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The presence of *Scinax altae* (Dunn, 1933) in Costa Rica, with redescription of its advertisement call

Presencia de *Scinax altae* (Dunn, 1933) en Costa Rica, con redescripción de su canto de advertencia

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ABSTRACT

Scinax altae is a treefrog hitherto known to occur only in Panama. Herein, we report two populations referred as *S. altae* by morphology, bioacoustics and genetics in the Southeastern Pacific region of Costa Rica. We present new maps showing the geographic distribution and hiatus between *S. staufferi* and *S. altae*. In addition, we aimed to redescribe the advertisement calls of *Scinax altae* and *Scinax staufferi*, based on new bioacoustic analysis and interpretation techniques, in order to evaluate their differential diagnosis.

Keywords: bioacoustics analysis, biogeography, distribution extension, phylogenetic analyses, treefrog.

RESUMEN

Hasta el presente, la rana arborícola *Scinax altae* se conocía solo de Panamá; sin embargo el estudio de ejemplares de *Scinax* provenientes de la vertiente Pacífica en el Sureste de Costa Rica, a los cuales se le examinó su morfología, bioacústica y genética, resultaron pertenecer inequívocamente a esta especie. Además de señalar la presencia de *S. altae* para Costa Rica, presentamos mapas que muestran la distribución e hiato geográfico entre *S. staufferi* y *S. altae* y analizamos los cantos de ambas especies, basándonos en nuevas técnicas de análisis e interpretación bioacústica, con el fin apoyar la diagnosis de ambas especies.

Palabras clave: ampliación de distribución, análisis bioacústico, análisis filogenético, biogeografía, rana arborícola.

INTRODUCTION

The treefrog *Scinax altae* (Dunn, 1933) (Anura: Hylidae) was described from the Canal Zone in Panama as *Hyla altae* without comparing it directly to the most similar species, *S. staufferi* (Cope, 1865). In a dichotomous key Dunn (1933) mentions *Hyla culex* Dunn, 1932 (a synonym of *S. staufferi* according to Taylor, 1952), which is differentiated from *S. altae* only by coloration (*H. altae* with four stripes on body and no barring on legs, vs. no body stripes and barred legs on *Hyla culex*). León (1969) and Duellman (1970) considered *Hyla altae* a subspecies of *H. staufferi* restricted to Panama, while the nominal subspecies was considered distributed from northwestern Costa Rica to Mexico (Savage 2002). León (1969) distinguished the two proposed subspecies of *Hyla staufferi* as follows: *H. s. staufferi* has irregular stripes and interrupted paravertebral stripes on the dorsum, two transverse bars on shanks and an interorbital bar, while *H. s. altae* has complete dorsolateral and paravertebral stripes, longitudinal stripe on shanks and absence of interorbital bar. Fouquette & Delahoussaye (1977) assigned most species of the *Hyla ruber* group, including *staufferi,* to the genus *Ololygon* (Fitzinger, 1843), based on sperm morphology, skipping *H. altae* in the process, which was considered a subspecies of *staufferi*. Duellman & Wiens (1992) placed *Ololygon* in synomymy of *Scinax*, including *S. altae* as a synonym of *S. staufferi*. Subsequently Duellman (2001) returned *S. altae* to species status, justifying this because no more specimens were collected in the hiatus between both species' distributions. Araujo-Vieira *et al.* (2023) found a 3% uncorrected pair distance (UPD) among *S. staufferi* from Teculután, Zacapa, Guatemala, and *S. altae* from Los Algarrobos, Chiriquí, Panama, placing both species in the *S. cruentomma* group. León (1969) had already made a basic description of the mating calls of *S. altae* and *S. staufferi*. No more published information is available about *S. altae*.

Scinax staufferi is distributed from southern Tamaulipas and Queretaro in Mexico (with its type locality in Orizaba, Veracruz, Mexico) to NW Costa Rica, in subhumid and dry forests (Frost 2023). In Costa Rica, *Scinax staufferi* is restricted to the northwestern Pacific lowlands, with no reports from the central and southern Pacific slopes; this leaves a gap of approximately 260 km between the southernmost locality of *S. staufferi* in Costa Rica and the closest known locality of *S. altae* in Panama (Savage 2002). Considering that Costa Rica is one of the best explored countries for amphibians (Savage 2002, Leenders 2016) and the species is abundant where it occurs, we are confident that the hiatus is not due to a lack of prospection. It is indeed important to dig deeper into the distinctiveness among *S. altae* and *S. staufferi*, especially updating the known distribution of both species, including localities on the central and southern Pacific slopes of Costa Rica. In addition, it is necessary to update data regarding the advertisement calls, using modern recording equipment and tools for analysis.

We recorded two populations of *Scinax,* morphologically referred to as *S. altae,* along the southeastern Pacific region of Costa Rica, within the hiatus between *S. staufferi* and *S. altae.* Specimens UCR24262-63 fall entirely into the described morphology and pattern for *S. altae* (Dunn 1933, León 1969, Duellman 1970, Kohler 2011; Fig 1A, B), presenting the aforementioned specific characteristics. Herein, we aim to redescribe the advertisement calls of *Scinax altae* and *Scinax staufferi*, based on new bioacoustic analysis and interpretation techniques, in order to evaluate their differential diagnosis.

MATERIALS AND METHODS

Fieldwork

In 2015, Edgar Chinchilla informed one of us (CBA) about a strange frog in Buenos Aires, Puntarenas province, Costa Rica (9° 9'41.46"N, 83°19'55.33"W; 372 m asl; Fig. 2). CBA and RS checked the locality and found a

