbi*, *C. aff. persimilis*, and *C. underwoodi*. Lastly, we present several miscellaneous call types (up-sweep, down-sweep, pulsatile-harmonic, and chain-chirp). Our quantitative analyses revealed differences in taxonomically important call variables such as call duration and dominant frequency. Formal descriptions of frog vocalizations are important in facilitating species identification in the field and may contribute to the recognition of hidden species diversity in morphologically similar species groups.

Key Words.—amphibians; bioacoustics; Central America; *Craugastor gabbi*; *Craugastor aff. persimilis*; *Craugastor stejnegerianus*; *Craugastor underwoodi*

Resumen.—Ciertos grupos taxonómicos poseen similitudes superficiales que pueden dificultar la identificación de especies y encubrir su diversidad real. En tales casos, las vocalizaciones pueden ser características distintivas importantes, particularmente para los anuros. Uno de estos conjuntos de anuros es el grupo de especies *Craugastor podiciferus*, en el que no existen descripciones de las vocalizaciones de muchas de sus especies. Por lo tanto, nuestro objetivo fue describir cuantitativamente por primera vez los cantos de anuncio de tres especies de este grupo (*Craugastor gabbi* [Rana de Hojarasca de Gabb], *C. aff. persimilis* [Rana de Hojarasca Similar], y *C. underwoodi* [Rana de Hojarasca de Underwood]) y proveer información de vocalizaciones emitidas por otras dos especies (*C. podiciferus* [Rana de Hojarasca Cerdito] y *C. stejnegerianus* [Rana de Hojarasca de Stejneger]) de localidades adicionales. Registramos 510 llamadas de 29 individuos en cinco localidades de Costa Rica. Confirmamos que las cinco especies tienen una llamada tonal que consiste en una nota corta (0.10–0.79 s), simple, con una frecuencia dominante que varía de 2,483.5–6,175.7 Hz. Además, confirmamos que *C. podiciferus* produce llamadas pulsada y documentamos llamadas similares para *C. gabbi*, *C. aff. persimilis* y *C. underwoodi*. Por último, presentamos varios otros tipos de llamadas (ascendentes, descendentes, armónicos pulsados y chirridos en cadena). Nuestros análisis cuantitativos revelan diferencias en las variables acústicas de importancia taxonómica, como la duración de la llamada y la frecuencia dominante. Las descripciones formales de vocalizaciones de anuros son importantes para facilitar la identificación de especies en el campo y pueden contribuir al reconocimiento de la diversidad de especies ocultas en grupos de especies morfológicamente similares.

Palabras Clave.—anfibios, bioacústica, Centroamérica; *Craugastor gabbi*; *Craugastor persimilis*; *Craugastor stejnegerianus*; *Craugastor underwoodi*

INTRODUCTION

Taxonomically, cryptic species are those nominally classified as a single species based on morphological similarities, but which may actually contain multiple

species when other diagnostic features are considered (Bickford et al. 2006; Vieites et al. 2009). For example, using molecular data, notable hidden species diversity has been uncovered (Stuart et al. 2006; Fouquet et al. 2007). Alternatively, because frog vocalizations

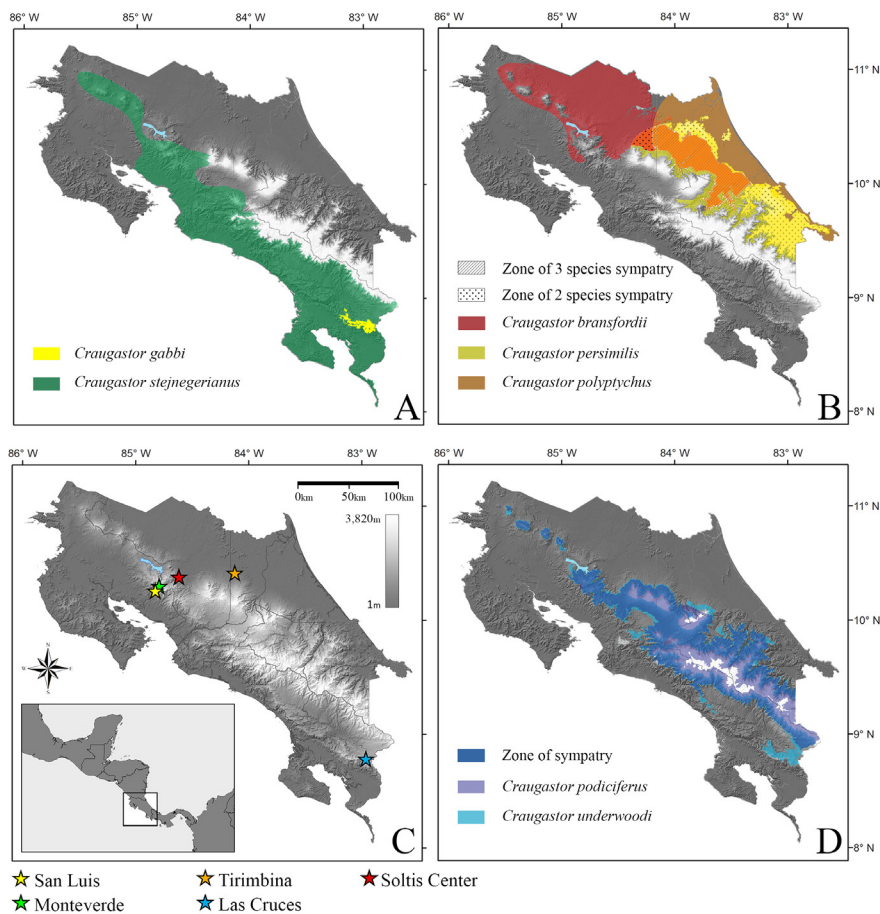


FIGURE 1. Range maps of the seven frog species in the *Craugastor podiciferus* species group that occur in Costa Rica (adapted from Cossel and Kubicki 2017), and locations of study sites. (A) Extensive regions of potential sympatry exist between Pacific slope species, (B) Caribbean slope species, and (D) montane species. (C) Location of sites in Costa Rica (details in Table 1) where we obtained digital audio recordings of the vocalizations of five species of frogs in the *C. podiciferus* species group.

are usually unique to each species, bioacoustical and behavioral data have also been used to delimit cryptic frog species, often in conjunction with genetic analysis (Angulo and Reichle 2008; Elmer and Cannatella 2008; Padial et al. 2008; Funk et al. 2012). A ramification of cryptic species identification for amphibian conservation is that the number of frog species in some taxonomic groups may be underrepresented if classification is based on morphology alone (Bickford et al. 2006; Pérez-Ponce de León and Poulin 2016). According to Funk et al. (2012), correctly identifying cryptic species will be important for identifying regions with high levels of species richness that merit additional protection. Some species currently considered a single species with a broad geographic range may actually constitute several species with smaller and more isolated ranges, each potentially warranting a higher conservation status (Funk et al. 2012; Gehara et al. 2014).

One group of frogs that should be further investigated for the presence of taxonomically cryptic species is the *Craugastor podiciferus* species group (Hedges et al. 2008). This species group belongs to the family

Craugastoridae, a diverse group of frogs containing 20 genera and 823 species (Frost, D.R. 2018. *Amphibian Species of the World: An Online Reference*. Version 6.0. American Museum of Natural History, New York, USA. Available from <http://research.amnh.org/herpetology/amphibia/index.html>. [Accessed 1 January 2018]). As described by Hedges et al. (2008), Padial et al. (2014), and Arias et al. (2016), the *Craugastor podiciferus* species group includes nine frogs: *Craugastor bransfordii* (Bransford's Flesh-bellied Frog), *C. gabbi* (Gabb's Flesh-bellied Frog), *C. jota* (Rio Changua Flesh-bellied Frog), *C. lauraster*, *C. persimilis* (Similar Flesh-bellied Frog), *C. podiciferus* (Piglet Flesh-bellied Frog), *C. polyptychus* (Many Folds Flesh-bellied Frog), *C. stejnegerianus* (Stejneger's Flesh-bellied Frog), and *C. underwoodi* (Underwood's Flesh-bellied Frog). These frogs are all similar in appearance and occur in similar habitats (e.g., leaf litter) in Central America, ranging from Honduras to Panama (Savage 2002); however, the majority of the species in this species group occur in Costa Rica (seven of nine species), with numerous locations of sympatry (Fig. 1).

