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The advertisement call of *Espadarana prosoblepon* (Anura: Centrolenidae) from a population in the Central Andes of Colombia

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Abstract

The advertisement call of *Espadarana prosoblepon* (Anura: Centrolenidae) from a population in the Central Andes of Colombia. The advertisement call of the glass frog *Espadarana prosoblepon* has been described for populations from Costa Rica in Central America and Ecuador in South America, but not for populations in Colombia. Because having a detailed description of the advertisement call of several populations of the same species is important for taxonomy, phylogenetics, ecology, behavior, and evolution, we describe the advertisement call of a population of *E. prosoblepon* in Colombia, South America. We analyzed 58 temporal and spectral features of 220 advertisement calls emitted by 31 males recorded between October–December 2023 at the "Cedro Rosado" Botanical Garden in Armenia, Department of Quindío, Central Andes of Colombia. In general, the advertisement call of *E. prosoblepon* in our study population consisted of 2–4 pulsed notes (beeps) with modulated frequency. The mean duration of the call was 230 ms (range 190–430 ms) and of the notes was 40.1 ms (31.0–48.7 ms), separated by intervals of 143.5 ms (71.5–181 ms). The dominant frequency of the call was 5.2 kHz (4.9–5.6 kHz). Our results suggest slight differences in note duration between the studied population in Colombia and populations of *E. prosoblepon* in Costa Rica and Ecuador. Further studies are necessary to test for potential effects of temperature and body size on call features both at the intra- and interpopulation levels. Given the wide latitudinal and altitudinal geographic distribution of *E. prosoblepon*, intraspecific geographic variation in its call is possible.

Keywords: Acoustic communication, Advertisement call, Amphibians, bioacoustics, intraspecific geographical variation.

Resumo

Canto de anúncio de *Espadarana prosoblepon* (Anura: Centrolenidae) de uma população dos Andes Centrais da Colômbia. O canto de anúncio da rã-de-vidro *Espadarana prosoblepon* foi descrito para populações da Costa Rica, na América Central, e do Equador, na América do Sul, mas não para populações da Colômbia. Como ter uma descrição detalhada do canto de anúncio de várias

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populações da mesma espécie é importante para a taxonomia, filogenia, ecologia, comportamento e evolução, descrevemos o canto de anúncio de uma população de *E. prosoblepon* da Colômbia. Analisamos 58 caraterísticas temporais e espectrais de 220 cantos de anúncio emitidas por 31 machos gravados entre outubro e dezembro de 2023 no Jardim Botânico "Cedro Rosado", em Armenia, Departamento de Quindío, Andes Centrais da Colômbia. Em geral, o canto de anúncio de *E. prosoblepon* da população estudada consistiu de 2–4 notas pulsadas (bipes) com frequência modulada. A duração média do canto foi de 230 ms (intervalo 190−430 ms) e das notas foi de 40,1 ms (31,0−48,7 ms), separados por intervalos de 143,5 ms (71,5−181 ms). A frequência dominante do canto foi de 5,2 kHz (4,9−5,6 kHz). Nossos resultados sugerem pequenas diferenças na duração da nota entre a população estudada na Colômbia e as populações de *E. prosoblepon* da Costa Rica e do Equador. São necessários mais estudos para testar os potenciais efeitos da temperatura e do tamanho do corpo nas caraterísticas do canto, tanto intra como interpopulacional. Dada a ampla distribuição geográfica latitudinal e altitudinal de *E. prosoblepon*, é possível que haja variação geográfica intraespecífica em seu canto.

Palavra-chave: Anfíbios, Bioacústica, Canto nupcial, Comunicação acústica, Variação geográfica intraespecífica.