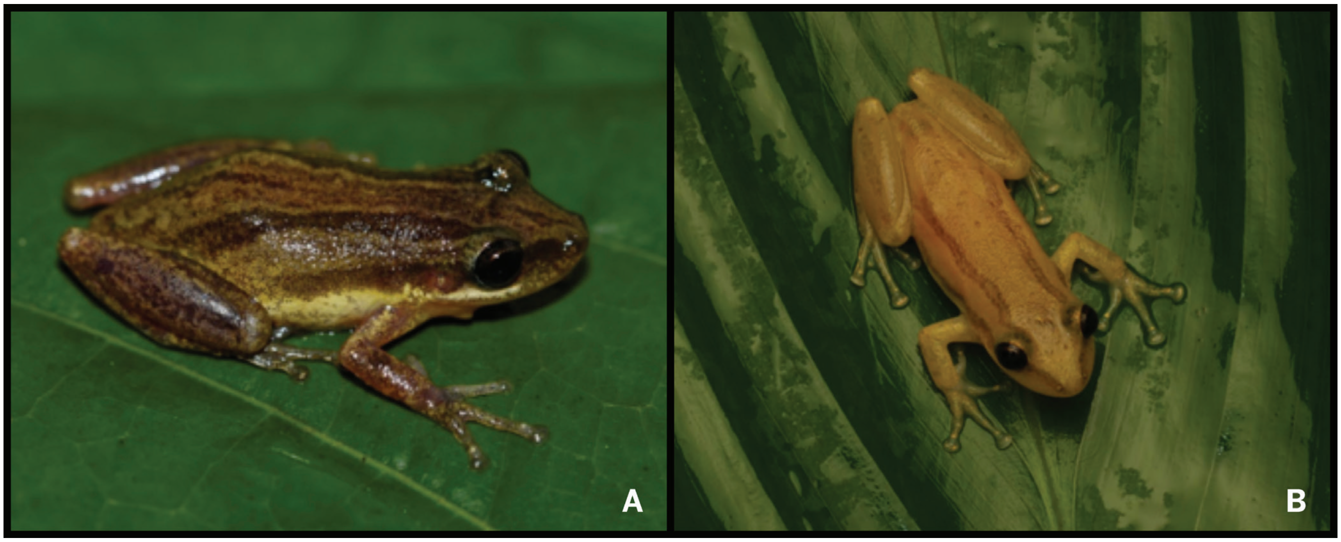

Figure 1. *Scinax altae* from Costa Rica. **A.** Buenos Aires, Puntarenas (day coloration). Photo: Remco Stuster. **B.** La Florida, near Golfito, Puntarenas (night coloration). Photo: Mike Canzoneri.

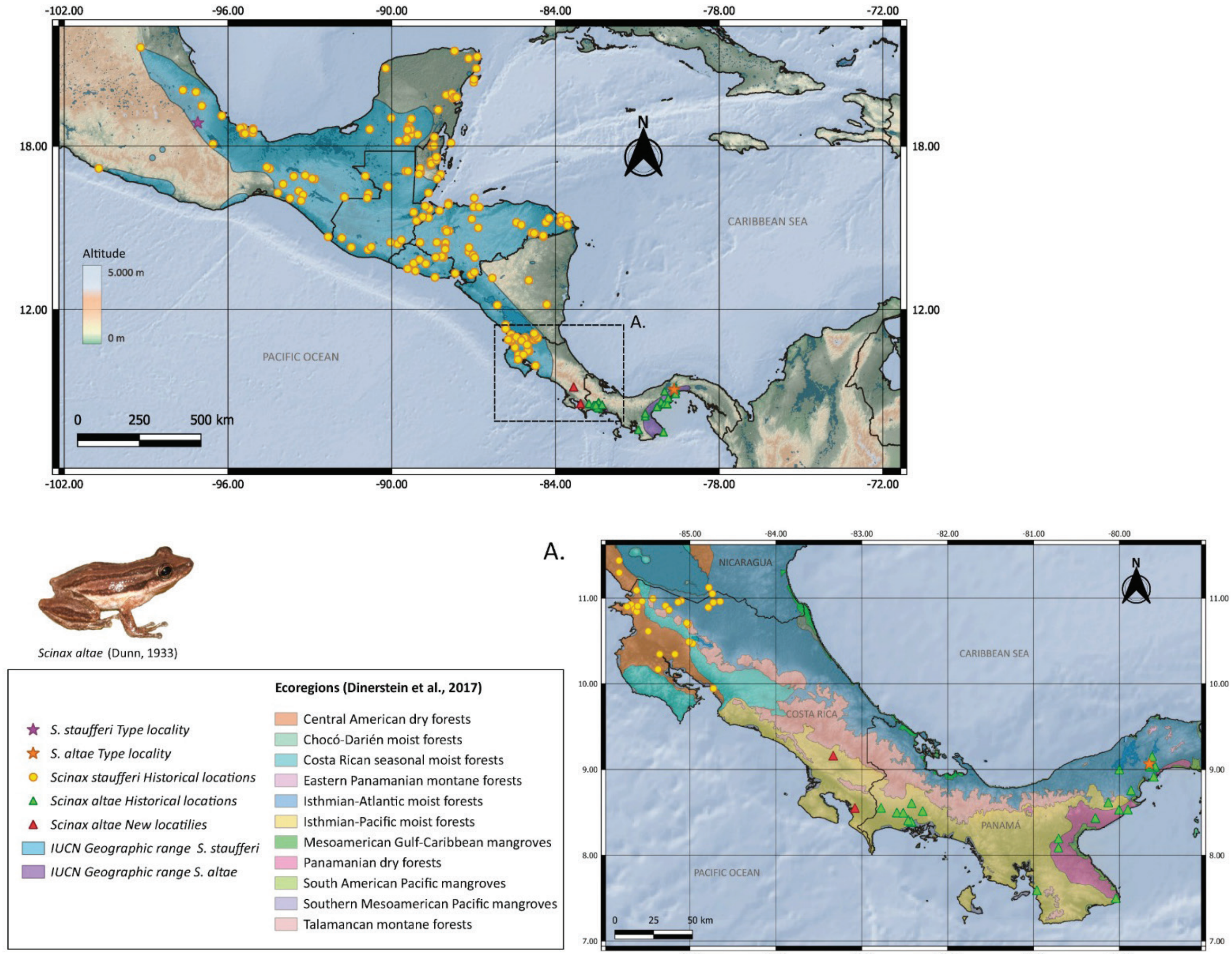

Figure 2. Map showing the known distribution range for *Scinax altae*, including two new localities reported in this work (red triangles) and its close relative *S. staufferi*. The inset map highlights the distribution in Costa Rica and western Panama. The distribution range is courtesy of the IUCN Red List (2022). The ecoregions follow Dinerstein *et al.* (2017).

few individuals (UCR 24262-63; Fig. 1A). However, the recorded calls were lost and needed to be recorded again. In the meantime, MC found the species at La Florida, La Mona, near Golfito (8°33'6.18"N, 83° 4'46.25"W; 150 m asl) and recorded two different animals (Fig. 1B).

Distribution

Distribution map generated with QGIS 3.22.6 (QGIS Development Team, 2023), distribution localities for both species were recovered from literature (Dunn 1934, León 1969, Duellman 1970, Savage 2002), Herpetology Department, Museo de Zoología, Universidad de Costa Rica (UCR) database and GBIF species occurrence (GBIF.org 2023); IUCN geographic ranges were obtained from IUCN Red List shape files (IUCN 2022); and the ecoregions follow Dinerstein *et al.* (2017).