TABLE 1. Locality details of sites in Costa Rica where vocalizations of five frog species in the *Craugastor podiciferus* species group, were recorded. Climate data are ¹Zahawi et al. (2017); ²<http://soltiscentercostarica.tamu.edu/>, accessed 3 March 2019; ³<https://weather-and-climate.com/>, accessed 3 March 2019; ⁴<http://www.reservamonteverde.com/climate.html>, accessed 3 March 2019; and ⁵Méndez Corrales (2009). We determined forest type by visual inspection.

Species	Site Name (ownership)	Coordinates	Elevation (ASL)	Holdridge Life Zone	Climate	Forest Type
<i>Craugastor gabbi</i> (Gabb's Flesh-bellied Frog)	Las Cruces Biological Station (Organization for Tropical Studies)	8.7857°N 82.9630°W	1,150 m	Tropical pre-montane wet forest	Average annual temp. of about 21° C; Average annual rainfall of 3,500–4,000 mm ¹	Secondary
<i>Craugastor aff. persimilis</i> (Similar Flesh-bellied Frog)	Soltis Center (Texas A&M University)	10.3835°N 84.6195°W	500 m	Transitional tropical pre-montane moist forest	Average annual temp. of 24° C; average relative humidity of 85%; average estimated annual rainfall of 4,200 mm ²	Secondary
<i>Craugastor aff. persimilis</i> (Similar Flesh-bellied Frog)	Tirimina Biological Reserve (private reserve)	10.4173°N 84.1256°W	200 m	Very humid tropical forest	Average annual temp. of 25.3° C; average annual precipitation of 3,777 mm ³	Gardens
<i>Craugastor podiciferus</i> (Piglet Flesh-bellied Frog) and <i>Craugastor underwoodi</i> (Underwood's Flesh-bellied Frog)	Monteverde Cloud Forest Preserve (Tropical Science Center)	10.3000°N 84.8167°W	1,300– 1,500 m	Low montane rainforest; low montane wet forest	Average annual temp of 18.8° C; average annual precipitation of 2,579 mm ⁴	Primary, secondary
<i>C. stejnegerianus</i> (Stejneger's Flesh-bellied Frog)	San Luis Biological Reserve (Tropical Science Center)	10.2607°N 84.8276°W	700 m	Transitional pre-montane wet forest	Average annual temp. of 23° C; average annual precipitation of 2,500–3,000 mm ⁵	Secondary

This species group has a confusing taxonomic history due to the morphological similarities between species. For instance, in the seminal work of Taylor (1952) on Costa Rican amphibians, he recognized eight distinct species; seven in the genus *Microbatrachylus* (*M. bransfordii*, *M. costaricensis*, *M. persimilis*, *M. polyptychus*, *M. rearki*, *M. stejnegerianus*, and *M. underwoodi*) and also *Eleutherodactylus podiciferus*. By contrast, Savage and Emerson (1970) synonymized this entire group into two species: the widely polymorphic species *E. bransfordii*, and *E. podiciferus*. Later, relying on molecular data, Miyamoto (1983) found that *E. bransfordii* and *E. stejnegerianus* were genetically distinct taxa. Subsequently, other workers have demonstrated genetic divergence within the *C. podiciferus* species group (Crawford 2003; Chen 2005; Crawford and Smith 2005; Streicher et al. 2009; Arias 2019). Savage (2002) resurrected *C. persimilis*, *C. polyptychus*, and *C. underwoodi*, leaving *C. rearki* and *C. costaricensis* under *C. bransfordii*, and *C. blairi*, *C. muricinus*, and *C. habenatus* under *C. podiciferus*. Previous to Savage (2002) two additional species were named, *C. jota* (Lynch 1980) and *C. lauraster* (Savage et al. 1996). Arias et al. (2016) identified an instance of cryptic diversity by recognizing *C. gabbi* as distinct from *C. stejnegerianus*. Most recently, Arias (2019) demonstrated substantial genetic diversity remaining within this species group, including evidence that the range of *C. persimilis* does not extend to the northern Caribbean slope where we obtained our samples, hence our use of *C. aff. persimilis* to minimize future taxonomic confusion.

The natural history and behaviors of many of the frogs in this group are poorly known, including the vocalizations (or calls) made by these frogs.

Qualitatively, known calls for frogs in this group are generally described as squeaks, trills, or chirps (Savage 2002; Hedges et al. 2008; Cossel and Kubicki 2017). Formal quantitative descriptions only exist for two species. Schlaepfer and Figueroa-Sandi (1998) formally described three vocalizations of *Craugastor podiciferus*: a squeak-like advertisement call, a trill, and a female reciprocal call. Twining and Cossel (2017) recently described the advertisement call, trill, and down-sweeping call of *C. stejnegerianus*.

In this paper, we compare vocalizations for five species in the *Craugastor podiciferus* species group: *C. gabbi*, *C. aff. persimilis*, *C. podiciferus*, *C. stejnegerianus*, and *C. underwoodi*. For the first time we quantitatively describe the tonal (squeak-like) calls of three species: *Craugastor gabbi*, *C. aff. persimilis*, and *C. underwoodi*. We also describe additional vocalization types including pulsed calls (trills), and other novel call types (down-sweep, up-sweep, pulsatile, and chain-chirp). Additionally, we compare the temporal and spectral properties of the calls of these five species to evaluate the potential for using bioacoustics to distinguish between these frogs in the field in areas where their ranges overlap. Our results may inform future taxonomic efforts at identifying suspected cryptic species still remaining within this group.

MATERIALS AND METHODS

Study sites.—All of our study sites were within the Republic of Costa Rica (Fig. 1). The study areas and the species recorded at each site were varied (Table 1); by elevation, Holdridge life zones (Holdridge 1967), forest types, and climatic variables (mean temperatures and precipitation levels).

Field measurements.—At each site, we located frogs by listening for vocalizing individuals in suitable habitat. We conducted searches primarily after dusk; however, we did record calls of a *Craugastor gabbi* male before sunset. On some occasions, we attempted to elicit a call response from frogs by mimicking their calls (producing a squeak). We also on occasion used playback via a Bluetooth (Bluetooth Special Interest Group, Kirkland, Washington, USA) speaker and/or phone, with pre-recorded audio files of the target species or a closely related species from the *Craugastor podiciferus* species group; for example, playing audio of *C. stejnegerianus* to elicit a response from *C. gabbi*. Once located, we visually confirmed the identity of the vocalizing frogs using white light, and subsequently obtained recordings in ambient or red light to minimize disturbance.

We recorded calls using the following combination of digital audio recorders and shotgun microphones: (1) Tascam® DR-05 (Tascam, Montebello, California, USA; sample rate = 44.1 kHz, 16-bit resolution, WAV format) with an Audio-Technica ATR-6550 shotgun microphone (Audio-Technica, Stow, Ohio, USA); (2) Tascam® DR-100 recorder (sample rate = 44.1 KHz, 24-bit resolution, WAV format) with a Sennheiser MKE-600 shotgun microphone (Sennheiser Electronic Corporation, Old Lyme, Connecticut, USA); and (3) Tascam® HD-P2 (sample rate = 44.1 KHz, 24-bit resolution, WAV format), coupled with a Sennheiser MKE-600 shotgun microphone. We made all recordings with the microphone at a distance of 1 m or less from the frogs. In a few instances we were able to use an Extech Sound Level Meter Model 407730 (FLIR Commercial Systems Inc., Nashua, New Hampshire, USA; ± 2 dB accuracy with 0.1 dB resolution) to record the sound intensity or power level (i.e., loudness) of frog vocalizations.