Introduction

Centrolenidae is a Neotropical clade of 166 species of arboreal frogs that have nocturnal habits and breed along streams (Frost 2024). Among centrolenids, one of the most studied species is *Espadarana prosoblepon* (Boettger, 1892) (Figure 1). This species is distributed from sea level to approximately 1800 m elevation from the eastern region of Honduras in Central America to Colombia and Ecuador in South America (Basto-Riascos *et al.* 2017a, Frost 2024, MR pers. obs.). Adult females of this species have a body size (snout–vent length, SVL) between 25−31 mm, while in adult males it varies between 23−28 mm (Basto-Riascos *et al.* 2017a). Although variation in morphological characters includes a dorsal color pattern that is uniformly green or has blue and yellow spots (Guayasamin *et al*. 2020), the most significant diagnostic character of *E. prosoblepon* is the presence of a well-developed blade-shaped *crista dorsalis* of the humerus in adult males (Savage 2002, Kubicki 2007, Guayasamin *et al*. 2020; Figure 1). Studies on *Espadarana prosoblepon* cover aspects of phylogenetic relationships (Guayasamin *et al.* 2009, 2020), population dynamics (Robertson *et al.* 2008, Angeli *et al.* 2015), male-male agonistic behavior (Krohn and Voyles 2014, Hedman and Hughey 2015, Rios-Soto *et al.* 2017), sexual selection (Jacobson 1985, Basto-Riascos *et al.* 2017b, Goyes-Vallejos *et al.* 2021), parental care (Goyes-Vallejos *et al.* 2022, 2024), and oviposition site preference and external morphological attributes of embryos through ontogeny (Salazar-Nicholls and Del Pino 2015, Ortiz-Ross *et al.* 2020). This species has been included in macroevolutionary studies about diversification and ecology (Hutter *et al.* 2013, Castroviejo-Fisher *et al.* 2014, Delia *et al.* 2017, Escalona *et al.* 2019, Mendoza-Henao *et al.* 2023, Valencia-Aguilar *et al.* 2024, Vargas-Salinas *et al.* 2024).

The advertisement call of *E. prosoblepon* has been described based on populations from Costa Rica in Central America (Jacobson 1985, Savage 2002, Kubicki 2007) and Ecuador in South America (Freile *et al.* 2020, Guayasamin *et al.* 2020), but those studies only provide a brief description of a few of call features and its variation of relatively few individuals. Despite being one of the most widely distributed glass frog species, no quantitative and detailed call description based on Colombian populations exists (Rivera-Correa *et al.* 2021, Duarte-Marín *et al.* 2022). Call descriptions based on

Figure 1. An adult male *Espadarana prosoblepon* in the "Cedro Rosado" Botanical Garden in Armenia, Department of Quindío, Central Andes of Colombia. Note the pattern of black and yellow dots on the dorsum, which is distinctive among individuals, and the ventrolateral projection of the *crista ventralis* of the humerus (humeral spine) present only in reproductive males. Specimen not collected.

populations of Costa Rica and Ecuador have not examined the potential relationship of call features with body temperature and body size. Anurans are ectothermic vertebrates and the functioning of the muscles involved in air pumping for call production is influenced by temperature; in addition, body size determines spectral call features such as dominant frequency (Gerhardt and Huber 2002). Interspecific differences and variation in advertisement call features within and between populations of the same species may be a consequence of differences in environmental temperature and body size (Ryan and Wilczynski 1991, Gerhardt and Huber 2002, Lingnau and Bastos 2007).

Having a detailed description of the advertisement call from several populations of a frog species allows inferences to be made in taxonomy (Padial *et al.* 2008, Köhler *et al.* 2017), phylogenetics (Duellman 2007, Schneider and Sinsch 2007), ecology, behavior, and evolution (Cocroft and Ryan 1995, Bosch *et al.* 2003, Robillard *et al.* 2006, Both and Grant 2012). Furthermore, a detailed characterization of the advertisement call and its interpopulation variability allows optimization of the mathematical algorithms used for monitoring populations based on automated recordings (López-Baucells *et al.* 2019). Herein, we describe the advertisement call of *E. prosoblepon* from an urban forest fragment in the Central Andes of Colombia. We also test for the influence of temperature and body size in call features of the advertisement call.

Materials and Methods

Study Site

The study was conducted from 10 October to 19 December 2023, in an urban forest fragment known as the "Cedro Rosado" Botanical Garden (04°32'40" N, 75°46'13" W; 1490−1530 m a.s.l.), located at the University of Quindío, Armenia, Colombia. This forest has an area of 15 ha, with an average annual temperature of 19°C, relative humidity between 65–75%, and a bimodal rainfall distribution with an average annual precipitation of 2436 mm (Rodríguez 1999).

Recordings were made with a Sennheiser ME66/K6 unidirectional microphone located at 50−150 cm in front of calling males and connected to a Tascam DR-40X digital recorder. We recorded body size (snout–vent length, SVL) and temperature of the calling frog with a Mitutoyo digital caliper (precision ± 0.01) and an Extech Series 42510 infrared thermometer (precision 0.1°C), respectively. All recordings were performed between 19:00 − 00:00 h. We identified males individually based on the pattern of yellow and black dorsal dots to avoid pseudoreplication (Figure 1).