Amplification and sequencing

We extracted total genomic DNA from the preserved tissue sample using the phenol-chloroform standard protocol (Sambrook & Russell 2006). We included one specimen housed in the UCR (UCR24263) collection that was previously identified as *Scinax altae* from Buenos Aires, Puntarenas province, Costa Rica (9°9'41.46"N, 83°19'55.33"W; 372 m asl). Partial sequences of the large subunit ribosomal RNA (16S) and cytochrome oxidase I (COI) mitochondrial genes were determined for one specimen of *Scinax* from Costa Rica. The DNA extraction, amplification, sequencing, and editing protocols follow those of Arias *et al.* (2018). The sequences are available under GenBank accession numbers MT176435–MT176437. The lists of vouchers and GenBank accession numbers used in this study are provided in Appendix 1.

Phylogenetic analyses

The sequences obtained here were compared with those available in GenBank for 14 specimens of closely related species to *Scinax altae* following Araujo-Veira *et al.* (2023) and including *S. boulengeri* (Cope, 1887) and *S. elaeochrous* (Cope, 1875) because of their presence in Costa Rica. We used sequences of *S. acuminatus* (Cope, 1862) to root all trees based on the results of Araujo-Veira *et al.* (2023). The protocols for alignments, selection of the best partition scheme, selection of the best model sequence evolution, the maximum likelihood analysis, Bayesian analysis (MrBayes and BEAST), and the computation of genetic distances follow those of Arias *et al.* (2018).

Bioacoustics analysis

We recorded the advertisement call of two males for each location of the individuals referred to as *Scinax altae* in Costa Rica. A total of 55 calls were analyzed. The audio records were obtained with the digital recorders Zoom H1 (set at 44.1 kHz sampling rate, 16-bit resolution and WAVE file format) and with the application WavePad for Android. We compared, bioacoustically, these populations on the southwestern Pacific region of Costa Rica with those of individuals identified as *S. altae* and *S. staufferi*. We obtained from repositories and sound collections 97 calls corresponding to three males of *S. altae* from three locations in Panama and 138 calls corresponding to four male individuals of *S. staufferi* from four locations in Costa Rica, Nicaragua and Mexico (Table1).

The structural, spectral and temporal variables of the calls were obtained with the software Raven Pro 1.6.1 (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology, 2023). A pre inspection was performed with different FFT (Fast Fourier Transform) resolution settings (from low to high FFT resolution) to indicate the presence of false harmonics and their dependence on the variation of this parameter, and thus differentiate them from true harmonics. It was found that the best resolution to analyze and visualize the spectrograms was using the following parameters: Hann window at 90% overlap, window size of 190 samples; 3dB filter bandwidth of 334 Hz, hop size of 19 samples, Discrete Fourier Transform (DFT) size of 256 samples, and grid spacing at 172 Hz.

Following Köhler *et al.* (2017), the following acoustic parameters were measured: 1) Structural variables: Number of notes per call, Number of pulses per note, Number of calls per call series. 2) Temporal variables: Call duration (=note duration) 90% (s), Intercall interval (s), Pulse Duration 90% (ms), Pulse repetition rate (Pulses /s), Call series duration, Intercall series interval. 3) Spectral variables: Frequency 5% (Hz), Frequency 95% (Hz), Bandwidth 90% (Hz), Dominant frequency (Hz), Peak Time (s). The structural and temporal variables were measured from the oscillogram and the spectral variables from the spectrogram and the power spectrum.

According to Köhler *et al.* (2017), excluding 10% of the call frequency and duration reduces subjectivity as boundary limits are influenced by brightness and contrast configurations of the spectrogram. Therefore, in order to reduce the identification biases of the spectral limits, the "threshold method" on the Power Spectrum described in Ríos-Chelén *et al.* (2017) was used. After delimiting the basic boundaries, the automatic measurement tools of the software were used: Freq 5% (Minimum frequency at 5% of the energy) and Freq 95% (Maximum frequency at 95% of the energy), to obtain the frequency bandwidth, where 90% of the energy of the notes is concentrated. The dominant frequency was calculated from the Power Spectrum, using the "Peak Frequency" function of the software. In the same way, in order to reduce the identification biases of the temporal limits of the sound units, the "amplitude peak as a threshold method" on the oscillogram, described in Köhler *et al.* (2017) was used. After delimiting the ba-

Table 1. Call recording localities of the specimens of *Scinax altae* and *Scinax staufferi*, used for comparison with the populations in question from southeastern Costa Rica and to redescribe advertisement calls.

Species	Locality	Calls (n)	Sources
Scinax altae	Chiriquí Viejo River, Hydroelectric project Burica, Bugaba, Panama	38	AB478. Present study
	Barro Colorado Nature Monument, Soberania National Park, Panama	20	Ibáñez *et al.* 1999
	Chepo, Panamá Province, Panama	39	ML194038 - Macaulay Library
Scinax staufferi	NW Costa Rica	27	UCR. Present study
	Amak community, MSB, BOSAWAS, Nicaragua	26	Present study
	Telchac, Yucatán, Mexico	51	Present study
	Mante, Tamaulipas, Mexico	34	ML208996 - Macaulay Library

sic time boundaries, the automatic measurement tools of the software were used: Time 5% (initial time at 5% of the energy) and Time 95% (final time at 95% of the energy) to obtain delta time where 90% of the energy of the sound units concentrates. The time where the highest energy or amplitude peak occurs within each note (Peak Time) was calculated from the oscillogram using the "Peak Time Relative" function of the software. The Pulse repetition rate (pulses/s) was calculated by dividing the number of counted pulses by the pulse period, as suggested by Köhler *et al*. (2017).

The terminology for the classification and description of calls was based on Köhler *et al*. (2017) and Emmrich *et al*. (2020).

The graphic representation of the spectrograms, oscillograms and power spectra were generated using the Seewave v 2.2.0 package (Sueur *et al.* 2008, Sueur *et al.* 2018) from RStudio version R 4.3.0 (R Core Team 2023), with the spectro, oscillo and meanspec functions respectively, using a Hanning window, 190 points of resolution FFT and an overlap of 90%.

In order to determine the existence of statistically significant differences and to examine which of the two species (*Scinax altae* or *Scinax staufferi*) better fit the bioacoustic characteristics of the Costa Rican populations in question, a one-way ANOVA test was applied for 13 variables. after corroborating the normality and homoscedasticity (equality of variances) of the data sets. To assess normality, a Shapiro-Wilk Test was performed and to check the homoscedasticity of the data, a Levene Test was performed. Subsequently, a post hoc Tukey HSD test of pairwise multiple comparisons was performed to examine the differences or similarities between the three data sets. These tests were performed using the R packages: rstatix v 0.7.2 (Kassambara 2023), stats (R Core Team 2023) and car v 3.1-2 (Fox & Weisberg 2019). Once the post hoc test showed to which species the bioacoustic characteristics of the populations of Costa Rica in question were more adjusted, the values of the acoustic variables were added to this species, and with this data, we made a differential diagnosis of the bioacoustic characteristics of both species.