When possible, we captured frogs after recording to confirm species identity. We relied on morphological characteristics, dichotomous keys, and range maps in Savage (2002) and Arias et al. (2016). We also obtained morphometric data, using calipers to measure snout-vent length (SVL), and Pesola® spring scales (Pesola AG, Schindellegi, Switzerland) to measure body mass. We collected photo and audio vouchers of all species, and we archived these at the Fonoteca Zoológica Animal Sound Library at the Museo Nacional de Ciencias Naturales of Madrid, Spain (www.fonozoo.com; accession numbers 11178–11188). Our permit allowed for the collection of museum vouchers of *C. aff. persimilis* and we deposited two individuals in Museo de Zoología at Universidad de Costa Rica (Tirimina - UCR #22515; Soltis - UCR #22516). In addition to frog morphometrics, we noted microhabitat details, such as calling height, perch type, and substrate. We

also recorded ambient weather conditions, such as cloud cover and precipitation, and when possible, we recorded wind speed, air temperature, and relative humidity using a Kestrel® Model 3000 weather meter (Kestrel Instruments, Boothwyn, Pennsylvania, USA).

Data analysis.—We used Raven Pro sound analysis software version 1.5 (<http://www.birds.cornell.edu/raven>) to analyze time waveforms and spectrograms for each call, and the package Seewave in R (Sueur et al. 2008) to graphically construct time waveforms and spectrograms of representative calls at a sensitivity threshold of 55 dB. We used the terminology of Köhler et al. (2017) to categorize vocalization types, temporal variables, and spectral variables. We scored call quality by visually referring to the spectrogram (noise level, harmonic structure, and number of harmonics visible), and generally only used those considered good to excellent for spectral analysis, whereas we used all calls for temporal parameters. Using Raven Pro, we manually selected each call to determine note duration (delta time) and call interval (difference in time between two successive calls). For vocalizations composed of discrete pulses (e.g., trills), we determined the number of pulses and pulse duration manually. When calls were organized into call groups, sometimes referred to as bouts, we measured the number of calls in the call group and the intervals between these calls.

Using the same selections, we completed spectral analysis using Raven Pro with the following presets: Fast Fourier Transform = 1024; time grids with a hop size of 128 samples, with 50% overlap; frequency grid Discrete Fourier Transform 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. For each call, we manually determined the lowest and highest frequencies, and when visible in the spectrogram we counted the number of harmonics. We used the selection spectrum function in Raven Pro, which measures the average spectrum of the call over the selected time interval, to determine fundamental frequency (the frequency of the first harmonic in the call spectrum), dominant frequency (the frequency with the highest amplitude in the call spectrum), and the emphasized harmonic (harmonic that includes the dominant frequency). In some cases, for example with pulsed calls (trills), we were unable to determine a fundamental frequency. To facilitate comparisons between northern and southern populations of *Craugastor stejnegerianus* (northern = San Luis, present study; southern = Hacienda Baru [Twining and Cossel 2017]), we used non-paired, two-tailed, unequal variance *t*-tests ($\alpha = 0.05$) to evaluate mean values of individual frogs for the call parameters of note duration and dominant frequency, both described by Köhler et al. (2017) as being taxonomically informative.

TABLE 2. Temporal and spectral parameters of tonal (squeak-like) calls of frogs in the *Craugastor podiciferus* species group recorded from various localities in Costa Rica (Table 1). Values are reported as mean (\pm 1 SD). The advertisement call of *Craugastor underwoodi* consists of a single note that occurs in call groups of 2–4 notes. Consequently, the value reported for call interval for *C. underwoodi* represents the interval between call groups, whereas the mean inter-call interval is 2.0 (0.8) s. Headings are Species (n) = Species and sample size (number of frogs) for each type of analysis, NC = number of calls analyzed, ND = note duration (in seconds), CI = Call interval (in seconds), NH = number of harmonics, HE = harmonic emphasize (percentage occurrence), MinF = minimum frequency (Hz), MaxF = maximum frequency (Hz), FF = fundamental frequency (Hz), and DF = dominant frequency (Hz).

Species (n)	NC	ND	CI	NH	HE	MinF	MaxF	FF	DF
<i>Craugastor gabbi</i> (Gabb's Flesh-bellied Frog; n = 4 temporal and spectral)	14	0.079 (0.014)	43.2 (18.3)	11.5 (2.1)	1 (14%) 2 (86%)	1,430.1 (407.6)	21,485.9 (671.4)	1,929.2 (425.6)	3,253.9 (255.3)
<i>Craugastor</i> aff. <i>persimilis</i> (Similar Flesh-bellied Frog; n = 11 temporal; n = 4 spectral)	44	0.079 (0.034)	51.1 (65.9)	11.9 (2.0)	1 (23%) 2 (48%) 3 (25%)	1,867.5 (745.6)	20,843.5 (2,115.3)	2,183.8 (673.2)	4,503.5 (1,805.8)
<i>Craugastor podiciferus</i> (Piglet Flesh-bellied Frog; n = 1 temporal and spectral)	67	0.057 (0.006)	6.5 (5.5)	7.1 (1.6)	1 (48%) 2 (52%)	1,014.4 (401.7)	13,862.5 (3,768.9)	2,066.3 (170)	2,483.5 (784.5)
<i>Craugastor stejnegermanus</i> (Stejneger's Flesh-bellied Frog; n = 9 temporal; n = 8 spectral)	118	0.070 (0.011)	47.3 (29.8)	10.6 (1.3)	2 (64%) 3 (22%)	1,255.0 (411.5)	20,207.8 (2,060.5)	1,940.1 (393.0)	4,617.6 (1,787.7)
<i>Craugastor underwoodi</i> (Underwood's Flesh-bellied Frog; n = 4 temporal and spectral)	60	0.10 (0.02)	114.2 (56.7)	9.9 (3.2)	3 (83%) 4 (13%)	1,260.2 (295.0)	20,484.8 (2,365.7)	1,948.2 (278.2)	6,175.7 (732.1)

RESULTS

We recorded 510 vocalizations from 29 individuals, which we analyzed for temporal properties. Of these 510 vocalizations, we analyzed 348 for their spectral properties. Most of the vocalizations we analyzed were presumed to be advertisement calls (squeaks), consisting of a single tonal note (n = 457), and approximately two thirds of these (n = 303) were of sufficient quality to permit spectral analyses. The second most commonly recorded vocalizations (n = 45) were pulsed (trills), and all were of sufficient quality to permit spectral analyses. Pulsed vocalizations consisted of a single note subdivided into a series of pulses with notable amplitude modulation, varying in pulse number and rate for each species. We also recorded a limited number (n = 8) of other calls that did not fit into the advertisement call (squeak) or pulsed vocalization (trill) categories. Due to the small sample size, we present the characteristics of these calls in Appendix Table, Appendix Figure. For all temporal and spectral properties, we report these data as mean \pm standard deviation (\pm SD).

Based on our observations, the five focal species recorded from the *Craugastor podiciferus* species group call from sites in the leaf litter primarily, or from perches < 1 m (and often < 0.5 m) above the forest floor. Calling locations may be either exposed or concealed, and from a variety of substrates. The calling position for all species is principally horizontal, with head and torso elevated. We observed that frogs would sometimes move short

distances between calls at irregular time intervals, and/or adjust the orientation of their calling positions. They would often align themselves toward mimicked calls or audio playback of calls and would usually approach the source in what appeared to be an investigative manner.

The presumed advertisement calls of all five species consist of a single tonal note, exhibiting frequency modulation in an ascending then descending pattern, producing harmonics having an inverted U-shaped curve (Fig. 2). The intervals between the advertisement calls (inter-call intervals) vary depending on the species and environmental conditions (Table 2). We noted that the calls of all focal species were very faint, usually only audible from short distances (\leq 3 m).