Calls were recorded in .wav format, digitized at 16 bits resolution and 44.1 kHz sampling rate; measurements of call features were made using the software RAVEN Pro 1.6 (Yang 2024). Spectrograms were created with a Fast Fourier Transformation window of 256 points and Blackman algorithm. The following call features were quantified (*sensu* Köhler *et al.* 2017): call duration (ms), number of notes, note duration (ms), interval between notes (ms), note emission rate (number of notes/call duration), dominant frequency of the note (kHz), and low and high call frequency of the note (kHz). Low and high frequencies of the note were measured at 20 dB (re 20 mPA) below the peak intensity of the dominant frequency, which is the value at which the signal energy could still be clearly distinguished from the background noise. We recorded dominant, low, and high frequency at the initial, middle, and final part of each note. Harmonic bands were evident in some recordings; we recorded the frequency of them when applicable. We recorded mean ± standard deviation and range obtained from all calls per male. None of the recorded individuals was collected. Copies of recordings obtained in this study were deposited in the Environmental Sound Collection of the Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH-CSA), Villa de Leyva, Boyacá, Colombia, and the codes attached to the recordings range from IAvH-CSA-39409 to IAvH-CSA-39439 (http://colecciones.humboldt.org.co/sonidos/).

Call features of advertisement calls of anurans are generally inter-correlated; therefore, after verifying such inter-correlation in our data, the number of variables (spectral and call features) was reduced by conducting a principal component analysis (PCA) with Varimax-rotation (Johnson and Wichern 2014). Since we detected and quantified the frequency of harmonics for some males but not others, these call features were not included in the PCA. The number of resulting components was determined considering an explained variance greater than 80% and eigenvalues > 1 (Cliff 1988, Peres-Neto 2005). The principal components (PCs) obtained from this analysis were used as dependent variables in four multiple linear regressions (Zar 1984). Each of these regressions was performed to test the relationship between call features in the PCs with temperature and body size as independent variables. Both the PCA and the multiple regression analysis were performed using the software SPSS v.21.0.0.0 (SPSS Inc 1999).

Results

The following description is based on 220 calls recorded from 31 males of *Espadarana prosoblepon*. The advertisement call of this species consists of 2.1 ± 0.2 "beeps" in average (range 2–4 beeps) pulsatile notes with amplitude and frequency modulation (Figure 2). Notes last 40.1 ± 4.8 ms (range 31.0–48.7 ms; N = 31 males), and consecutive notes are separated by silent intervals of 143.5 ± 18.7 ms (71.5–181 ms). Mean call duration was 230 ± 40 ms (190–430 ms), and call duration increased with the number of notes per call (Pearson correlation: $r = 0.81$, $p < 0.001$). Note emission rate was 9.2 ± 0.9 calls/s (7.4–11 calls/s). The dominant frequency of the first note was 5.3 ± 0.2 kHz (4.9–5.6 kHz), of the second note was 5.2 ± 0.2 kHz (4.9–5.6 kHz), and of the third note was 5.1 ± 0.1 kHz (4.9–5.2 kHz; N = 3). Only one male emitted a call with four notes; the mean dominant frequency of this fourth note was 5.1 kHz. The low frequency of the first note was 4.8 ± 0.2 kHz (4.5–5.2 kHz; N = 31), and the high frequency was 5.8 ± 0.2 kHz (5.6–6.1 kHz); for the second note the low frequency was 4.8 ± 0.2 kHz (4.4–5.1 kHz), and the high frequency was 5.8 ± 0.2 kHz (5.5–6.1 kHz); and for the third note the low frequency was 4.8 ± 0.2 kHz (4.6–5.0 kHz; N = 3), and the high frequency was 5.6 ± 0.1 kHz (5.5–5.8 kHz; N = 3). The low and high frequency for the fourth note was 4.8 kHz and 5.9 kHz, respectively (N = 1 male). After pooling the data of dominant, low, and high frequency for all the notes and males, we determined that the mean dominant call frequency of *E. prosoblepon* in the studied population was 5.2 ± 0.2 kHz (4.9–5.6 kHz; N = 31), the low frequency was 4.8 ± 0.2 kHz (4.5–5.2 kHz), and the high frequency was 5.8 ± 0.2 kHz (5.6–6.1 kHz). Detailed data of the dominant, low, and high frequency at the initial, middle, and final part of each note is summarized in Table 1. We recorded up to three harmonics in the call of some males; the first harmonic was at 10.3 ± 0.4 kHz (9.7–

11.1 kHz; $N = 30$), the second harmonic was at 15.6 ± 0.6 kHz (14.3–16.7 kHz; $N = 26$), and the third harmonic was at 14.9 kHz in only one male.