Our sampling unit for the different statistical analysis was the recorded male, that is, we calculated for each male the mean value of the different acoustic variables and call characteristics. After clarifying to which species the populations from southeastern Costa Rica were more appropriate and adding their data to it, a new mean ± standard deviation (SD) was calculated, followed by the range of variation (minimum-maximum). A t-student test was performed for two independent samples of the 13 acoustic variables examined, in order to examine the differences between the advertisement calls of *Scinax altae* and *Scinax staufferi*, where Values of $p \leq 0.05$ were considered to be statistically significant. Finally, to visualize the variability of the acoustic parameters, box-and-whisker plots performed using the R package ggplot2 v 3.4.3 (Wickham 2016).

RESULTS

New records

COSTA RICA: Provincia de Puntarenas, Cantón de Buenos Aires, Distrito de Buenos Aires, Buenos Aires town (9° 9'41.46"N, 83°19'55.33"W; 372 m asl). Two adult males (UCR24262–63) 2015 (Fig. 1A). A disjunct locality is: Provincia de Puntarenas, Cantón de Golfito, Distrito de Golfito, La Florida near La Mona (8°33'6.18"N, 83° 4'46.25"W; 150 masl) in 2023 (Fig. 1B).

Distribution

Following Dinerstein *et al.* (2017), the presence of *S. altae* is expanded to the Isthmian Pacific moist forest (11 of 30 localities, including the 2 new Costa Rican ones), Panamanian dry forest (6/30), Isthmian Atlantic moist forest (9/30) which includes the type locality and 4/30 in the Southern Mesoamerican Pacific mangroves (Fig. 2). From North to south, *Scinax staufferi* has 185 locality records, distributed following Dinerstein *et al.* (2017) as follows: 4 from Veracruz moist forests, 37 from Petén-Veracruz moist forests, including the type locality, 1 from Veracruz dry forests, 3 from the Sierra de los Tuxtlas, 3 from Southern Pacific dry forests, 33 from Central American pine-oak forests, 5 from Chiapas Depression dry forests, 25 from Yucatan moist forests, 24 from Central American Atlantic moist forests, 25 from Central American dry forests, 10 from Mesoamerican Gulf-Caribbean mangroves, 13 from Isthmian-Atlantic moist forests and 2 from Costa Rican seasonal moist forests (Fig. 2).

Molecular phylogenetic

The resulting data matrix had a total sequence length of 1,226 bp, including gaps; 580 bp for 16S and 646 for COI. Three partitions were identified with the following substitution models: SYM+I+G for 16S + COI codon position 2, HKY for codon position 1 of COI, and F81 for COI codon position 3. The phylogenies from RAxML and MrBayes were almost identical in topology (Fig. 3). The individual from the south Pacific slope of Costa Rica is grouped within *S. altae* specimens from Panama. The phylogenetic analysis found *Scinax boulengeri* as sister clade to other species. *Scinax elaechroa* was inferred as sis-

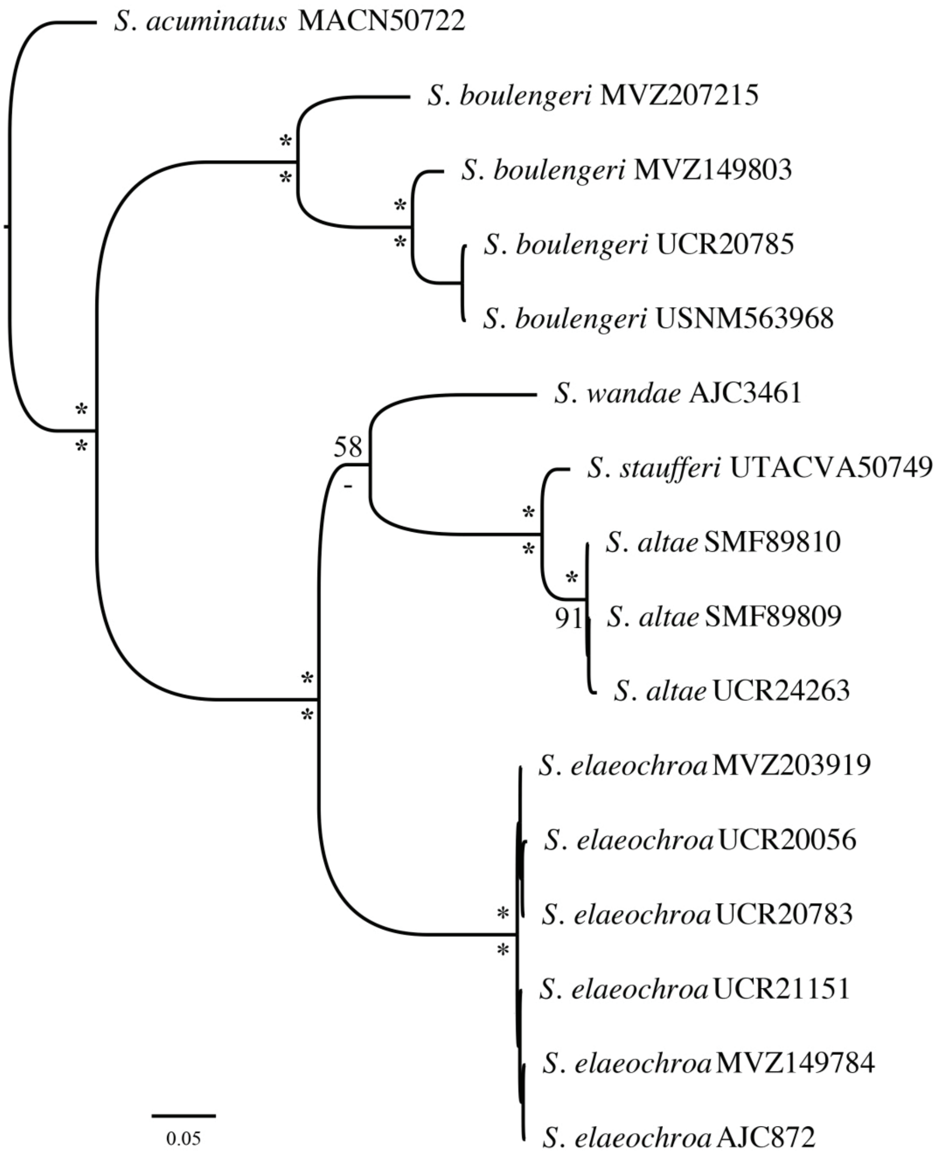

Figure 3. Bayesian phylogenetic inference shows the relationships of the *Scinax* species occurring in Costa Rica based on the 16S and COI mitochondrial DNA gene fragments. Posterior probabilities (multiplied by 100) from BEAST analysis are shown above branches. Below the branches are shown bootstrap proportions from RAxML analysis. The scale bar refers to the estimated substitutions per site. The support values of any node within the species are not shown. The asterisks represent support > 99.

ter clade to the clade formed by *Scinax cf. wandae* + [*S. altae* + *S. staufferi*]. Genetic distances between the *S. altae* and *S. staufferi* are 2.97–3.14 % for 16S.