***Craugastor gabbi*.**—We obtained in situ recordings for this species at the Las Cruces site on 12 May 2017 between 1700–2345. During recordings, the temperature ranged from 23.9–25.4° C, with 79–98% relative humidity, no wind, and no precipitation. Frogs were calling from semi-concealed locations in the leaf litter, or from clumps of vegetation on the forest floor, with no individual observed calling from higher than 10 cm. Similar to other focal species, advertisement calls of *C. gabbi* were audible at no greater than 2–3 m, and trills were even more faint, inaudible at distances of greater than about 1 m. We captured four frogs to obtain morphometric measurements, which ranged in size from 14.1–17.1 mm SVL, and in mass from 0.6–1.2 g.

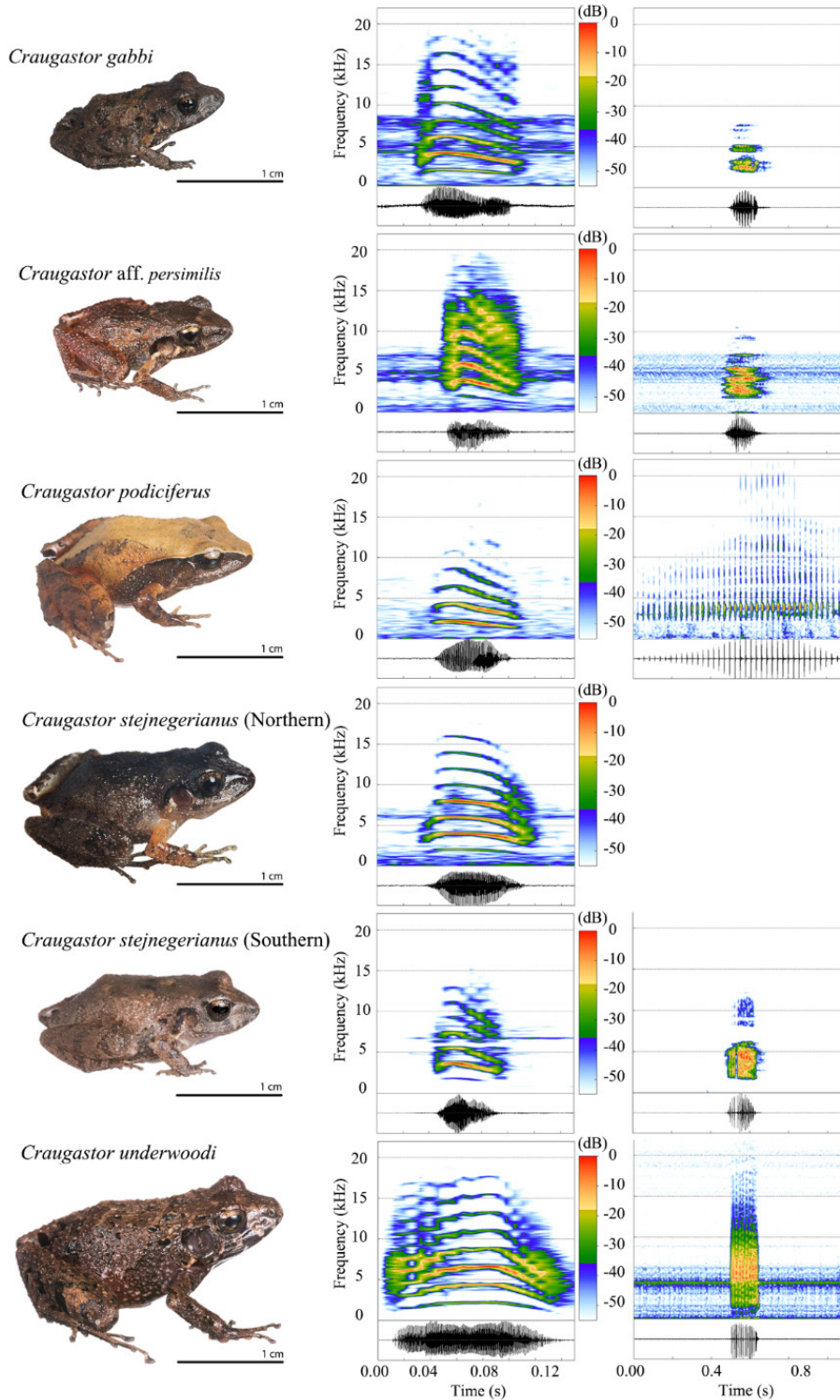


FIGURE 2. Representative individuals from each of five species in the *Craugastor podiciferus* species group, with spectrograms (above, in color) and waveforms (below, in black) for tonal, squeak-like calls (left column), and pulsed calls (right column), respectively. Calls were graphically constructed using Seewave for R (Sueur et al. 2008) at a sensitivity level of 55 dB (vocalization details; Tables 2 and 3). *Craugastor stejnegerianus* (Northern) represents recordings obtained at San Luis, Costa Rica, and *C. stejnegerianus* (Southern) is based on recordings obtained at Hacienda Baru, Costa Rica and described by Twining and Cossel (2017). (Photographed by John O. Cossel, Jr.)

TABLE 3. Temporal and spectral parameters of pulsed calls (trills) of frogs in the *Craugastor podiciferus* species group recorded from various localities in Costa Rica (Table 1). Values are reported as mean (\pm SD). Headings are Species (n) = Species and sample size (number of frogs) for each type of analysis, NC = number of calls analyzed, CND = Call (note) duration (in seconds), PPN = pulses per note, PR = pulse rate (pulses per second), MinF = minimum frequency (Hz), MaxF = maximum frequency (Hz), and DF = dominant frequency (Hz). For *Craugastor stejnegerianus*, information is from Twining and Cossel (2017).

Species (n)	NC	CND	PPN	PR	MinF	MaxF	DF
<i>Craugastor gabbi</i> (Gabb's Flesh-bellied Frog; n = 1)	13	0.14 (0.02)	9.385 (1.1)	67.19 (3.3)	1,573.7 (213.8)	8,294.3 (562.7)	4,665.3 (316.4)
<i>Craugastor aff. persimilis</i> (Similar Flesh-bellied Frog; n = 2)	12	0.14 (0.02)	13.0 (2.0)	93.50 (14.1)	1,907.5 (357.0)	12,681.3 (1,932.1)	4,565.0 (1000.4)
<i>Craugastor podiciferus</i> (Piglet Flesh-bellied Frog; n = 1)	16	0.97 (0.2)	35.13 (5.7)	36.48 (1.5)	1,888.3 (266.3)	21,509.01 (1,419.2)	3,606.84 (171.9)
<i>Craugastor stejnegerianus</i> (Stejneger's Flesh-bellied Frog; n = 1)	2	0.12 (0.02)	11–14	91–117	1,337.2 (38.6)	19,649.0 (578.9)	4,392.8 (121.8)
<i>Craugastor underwoodi</i> (Underwood's Flesh-bellied Frog; n = 1)	4	0.12 (0.01)	11.25 (3.0)	91.35 (15.1)	0	18,149.6 (3,266.1)	6,029.3 (730.86)

Craugastor gabbi has a short, tonal (squeak-like) advertisement call with short pauses between squeaks (Table 2). Each call contained multiple harmonics, and generally the second harmonic was emphasized, but occasionally the first harmonic was emphasized (Table 2). The tonal (squeak-like) vocalization of *C. gabbi* was frequency modulated across all harmonics with asymmetrical or positively-skewed harmonics (Fig. 2). The modulation of a typical call from *C. gabbi* had an initial frequency of 2,542 Hz, rising to 3,873 Hz, and then falling to 2,792 Hz in the emphasized harmonic (2nd; Fig. 2). We found that the mean fundamental frequency = $1,929.2 \pm 425.6$ Hz (range, 1,525–2,912 Hz), and the mean dominant frequency = $3,253.9 \pm 255.3$ Hz (range, 2,912–3,984 Hz; Fig. 2).