The variability in the 26 advertisement call features included in the PCA was summarized into four PCs (Table 2). PC1 and PC2 grouped all the spectral features of the call, while PC3 and PC4 grouped temporal features of the call. When we tested the relationship of these PCs with temperature and body size (Figure 3), we found that temperature does not relate to PC1 (β = 0.001, t = 0.006, $p = 0.995$), PC2 (β = 0.018, t = 0.097, $p = 0.923$), and PC3 (β = -0.017, t = -0.1, $p = 0.921$), but relates in a negative way with PC4 (β = -0.58, t = -3.83, $p = 0.001$), while body size does not relate to PC1 (β = -0.073, t = -0.388, $p = 0.701$), PC2 (β = -0.043, t = -0.229, $p = 0.820$) and PC4 (β = 0.166, t = 1.094, $p = 0.283$), but relates in a negative way with PC3 (β = -0.455, t = -2.703, $p = 0.012$).

Discussion

The genus *Espadarana* is composed of five species (Frost 2024). The advertisement call has been described for *E. prosoblepon* (Jacobson 1985, Savage 2002, Kubicki 2007, Freile *et al.* 2020, Guayasamín *et al.* 2020), *E. andina*

Table 1. Summary of the dominant, low, and high frequency (kHz) values per note of the advertisement call of the glass frog *Espadarana prosoblepon* in the "Cedro Rosado" Botanical Garden in Armenia, Department of Quindío, Central Andes of Colombia. Data are presented as mean ± standard deviation and range. Sample size (number of males recorded, *N*) varies because not all males emitted advertisement calls with the same number of notes. See text for other temporal and spectral features of the calls.

	Note 1 *N* = 31	**Note 2** *N* = 31	**Note 3** *N* = 5	**Note 4** *N* = 1
Initial part of the note				
Dominant frequency	5.23 ± 0.17 (4.91 – 5.51)	5.22 ± 0.17 (4.99 – 5.49)	5.12 ± 0.14 (4.99 – 5.34)	5.17 ± 5.17
Low frequency	4.81 ± 0.19 (4.48 – 5.20)	4.82 ± 0.18 (4.40 – 5.24)	4.73 ± 0.15 (4.59 – 4.99)	4.68
High frequency	5.67 ± 0.18 (5.37 – 6.05)	5.64 ± 0.19 (5.35 – 6.03)	5.54 ± 0.09 (5.43 – 5.68)	5.55
Middle part of the note				
Dominant frequency	5.45 ± 0.16 (5.17 – 5.81)	5.43 ± 0.17 (5.17 – 5.76)	5.20 ± 0.08 (5.17 – 5.34)	5.68
Low frequency	5.06 ± 0.15 (4.82 – 5.42)	5.01 ± 0.16 (4.65 – 5.37)	4.81 ± 0.15 (4.59 – 4.98)	4.78
High frequency	5.87 ± 0.17 (5.56 – 6.21)	5.85 ± 0.19 (5.53 – 6.15)	5.66 ± 0.11 (5.60 – 5.85)	5.99
Final part of the note				
Dominant frequency	5.53 ± 0.18 (5.26 – 5.97)	5.45 ± 0.22 (5.10 – 5.98)	5.23 ± 0.16 (5.11 – 5.51)	5.51
Low frequency	5.06 ± 0.20 (4.79 – 5.47)	4.96 ± 0.18 (4.62 – 5.36)	4.76 ± 0.16 (4.64 – 5.05)	4.63
High frequency	5.96 ± 0.22 (5.65 – 6.42)	5.94 ± 0.24 (5.60 – 6.53)	5.66 ± 0.11 (5.55 – 5.83)	6.00