Bioacoustics analysis

The one-way ANOVA test for 13 acoustic variables of the three data sets showed significant differences for certain bioacoustic variables. When examining the multiple comparisons using the Tukey HSD tests, it was found that the characteristics of the calls of the populations of southeastern Costa Rica better fit the characteristics of the species *Scinax altae*, since no statistical differences were found in comparison with that species; while in contrast to *Scinax staufferi*, significant differences were found in several of the acoustic characteristics (Table 2). Once the identity of the specimens from southeastern Costa Rica was ascertained, we added them to the *Scinax altae* data, leaving a total of 152 calls analyzed for this species. The summary of call measurements is presented in Table 3.

Using the note-centered approach, both advertisement calls share a single pulsed-type note, that is, each note is subdivided into acoustic units (pulses) separated from each other by modulations of amplitude less than 100% and duration less than 50 milliseconds (ms) (Fig. 4). These

Table 2. One-way ANOVA and Tukey post-hoc test for 13 bioacoustic variables in the three data sets: (1) Populations in question from southeastern Costa Rica, (2) *Scinax altae,* (3) *Scinax staufferi*. Statistically significant results ($p \leq 0.05$) are highlighted in bold.

Acoustic measurements	One-way ANOVA	Tukey HSD		
		2-1	3-1	3-2
Call (Note) duration 90% (s)	F (2,6) = 0,218 p = 0,81 η^2 = 0,068	0,848	0,811	0,999
Intercall interval	F (2,6) = 0,514 p = 0,622 η^2 = 0,146	0,959	0,835	0,610
Num. Call/ Call series	F (2,6) = 11,07 **p = 0,0097** η^2 = 0,787	0,919	0,019	0,018
Call series duration	F (2,6) = 13,85 **p = 0,006** η^2 = 0,822	0,803	0,010	0,012
Intercall series interval	F (2,6) = 12,54 **p = 0,019** η^2 = 0,862	0,928	0,026	0,025
Bandwidth 90% (Hz)	F (2,6) = 2,695 p = 0,146 η^2 = 0,473	0,248	0,136	0,905
Frequency 5% (Hz)	F (2,6) = 6,86 **p = 0,028** η^2 = 0,696	0,991	0,071	0,037
Frequency 95% (Hz)	F (2,6) = 5,782 **p = 0,040** η^2 = 0,658	0,222	0,034	0,324
Dominant frequency (Hz)	F (2,6) = 7,315 **p = 0,025** η^2 = 0,709	0,769	0,107	0,025
Peak Time (s)	F (2,6) = 2,382 p = 0,173 η^2 = 0,443	0,870	0,435	0,168
Pulse Duration 90% (ms)	F (2,6) = 0,238 p = 0,796 η^2 = 0,073	0,999	0,843	0,836
Num. of pulses/call-note	F (2,6) = 1,737 p = 0,254 η^2 = 0,367	0,313	0,956	0,317
Pulse repetition rate (Pulses /s)	F (2,6) = 0,579 p = 0,589 η^2 = 0,162	0,824	0,564	0,876

Table 3. Summary and results of statistical comparisons (t Student results) for the 13 bioacoustic characteristics measured in the calls between *Scinax altae* and *Scinax staufferi*. Values are reported a mean ± standard deviation, (minimum– maximum). n = Sample size. Statistically significant results (p ≤ 0.05) are highlighted in bold.

Acoustic measurements	*Scinax altae* (n=152)	*Scinax staufferi* (n=138)	t-student Test
Num. Notes/call	1	1	-
Call duration 90% (s)	0,123 ± 0,007 (0,115- 0,133)	0,127±0,020 (0,101-0,150)	t=-0,377 p= 0,718
Intercall interval (s)	0,487±0,078 (0,428-0,619)	0,425±0,101 (0,335-0,567)	t=1,047 p= 0,330
Num. Call/ Call series	6,079±1,363 (2-8)	24,333±8,165 (17-35)	t= -4,999 **p= 0,002**
Call series duration (s)	3,550±1,018 (2,217-4,736)	13,651±4,048 (8,763-17,739)	t= -5,457 **p= 0,0009**
Intercall series interval (s)	3,358±0,642 (2,633-4,205)	6,140±0,429 (5,837-6,443)	t= -5,490 **p= 0,003**
Frequency 5% (Hz)	1855,739±32,621 (1815,589-1899,229)	1622,684±127,448 (1528,846-1808,789)	t= 3,993 **p= 0,005**
Frequency 95% (Hz)	4645,477±397,300 (4086,538-4968,297)	4118,188±189,966 (3937,500-4319,712)	t= 2,418 **p= 0,046**
Bandwidth 90% (Hz)	2789,739±419,009 (2221,154-3136,017)	2495,504±2426,457 (2338,235-2790,865)	t= 1,279 p= 0,242
Dominant frequency (Hz)	2424,093±431,496 (1981,055-2885,449)	3542,758±421,957 (2918,179-3837,696)	t= -3,901 **p= 0,006**
Peak Time (s)	0,362±0,153 (0,219-0,540)	0,593±0,154 (0,362-0,680)	t= -2,245 p= 0,060
Pulse Duration 90% (ms)	4,358± 0,624 (3,462- 5,100)	4,652±0,543 (3,890- 5,166)	t= -0,742 p= 0,482
Num. of pulses/call-note	21,591±0,885 (20,727-22,800)	20,998±1,217 (19,444-22,353)	t= 0,851 p= 0,423
Pulse repetition rate (Pulses /s)	131,734±5,336 (125,806-136,539	124,589±16,271 (105,676-145,200)	t= 0,935 p= 0,381

pulsed notes do not present frequency modulations during the course of the call. The pulses that make up the notes are distinct, partly fused, without silent intervals. The majority of calls had an ascendant amplitude modulation from the first to the third pulse, until reaching a plateau, which was sustained throughout the call duration, where the first pulse always had a lower intensity (Fig. 5).

In high FFT resolution, the spectrogram is automatically structured into frequency bands (similar to towers of coins) that should not be confused with harmonics. According to Jackson (1996) and Gerhardt (1998), these frequency bands are caused by high rates of emission of acoustic structures (pulses), and thus reflect the pulse rate.

Because each coherent entity of sound emission is separated by silent intervals longer than the duration of the units themselves, we do not consider them as a series of notes within a song, but as calls (consisting of one note) that are grouped together in call series with silent intervals between the series. Although the note-centered approach could consider the call series of songs as the coherent sound unit (call), Köhler *et al.* (2017) point out that it is important to compare homologous bioacoustic entities under a taxonomic perspective and apply the same name to them. In this way, comparative studies of advertisement calls of species in the genus *Scinax* (Carvalho *et al.* 2015, Bang & Giaretta 2016, Ferrão *et al.* 2022) coincide in considering the advertisement calls consisting of a single note, grouped into call series (Fig. 5).