We also recorded pulsed (trill) vocalizations that consisted of a single pulsed note subdivided into a pulse series with a mean number of pulses = 9.4 ± 1.1 pulses (range, 7–11 pulses; Table 3). Pulsed vocalizations were notably longer than advertisement calls, with a mean note duration = 0.140 ± 0.017 s (range, 0.100–0.157 s; Table 3). These vocalizations are pulsatile-harmonic sounds as described by Köhler et al. (2017), and we found that the mean dominant frequency = $4,665.3 \pm 316.4$ Hz (range, 3,984–4,993 Hz; Table 3; Fig. 2).

***Craugastor aff. persimilis*.**—On 14 and 15 June 2015, we obtained in situ recordings of *Craugastor aff. persimilis* at the Soltis and Tirimbina sites. Ambient conditions on 14 June (2000–2200) were 26.1° C with 92% relative humidity, and on 15 June (2000–2200) were 26.1° C with 91% relative humidity. We observed individuals calling from the ground as well as from low vegetation, < 10 cm above the forest floor. Focal frogs at Tirimbina were calling in close proximity (about 2–3 m) to each other and the voucher specimen was found calling approximately 0.5 m away from another individual, which was not vocalizing at the time of recording. The individual from Soltis was calling alone

with no other apparent individuals vocalizing nearby. We captured one frog from each site and preserved it as a voucher specimen (Tirimbina - UCR #22515; Soltis - UCR #22516). We obtained morphometric measurements from each frog, with the SVL of 15.5 and 17.4 mm and mass of 0.4 and 0.5 g.

We made additional recordings at the Soltis Center site on 28 May 2017 between 2200–2330. The temperature ranged from 23.5–24.5° C, with 100% relative humidity and no wind. It had rained during the day but had stopped by the time of recording. Frogs were again calling about 10 cm above the ground. We captured two frogs to obtain morphometric measurements, which ranged in SVL from 13.1–16.2 mm, and in mass from 0.35–0.5 g.

We recorded four types of vocalizations for *Craugastor aff. persimilis* (Tables 2 and 3; Fig. 2; Appendix Table, Appendix Figure). *Craugastor aff. persimilis* produce a short, tonal (squeak-like) advertisement call, with pauses between calls of nearly a minute (Table 2). We analyzed the calls of four frogs for their spectral properties. Similar to *C. gabbi*, the tonal call of *C. aff. persimilis* was frequency modulated across all harmonics, and the shape of the frequency distribution for a typical call is an asymmetrical or positively-skewed, inverted U-shaped curve (Fig. 2). The modulation of a typical call from *C. aff. persimilis* had an initial frequency of 3,293 Hz, rising to 3,940 Hz, and then falling to 2,117 Hz for the emphasized harmonic (2nd; Fig. 2). The advertisement calls had multiple harmonics, and the emphasized harmonic was generally the second (48%; Table 3). The mean fundamental frequency = $2,183.8 \pm 673.2$ Hz, and the mean dominant frequency = $4,503.5 \pm 1,805.8$ Hz (range, 3,101–13,781 Hz; Table 2).

Craugastor aff. persimilis also makes a pulsed vocalization (trill) consisting of a single note subdivided into a pulse series with a mean of 13.0 ± 2.0 pulses (range, 10–16 pulses), with a note duration = 0.14 ± 0.02

s (range, 0.11–0.17 s). The mean dominant frequency for the trills = $4,565.0 \pm 1,000.4$ Hz (range, 3,101–6,374 Hz; Table 3). In addition to the advertisement calls and pulsed vocalizations (trills), we recorded three other types of vocalizations with unknown functions. These include three up-sweeping vocalizations with ascending frequency modulation produced by a single individual, two down-sweeping vocalizations with descending frequency modulation produced by the same individual, and two pulsatile-harmonic vocalizations produced by a separate individual. The temporal and spectral properties of these vocalizations are presented in Appendix Table, Appendix Figure.

Craugastor podiciferus.—We obtained recordings in situ for two frogs in close proximity (< 1 m) at the Monteverde site on 2 June 2017 at 2200; however, recordings for only one of the frogs was of sufficient quality to allow spectral analysis. The temperature was 19.7° C, with 100% relative humidity and a wind speed of 2.3 km/h. It had rained during the day but had stopped by the time of recording. The frog was calling from a dead leaf on the forest floor. When making the squeak-like advertisement call and subsequent trills, the subgular vocal sac was distended, with the sac about twice the size for the trill as it was for the squeak. During the trill, we noted a slow, sustained compression of the flanks of the frog. The sound meter registered 43 dB for the advertisement call (squeak) at a distance of 5 cm from the frog; for the pulsed calls (trills) we registered 57 dB at a distance of 7.5 cm.

We confirmed that *Craugastor podiciferus* produces a short, tonal (squeak-like) vocalization with a short pause between calls (Table 2). The tonal calls are spectrally similar to those of other species in the *C. podiciferus* species group having an inverted U-shaped curve with ascending and descending frequency modulation across all harmonics (Table 2; Fig. 2). The modulation of a typical call from *C. podiciferus*, with the first harmonic emphasized, had an initial frequency of 1,802 Hz, rising to 2,223 Hz, and then falling to 1,202 Hz. (Fig. 2). The tonal calls had multiple harmonics = 7.1 ± 1.6 harmonics (range, 6–10 harmonics), with a mean fundamental frequency = $2,066.3 \pm 170.0$ Hz (range, 1,587–2,240 Hz), and the mean dominant frequency = $2,483.5 \pm 784.5$ Hz (range, 1,895–4,307 Hz; Table 2). However, we found that the emphasized harmonic was either the first (47.8%) or the second (52.2%). When the first harmonic was emphasized, the mean dominant frequency = $2,137.2 \pm 114.6$ Hz (range, 1,895–2,412 Hz). When the second harmonic was emphasized, the mean dominant frequency = $3,691.9 \pm 293.3$ Hz (range, 2,756–4,479 Hz). We also recorded pulsed (trill) vocalizations with a duration of nearly 1 s, that consisted of a single pulsed note subdivided into a pulse

series and had a mean number of pulses per note = 35.1 ± 5.7 pulses (range, 28–48 pulses; Table 3). The mean dominant frequency of the pulsed calls = $3,606.8 \pm 171.9$ Hz (range, 3,445–3,962 Hz; Table 3; Fig. 2).

We recorded a third type of call from one individual that we have designated a chain chirp (Appendix Table, Appendix Figure). The single call consisted of a group of 12 chirp-like notes with a call duration = 2.08 s. The mean note duration within the note group = 0.032 s, and the mean inter-note interval = 0.15 s. Each of the first eight notes in the group is slightly shorter than the previous note. Each note in the series has between seven and eight visible harmonics, with the first being emphasized. The fundamental and dominant frequencies = 1,894.9 Hz.

Craugastor stejnegerianus.—We obtained recordings for this species in situ at the San Luis site on 5 June 2017 between 1830–2100. The temperature ranged from 23.8 – 25.0° C, with 100% relative humidity and no wind. It had rained during the day but had stopped by the time of recording. Frogs were primarily calling from dead leaves and sticks on the forest floor, but also from green leaves and branches at heights ranging from 5–100 cm above the forest floor. The sound pressure level for one frog was 57 dB at a distance of 7.5 cm from the frog. Seven frogs had a mean SVL = 20.0 ± 1.3 mm (range, 18.3–21.7 mm), and a mean mass = 0.7 ± 0.1 g (range, 0.5–0.8 g).

Craugastor stejnegerianus produces a short, tonal (squeak-like) advertisement call with pauses between calls (Table 2). The call is frequency modulated across all harmonics, and the shape of the frequency distribution for a typical call is a nearly symmetrical inverted U-shaped curve, with a slight, positive skew. The modulation of a typical call from *C. stejnegerianus* had an initial frequency of 3,175 Hz, rising to 3,940 Hz, and then falling to 2,646 Hz for the emphasized harmonic (2nd; Fig. 2). The advertisement call has multiple harmonics, and the emphasized harmonic was generally the second (64%) or third (22%; Table 2). The mean fundamental frequency = $1,940.1 \pm 393.0$ Hz (range, 1,381–4,080 Hz), and the mean dominant frequency was $4,617.6 \pm 1,787.7$ Hz (range, 2,756–13,092 Hz; Tables 2 and 3; Fig. 2).

Craugastor underwoodi.—We obtained recordings for this species at the Monteverde site on 2 and 3 June 2015 between 2000–2200. The temperature ranged from 21 – 22° C, with 80–90% relative humidity and no wind. One frog was calling from the leaf litter, and the second from a branch within 10 cm of the forest floor. We captured one of the frogs, which had a SVL of 24.8 mm and mass of 3.2 g.

The advertisement calls of four *Craugastor underwoodi* consisted of a short, tonal (squeak-like) call comprised of a single note (Table 2). Calls occur in call groups (bouts) of two (40%), three (40%), or four (20%) calls with a mean inter-call interval = 2.0 ± 0.8 s (range, 0.2–3.3 s). These call groups are separated by a longer period of silence, with a mean interval between call groups of 114.2 ± 56.7 s (range, 8.1–242.4 s; Table 2; Fig. 2).

The tonal advertisement call is frequency modulated across all harmonics, and the shape of the spectrogram for typical calls are nearly symmetrical or positively-skewed, inverted U-shaped curve. The harmonic bands in the spectrogram, however, do not appear as smooth curves, but contain several rapid changes or breaks in pitch. The modulation of a typical call from *C. underwoodi* had an initial frequency of 3,392 Hz, rising to 6,685 Hz, and then falling to 2,745 Hz for the emphasized harmonic (3rd; Fig. 2). The advertisement call has multiple harmonics, and the emphasized harmonic is typically the third (83%) or fourth (13%; Table 2). The mean fundamental frequency = $1,948.2 \pm 278.2$ Hz (range, 1,195–2,926 Hz) and the mean dominant frequency = $6,175.7 \pm 732.1$ Hz (range, 3,445–7,407 Hz; Table 2).

Similar to other species in this species group, *Craugastor underwoodi* also has a pulsed call that sounds like a buzz-like trill, consisting of a short, single pulsed note subdivided into a pulse series (Table 3; Fig. 2). The call is composed of a series of 8–15 pulses (mean = 11.3 ± 3.0 pulses), with a pulse rate = 91.4 ± 15.1 pulses/s (range, 76.2–107.9 pulses/s; Table 3). The mean dominant frequency for the pulsed call = $6,029.3 \pm 730.9$ Hz (range, 5,340–6,891 Hz; Table 3).

DISCUSSION

We quantitatively described for the first time, vocalizations of *Craugastor gabbi*, *C. aff. persimilis*, and *C. underwoodi*, achieving the intended aims of our research. Furthermore, we confirmed that all five species in the *Craugastor podiciferus* species group considered herein produce a squeak-like call as stated by Schlaepfer and Figeroa-Sandi (1998), Savage (2002), Cossel and Kubicki (2017), and Twining and Cossel (2017), which may function as the advertisement call in some or all of these species. Although the temporal and spectral properties of *C. podiciferus* and *C. stejnegerianus* vocalizations have been previously described (Schlaepfer and Figeroa-Sandi 1998; Twining and Cossel 2017), our results add to the existing data and allow comparisons of the vocalizations of these five species. Furthermore, the additional data from *C. stejnegerianus* allowed comparison of the calls from a northern site in Costa Rica (San Luis), believed to be an

undescribed species (Crawford 2003; Arias et al. 2016), to those of a population from a site about 150 km to the southeast (Hacienda Baru), also in Costa Rica (Fig. 2).

We also corroborated the presence of a pulsed call (trill) in *Craugastor podiciferus* (Schlaepfer and Figeroa-Sandi 1998), and report for the first time, this type of call in *C. gabbi*, *C. aff. persimilis*, and *C. underwoodi* as well. The function of this call type is unclear and warrants further investigation. We have observed that they are generally produced much less frequently than the tonal, squeak-like call. We observed also that pulsed calls were produced in the context of two or more males in proximity, or the perceived proximity of a male due to mimicked/artificially produced squeaks.

The vocalizations of these frogs are all relatively faint, and this is likely due to the absence of vocal slits in four of the five species considered herein (Hedges et al. 2008), with *Craugastor podiciferus* being the exception. Another craugastorid frog lacking vocal slits, *Craugastor gollmeri*, is described as having a soft advertisement call inaudible at distances beyond 3–4 m (Ibáñez et al. 2012). By contrast, Salazar-Zúñiga and García-Rodríguez (2014) describe vocalizations from *Craugastor noblei* (also lacking vocal slits/sac) as being audible from a distance of about 30 m. For most species in this group, their inability to use a vocal sac as a resonator and/or amplifier likely explains calls of low amplitude. Nonetheless, they are of sufficient volume to provide a male spacing function, and to advertise their location to females in close proximity. In spite of the presence of a subgular vocal sac in *C. podiciferus*, we found that it was not greatly distended during tonal (squeak-like) calls, producing a faint call (43 dB at 5 cm). By contrast, the vocal sac was about twice as distended during pulsed call production, and correspondingly had a call of greater amplitude (57 dB at 7.5 cm).

Comparison of advertisement calls.—Among species compared, we found differences in note duration, spectrogram shape, number of harmonics, the emphasized harmonic, and dominant frequencies. According to Köhler et al. (2017), the call variables of most taxonomic value are call/note duration, dominant frequency, pulse rate, and call/note rate, listed in order of importance. Consequently, for advertisement calls of the five species under consideration, we primarily focused on note duration and dominant frequency (and by inference, the emphasized harmonic), and considered spectrogram shape and number of harmonics to be of secondary importance.

The shortest mean note duration is found in *Craugastor podiciferus*, and the longest duration is in *Craugastor underwoodi*, with a difference of 0.043 s. The mean note duration is virtually identical between *C.*

stejnegerianus and *C. aff. persimilis*, whereas the mean note duration in *C. gabbi* is intermediate between that of *C. podiciferus* and *C. stejnegerianus*. *Craugastor podiciferus* had the lowest mean dominant frequency, and *C. underwoodi* had the highest, with a difference of 3.7 kHz. The mean dominant frequencies of *C. aff. persimilis* and *C. stejnegerianus* are nearly identical, and the mean dominant frequency of *C. gabbi* is intermediate between *C. podiciferus* and *C. aff. persimilis*. We found very little difference in the fundamental frequency between the five frogs.

Another call characteristic that can be taxonomically informative and which is related to dominant frequency, is the emphasized harmonic. We found variability regarding which harmonic was emphasized, both within and among the species. For example, in *C. podiciferus*, the emphasized harmonic was nearly evenly split between the first (48%) and second (52%) harmonic; however, additional data should be obtained from more individuals, as these data are from a single male. The emphasized harmonic was most often the second harmonic for *C. gabbi* (86%), *C. aff. persimilis* (48%), and *C. stejnegerianus* (64%), which according to Arias et al. (2016) are most closely related. *Craugastor underwoodi*, which exists within a separate clade (Arias et al. 2016), generally emphasized the third harmonic (83%). Although dominant frequency may often be correlated to body size in anurans (Gingras et al. 2013), this is contrary to our observations for *C. underwoodi* which has the largest body size yet emphasized the third harmonic and had the highest dominant frequency.