The advertisement calls of *Scinax altae* are structured in call series composed of 2-8 calls per series ($\bar{x}$ = 6,079±1,363); their duration varied from 2,217-4,736 s ($\bar{x}$ =3,550±1,018), and they are separated by time in-

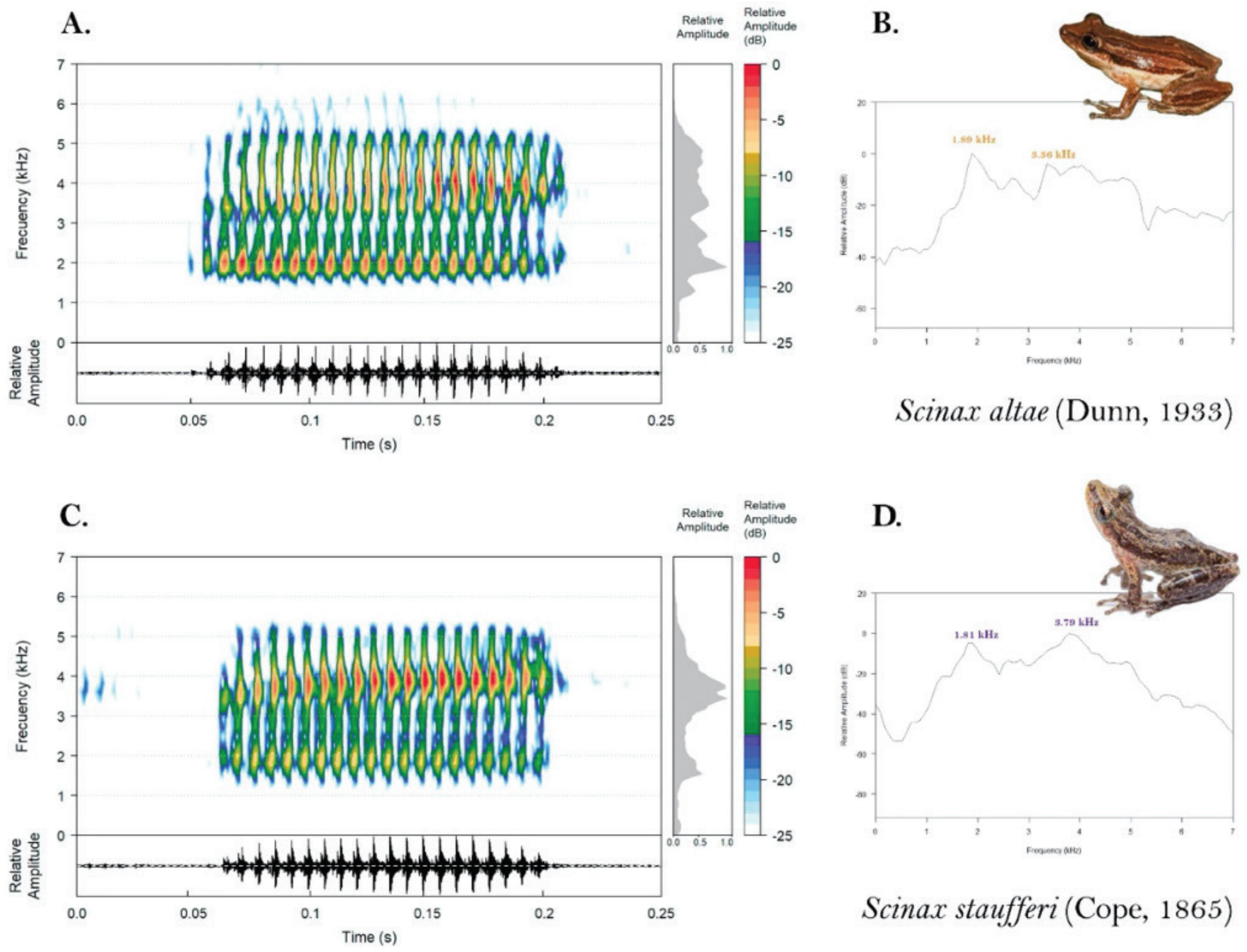

Figure 4. Comparison of the advertisement calls of *Scinax altae* and *Scinax staufferi*. Spectrogram, oscillogram (A) and mean frequency spectrum (B) of *S. altae*. Spectrogram, oscillogram (C) and mean frequency spectrum (B) of *S. staufferi*. Specimen photographs by Abel Batista and Victor Jiménez Arcos.

tervals between 2,633-4,205 s (x̄ =3,358±0,642). Call duration varied from 0,115- 0,133 s (x̄ =0,123 ± 0,007), with intervals of 0,428-0,619 s (x̄ =0,487±0,078). Calls had 20,7-22,8 pulses (x̄ =21,591±0,885), emitted at rates of 125,806-136,539 pulses per second (x̄ =131,734± 5,336). The pulse duration varied from 3,462-5,100 ms (x̄ =4,358± 0,624). Regarding the spectral characteristics, dominant frequency varied from 1981,055-2885,449 Hz (x̄ =2424,093±431,496); minimum frequency at 5% of the energy (Freq 5%) varied between 1815,589-1899,229 Hz (x̄ =1855,739±32,621) and maximum frequency at 95% (Freq 95%) between 4086,538-4968,297 Hz (x̄ =4645,477±397,300).

The advertisement calls of *Scinax staufferi* are structured in call series composed of 17 -35 calls per series ((x̄ =24,333±8,165); their duration varied from 8,763-17,739 s (x̄ =13,651±4,048), and were separated by time intervals between 5,837-6,443 s (x̄ =6,140±0,429). Call duration varied from 0,101-0,150 s (x̄ =0,127±0,020), with intervals of 0,335-0,567 s (x̄ =0,425±0,101). Calls had 19,444-22,353 pulses (x̄ =20,998±1,217), emitted at rates of 105,676-145,200 pulses per second (x̄ =124,589±16,271). The pulse duration varied from 3,890- 5,166 ms (x̄ =4,652±0,543). Regarding the spectral characteristics, dominant frequency varied from 2918,179-3837,696 Hz (x̄ =3542,758±421,957); minimum frequency at 5% of the energy (Freq 5%) varied between 1528,846-1808,789 Hz (x̄ =1622,684±127,448) and maximum frequency at 95% (Freq 95%) between 3937,500-4319,712 Hz (x̄ =4118,188±189,966).