The shapes of the spectrograms were similar for all but *C. underwoodi*, which had distinctive pitch-breaks. The shape of the spectrogram is important in this case, because *C. underwoodi* and *C. podiciferus* are syntopic at some sites, and the spectrogram shape can be used as diagnostic when comparing the calls of these two species where their ranges overlap. The number of harmonics were also similar for all focal species, with the exception of *C. podiciferus*, which had fewer harmonics. Although the number of detectable harmonics can be a function of recording quality, we feel our recordings of *C. podiciferus* were of high quality, and the number of harmonics reported by Schlaepfer and Figeroa-Sandi (1998) were also lower than values we found for this species.

When comparing our findings with other published accounts, we found subtle differences between our data and the description by Savage (2002) for the advertisement call of *Craugastor underwoodi*. Savage (2002) described the call of *C. underwoodi* as a “two-note call consisting of ‘squeak-squeak’ followed by a long pause.” Based on terminology proposed by Köhler et al. (2017), however, we found that *C. underwoodi* advertisement calls consist of single notes produced in

call groups of two to four calls that are approximately 2 s apart, and each call group is separated by an interval of nearly 2 min. None of the other frog species considered herein appear to have calls in groups. This call attribute is yet another characteristic that should allow field identification of *C. underwoodi* in instances of sympatry/syntopy with other *Craugastor* species.

Similarly, our findings for *Craugastor podiciferus* differed notably from those of Schlaepfer and Figeroa-Sandi (1998), who previously reported that the advertisement call of this species is a squeak-like sound lasting 44 ms and repeated every 10–20 s, with a dominant frequency of 2.7 kHz, and three harmonics. In contrast, we found that the mean note duration was approximately 57 ms and the calls occurred at intervals ranging from 0.33–31.4 s. In addition, our spectral analysis showed that the number of harmonics ranged from 6–10 (mean = 7.1), and the dominant frequency varies with the emphasized harmonic, ranging from 2.1 kHz to 3.7 kHz (mean = 3.1 kHz). These differences may be a consequence of our data being from a northern clade of *C. podiciferus* as described by Streicher et al. (2009), whereas the data reported by Schlaepfer and Figeroa-Sandi (1998) were from frogs found near San Vito, Costa Rica, which represents a southern clade of *C. podiciferus* as proposed by Streicher et al. (2009).

Lastly, the call parameters of *Craugastor stejnegerianus* we report herein also differ from findings reported by Twining and Cossel (2017), from Hacienda Baru, about 150 km southeast of our site at San Luis. We found that the fundamental frequency and number of harmonics were essentially the same; however, our statistical analyses demonstrated significant differences in call duration (0.051 s at Hacienda Baru versus 0.070 s at San Luis; $t = 7.126$, $df = 15$, $P < 0.001$), and dominant frequency (3,335.5 Hz at Hacienda Baru versus 4,617.6 Hz at San Luis; $t = 4.042$, $df = 9$, $P = 0.003$). The higher mean dominant frequency in our data is likely due to the third harmonic being emphasized 22% of the time in frogs from San Luis. These differences are noteworthy in light of suggestions by Köhler et al. (2017) that these variables are often of taxonomic importance. Further, our findings corroborate suggestions by Arias et al. (2016) that northern and southern populations of *C. stejnegerianus* may represent yet another case of taxonomic cypsis in this species (*sensu lato*).

Comparison of pulsed calls (trills).—For pulsed call comparisons, we primarily focused on note duration, dominant frequency, and pulse rate, as these were the variables of greatest taxonomic importance, as recommended by Köhler et al. (2017). It should be noted that although we are using the term pulsed calls to describe this set of vocalizations, there does appear to be some harmonic structure in the pulses,

which consequently may warrant the usage of the term pulsatile-harmonic as described by Köhler et al. (2017). *Craugastor podiciferus* had the longest note duration (0.97 ± 0.2 s), whereas the remaining species all had similar and notably shorter note durations ranging from 0.12 to 0.14 s. A consequence of this longer duration is that the pulse rate is notably slower (36.6 pulses/s). *Craugastor gabbi* (67.0 pulses/s) was intermediate in pulse rate between *C. podiciferus* and the remaining species, which were similar to values reported by Twining and Cossel (2017) for *C. stejnegerianus* (91–117 pulses/s).

With regard to the dominant frequency of the pulsed calls, we found that *Craugastor podiciferus* was again noticeably different with the lowest mean dominant frequency ($3,606.8 \pm 171.9$ Hz). Further, *C. underwoodi* had the highest mean dominant frequency ($6,029.3 \pm 730.9$ Hz). These differences should facilitate identification of these two species in areas where they are syntopic. The remaining species, *C. gabbi*, *C. aff. persimilis*, and *C. stejnegerianus* (Twining and Cossel 2017), all had similar dominant frequencies, ranging from 4,393–4,665 Hz. This similarity is to be expected based on their close phylogenetic relationships as presented by Arias et al. (2016).

Pulsed calls (trills) have been previously reported for a southern population (Cordillera de Talamanca) of *Craugastor podiciferus* by Schlaepfer and Figueroa-Sandi (1998), who reported that trills for this species consisted of 8–9 pulses with a mean frequency of 5.5 kHz and no harmonics. In our analysis of 13 pulsed calls (trills) from one frog of this species, we found a mean of 36 pulses, and a mean dominant frequency of 3.6 kHz. These differences support the notion of an undescribed species within *C. podiciferus* (*sensu lato*) as proposed by Streicher et al. (2009). Schlaepfer and Figueroa-Sandi (1998) described the production of trills in a male/female social context, implying a courtship function. We also observed pulsed calls produced by frogs in close proximity to each other; however, based on additional observations of *C. podiciferus* (unpubl. data) at Monteverde (June 2018), we have seen frogs producing pulsed calls in situations when they were not in close proximity to another frog, suggesting the pulsed call may have other functions. Consequently, the function of these calls needs to be determined.

Factors resulting in call variation.—Although anuran vocalizations are generally species specific (Vieites et al. 2009), there are also a number of other factors that may account for differences we observed both within and among species. For example, fluctuations in temperature, moisture, and other abiotic factors can influence calling rate and intensity (Gayou 1984; Brooke et al. 2000; Saenz et al. 2006; Grant et al.

2013). Frogs are also known to vary call duration and call frequency in response to the calls of other anurans or due to background noise (Wells and Schwartz 1984; Lopez et al. 1988; Narins 1992; Köhler et al. 2017). Female choice and proximity may be other factors that influence call variables including rate, amplitude, and pitch (Wells and Schwartz 1984; Gerhardt 1991, 1994; Köhler et al. 2017). Even body size may constrain some parameters such as dominant frequency (Gingras et al. 2013).

Limitations, importance, and future work.—Our study was limited to five of the nine species currently recognized within the *Craugastor podiciferus* species group. Future work should include quantitative descriptions of vocalizations from other species in this group, including *Craugastor bransfordii*, *C. polyptychus*, *C. jota*, and *C. lauraster*. Our work was also limited geographically, with only five study sites, four of which were clustered in the northern portion of Costa Rica in the Cordillera de Tilarán, and one in the southern region. Consequently, future work should include a larger data set with sampling of individuals across wider geographical and elevational gradients to allow statistical discrimination among populations and species in the group. In particular, additional effort should include recordings of multiple individuals of *C. podiciferus*, as our data represent a single individual.

Future sampling should not only include intensive bioacoustical surveys, but these audio recordings need to also be associated with vouchered museum specimens and tissue samples that will allow subsequent analyses (Pérez-Ponce de León and Nadler 2010). Using molecular data, Arias (2019) demonstrated significant cryptic diversity remaining in the *Craugastor podiciferus* species group. And, whereas species in this group are morphologically very similar, integrative approaches relying on paired bioacoustical and molecular data will be essential. For example, data reported herein coupled with molecular data should help elucidate the taxonomic status of the northern and southern populations of *C. stejnegerianus*.