Despite the similarity, the acoustic and statistical analyses (t-student test) show that there are significant differ-

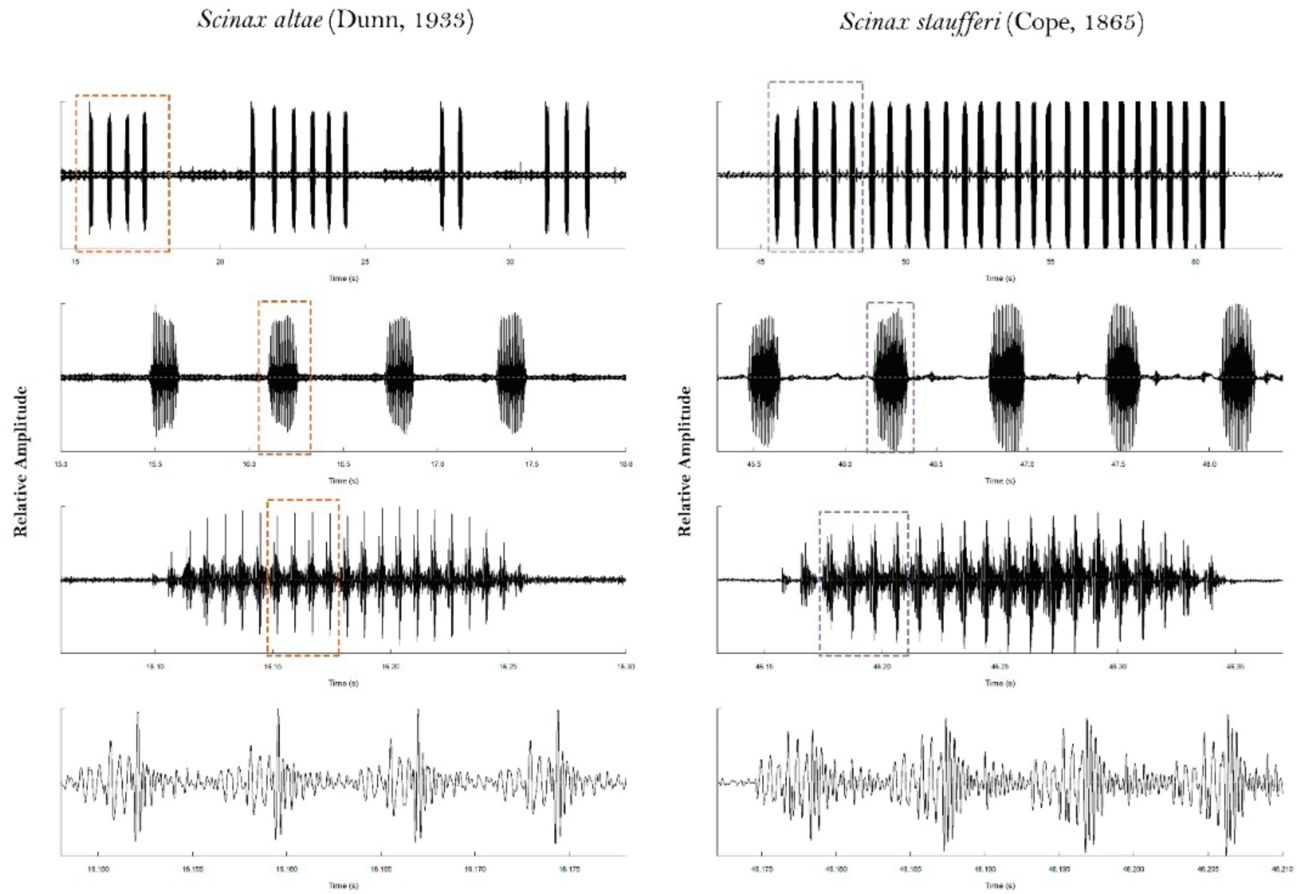

Figure 5. Advertisement calls structure. Oscillogram zooming (through shorter time slices) of *Scinax altae* and *Scinax staufferi*. Upper panel: Shown how advertisement calls are organized into call series. Short call series for *S. altae*, and very long call series for *S. staufferi*. Upper central panel: An approach to the structure of one of the call series for *S. altae* and its equivalence in time for a portion of the call series for *S. staufferi*. Lower central panel: an approach to the structure of the advertisement call, consisting of a single pulsed-type note. Lower panel: An approach to the structure of the pulses, which are distinct, partly fused, without silent intervals.

ences between the advertisement calls of these species for some structural variables (Number of notes per call); temporal variables (Call series duration, Intercall series interval); and spectral variables (Frequency 5%, Frequency 95%, Dominant frequency). The variability of the acoustic parameters can be visualized in box-and-whisker plots (Fig. 6). The most striking difference is in Dominant frequency, where *S, altae* shows 2424,093±431,496 Hz while *S. staufferi* is much higher (3542,758±421,957 Hz). Likewise, it was found that the way in which their calls are organized is different. Series of short calls (6,079±1,363 calls per series) characterize *S. altae*, while in *S. staufferi* calls are organized in series of greater numbers (24,333±8,165 calls per series), showing the importance of examining and integrating these types of structural characteristics of advertisement calls, in addition to the traditional temporal and spectral characteristics.

Previous call analyses of both species (León 1969, Duellman 1970) failed to provide modern statistical data.

Observations on natural history

The Buenos Aires population is in a rapidly reducing lagoon in the middle of the city. Many species called on the night of April 30, 2018, after heavy but short rainfall; other species calling included *Dendropsophus microcephalus* (Cope, 1886)*, D. ebraccatus* (Cope, 1874)*, Scinax elaeochrous, Leptodactylus poecilochilus* (Cope, 1862). *Scinax altae* call randomly every several minutes. The call is striking but difficult to locate among so many other loud calling species.

In La Florida, only two males were located by MC, and only once each time, when the photos and recordings were made. Both individuals were seen around an artificial pond made to attract local amphibians, such as *Agalych-*