We were able to identify differences in call variables between one syntopic species pair in this group: *Craugastor podiciferus* and *C. underwoodi*. Additional work is needed to evaluate whether these call differences hold across other regions of sympatry, and if similar differences exist for other syntopic species pairs (e.g., *C. bransfordii* and *C. polyptychus*). While we identified several different types of vocalizations in this study, the function of the various call types in this species group has not been determined and requires additional study. Further sampling would also help to identify other potential call types made within this species group that have not yet been recorded. Additionally, research is

also needed to identify the parameters that result in variations in call variables and intensities, such as weather conditions, proximity to neighbors, and other species calling in the area (acoustical partitioning). These efforts should include measuring amplitude of calls to determine if loudness plays a role in call function; for example, high amplitude calls for advertising, and low amplitude calls for courtship.

Although the advertisement calls of these five species consist of a squeak and qualitatively sound similar to the human ear, we have demonstrated that there are indeed subtle, and at times substantial, quantitative differences between the vocalizations of these species. Our work supports the idea that bioacoustical surveys may be an important tool for distinguishing between sister taxa, and when notable species-specific attributes occur, can be sufficient to distinguish between syntopic species. With additional studies, it may be possible to find similar call differences between other syntopic species pairs, and perhaps identify other taxonomically cryptic species within this species group.

Acknowledgments.—We would like to thank Carli Salvador, Larell Brown, and Jennifer Dewey (Northwest Nazarene University), Dayan Hernández Díaz, Wilson Salas Jiménez, and Geiner Alvarado Huertas (Monteverde Cloud Forest Preserve), and Edwin Méndez Cruz for their help in the field. We are thankful for the Spanish translation provided by Víctor Acosta Chaves and Adrián García-Rodríguez. We are also grateful to Yoryneth Mendez Corrales (Tropical Science Center) for help with permitting and field logistics. We would like to thank Lourdes Vargas Fallas (Sistema Nacional de Áreas de Conservación) for facilitating permits. This work was conducted under the Costa Rican Ministerio de Ambiente y Energía permit numbers SINAC-SE-GASP-PI- R-048-2015, SINAC-CUS- PI-R-072-2016, SINAC-CUS- PI-R-045-2017. We are grateful to Dan Nogales (Northwest Nazarene University) and Timothy Wooster (Eastern Nazarene College) for supporting this work administratively and for allocating funding for this project.

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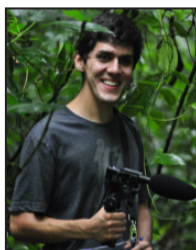


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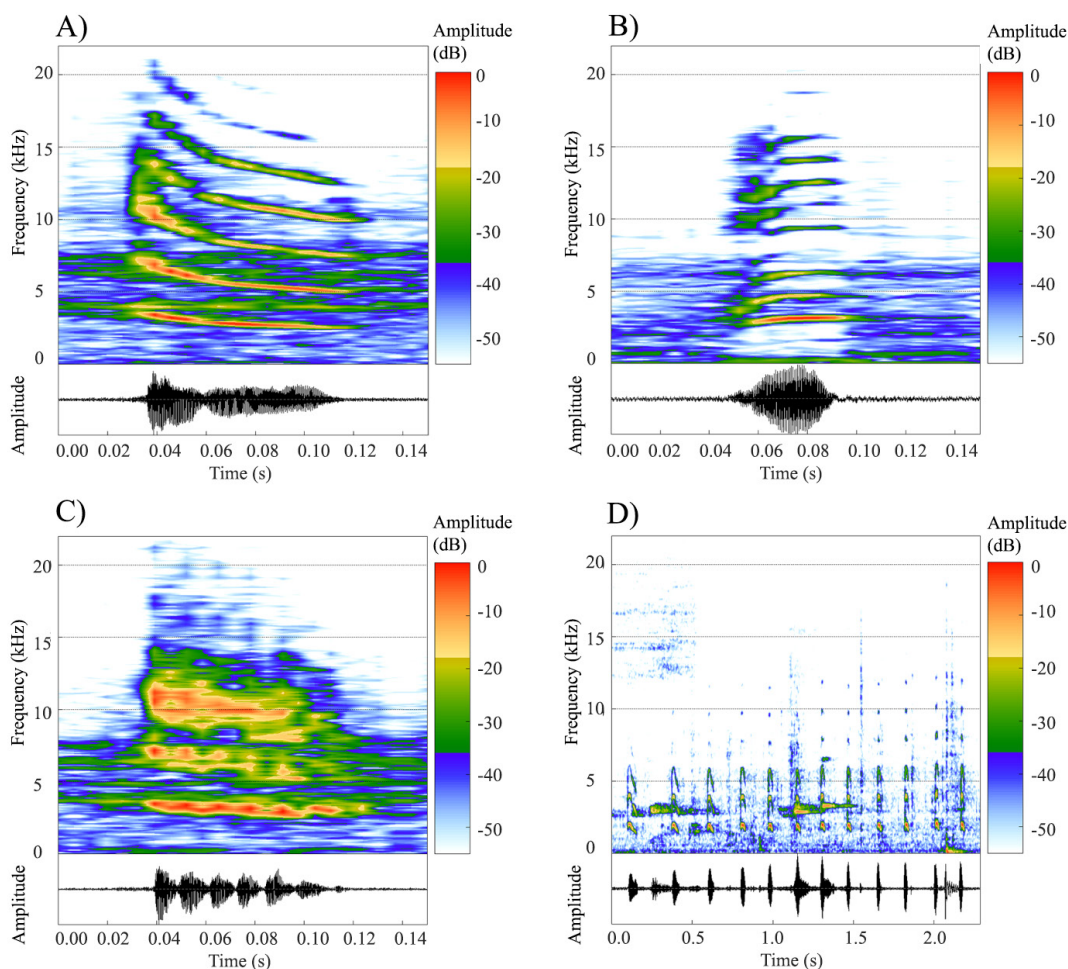
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APPENDIX TABLE. Temporal and spectral parameters of additional types of vocalizations (vocalization details; Appendix Figure) of frogs in the *Craugastor podiciferus* species group (*C. aff. persimilis*, Similar Flesh-bellied Frog, and *C. podiciferus*, Piglet Flesh-bellied Frog) recorded from various localities in Costa Rica (Table 1). Values are reported as mean (\pm SD).

Species	Call type	Number of calls analyzed	Call/Note duration (s)	Number Of harmonics	Harmonic emphasized	Pulses per note	Pulse rate (pulses/s)	Fundamental freq. (Hz)	Dominant freq. (Hz)
<i>Craugastor aff. persimilis</i>	Up-sweeping	3	0.045 (0.002)	12.33 (0.058)	2	-	-	1,960.93 (1,002.55)	3,215.6 (99.42)
<i>Craugastor aff. persimilis</i>	Down-sweeping	2	0.098 (0.011)	7	1	-	-	3,014.6 (365.43)	3,014.6 (365.43)
<i>Craugastor aff. persimilis</i>	Pulsatile harmonic	2	0.103 (0.013)	6	1	5–6	58.25	3,186.9 (121.76)	3,186.9 (121.76)
<i>Craugastor podiciferus</i>	Chain-chirp	1	2.077	7–8	1	-	-	1,894.9	1,894.90



APPENDIX FIGURE. Additional vocalizations from male frogs in the *Craugastor podiciferus* species group: *Craugastor aff. persimilis*, Similar Flesh-bellied Frog (A–C), and *Craugastor podiciferus*, Piglet Flesh-bellied Frog (D). Calls were graphically constructed using Seewave for R (Sueur et al. 2008) at a sensitivity level of 55 dB; for each graphic, spectrogram (color) is above and waveform (black) is below/black. Calls are described as (A) down-sweep, (B) up-sweep, (C) pulsatile call, and (D) a chain-chirp (vocalization details, Appendix Table).