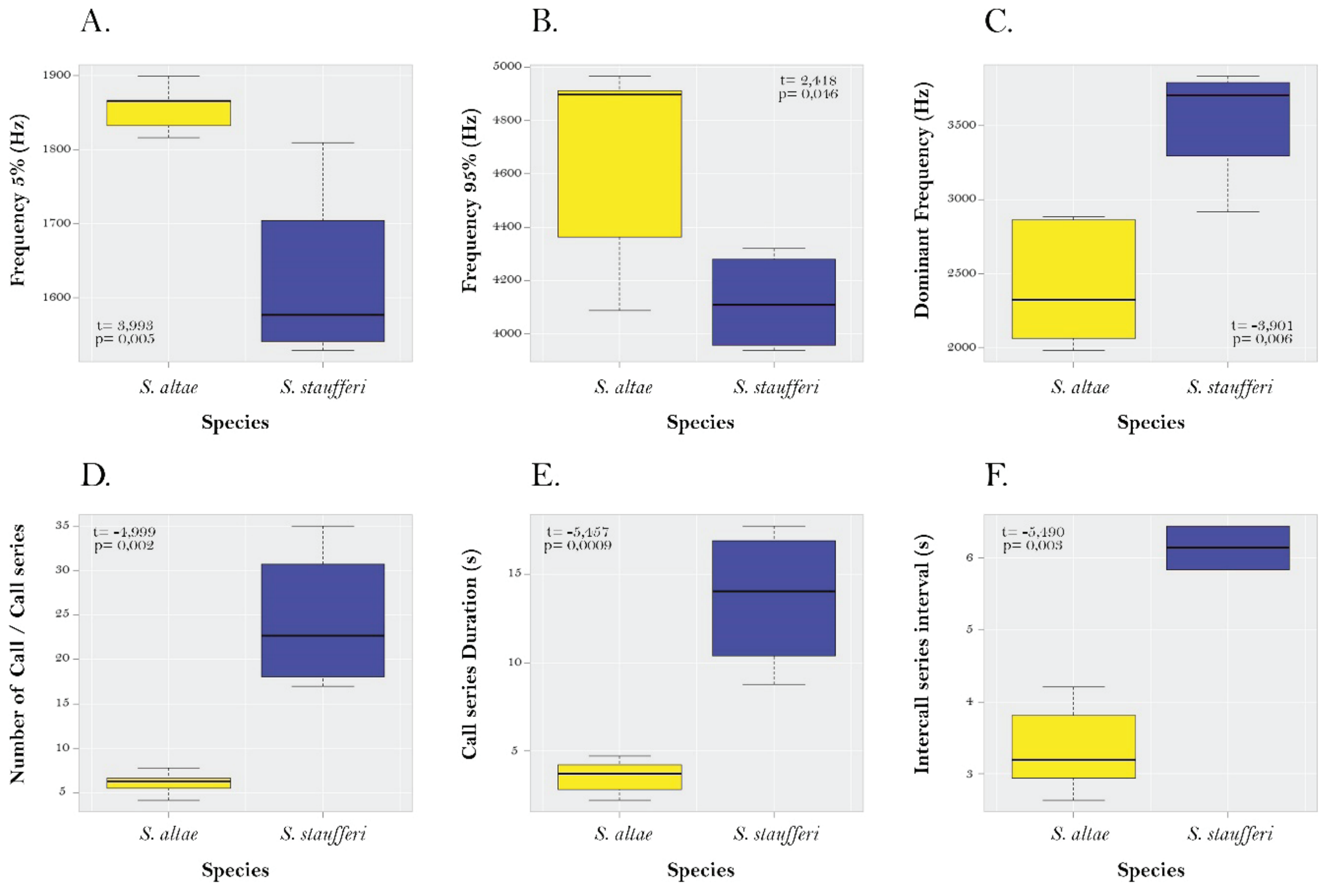

Figure 6. Box-and-whisker plots for six bioacoustic character measurements of the advertisement calls of *Scinax altae* and *Scinax staufferi.* that presented statistically significant results (p ≤ 0.05) from t-student Test. A. Frequency 5%. B. Frequency 95%. C. Dominant Frequency. D. Number of calls/call series. E. Call series duration. F. Intercall series interval.

nis callidryas, A. spurrelli, Scinax boulengeri, Engystomops pustulosus, and *Leptodactylus savagei*, among others. It appears that the species is a new arrival to the area, as there is not an apparent established population, but, rather, a few single males calling and probably dispersing.

DISCUSSION

The phylogenetic evidence suggests that the populations of *Scinax* from southwestern Pacific Costa Rica can be referred to *S. altae* with high confidence. The genetic distances among *S. altae* and *S. staufferi* are around the thresholds of 3% in 16S suggested by Fouquet *et al.* (2007) to separate among species or define candidate species. However, it is necessary to highlight that the only available sequence of *S. staufferi* is from Guatemala (Araujo-Vieira *et al.* 2023); therefore, it is possible that these relationships will change with new sequences throughout the distribution range of this species. It is necessary to include additional specimens of *S. staufferi* from Northwestern Costa Rica, to update the distribution range of this species and further delineate *S. altae* and *S. staufferi.*

At a bioacoustic level, despite the similarity that it seems to show, the acoustic and statistical analyses (t-student test) display that there are significant differences between the advertisement calls of these species, for some structural variables (number of notes per call); temporal variables (call series duration, intercall series interval) and spectral variables (frequency 5%, frequency 95%, dominant frequency). The variability of the acoustic parameters can be visualized in box-and-whisker plots (Fig. 6). The most striking difference is in Dominant frequency, where *S. altae* show 2424,093±431,496 Hz while *S. staufferi* is quite higher (3542,758±421,957 Hz). Furthermore, the way in which their calls are organized is different. Series of short calls (6,079±1,363 calls per series) characterize *S. altae*, while in *S. staufferi* calls are organized in series of greater numbers (24,333±8,165 calls per series), showing the importance of examining and integrating these types of structural characteristics of advertisement calls, in addition to the traditional temporal and spectral characteristics.

The record of this acoustically prominent species (*Scinax altae*) in the Pacific slopes of southern Costa Rica is highlighted due to the fact that this region has been historically well sampled and explored. This new record joins recent records of two frogs, *Pristimantis taeniatus* (Boulenger, 1912) (Arias *et al.* 2023) and *Elachistocleis pearsei* (Ruthven, 1914) (Vargas & Barrio-Amorós 2023) on the South Pacific versant of Costa Rica. Very little is known about the natural history and habitat use of these species in Costa Rica. It is necessary to evaluate whether these new records correspond to chance finds in unexplored microhabitats or if it is possible that recent expansion ranges explain their presence. The distribution ranges of these three species recently recorded for the Pacific slope of Costa Rica is limited due to big agricultural expansions (pineapple and oil palm). However, it seems possible that these species are dispersed via transportation of fruits or other agricultural items.

León (1969) and Duellman (1970) described the mating calls of *S. altae* and *S. staufferi* as a series of short nasals notes "ah-ah-ah-ah", which present a fundamental frequency of about 106 Hz for *S. staufferi* and around 112 Hz for *S. altae*.

Currently, the fundamental frequency to describe the advertisement calls is little used, because it can be rather difficult to identify which one is the base frequency produced by the vocal chords, therefore, pinpointing the fundamental frequency of secondary importance (Köhler *et al.* 2017).

Köhler *et al.* (2017) and Emmrich *et al.* (2020) developed a set of parameters for the description of advertisement calls. According to these authors, in general, the calls of these two species are better classified as a pulse repetition sound, since they consist of bursts of sound energy represented as several acoustic units.

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