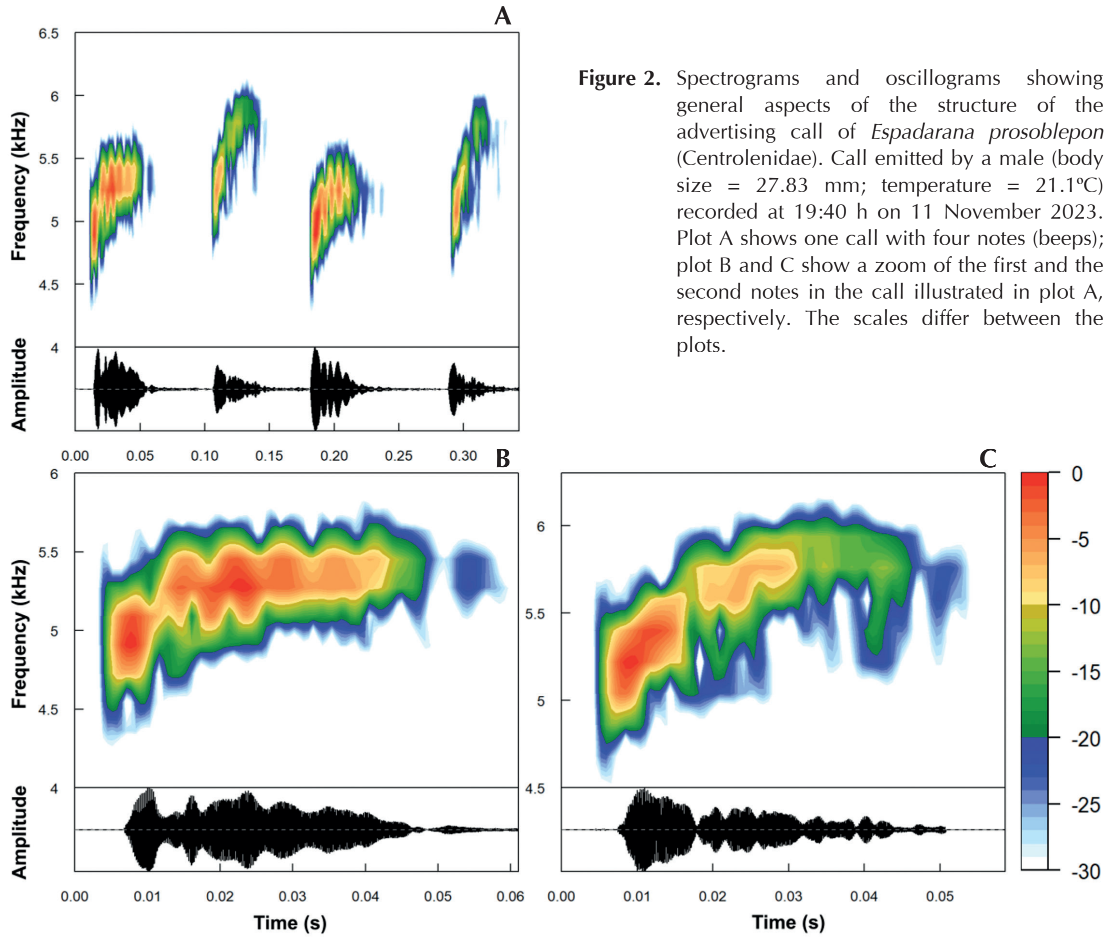

Figure 2. Spectrograms and oscillograms showing general aspects of the structure of the advertising call of *Espadarana prosoblepon* (Centrolenidae). Call emitted by a male (body size = 27.83 mm; temperature = 21.1ºC) recorded at 19:40 h on 11 November 2023. Plot A shows one call with four notes (beeps); plot B and C show a zoom of the first and the second notes in the call illustrated in plot A, respectively. The scales differ between the plots.

(Rivero, 1968) (Señaris and Ayarzaguena 2005, Cabanzo-Olarte and Ortega-Chinchilla 2017), *E. audax* (Lynch and Duellman, 1973) and *E. callistomma* (Guayasamin and Trueb, 2007) (Guayasamin *et al.* 2020), but not for *E. durrellorum* (Cisneros-Heredia, 2007) (Cisneros-Heredia 2007, Guayasamin *et al.* 2020). The advertisement call of *E. prosoblepon* follows a type trii call structure, as do other species in the genus *Espadarana* (Duarte-Marín *et al.* 2022). Some specific differences occur in features such as call duration, note duration, number of notes per call, and dominant frequency (Table 2). For instance, the number of notes per call in *E. prosoblepon* (2–5 notes) is similar between sister species, *i.e., E. callistomma* (3–4 notes), but higher than in *E. andina* and *E. audax* (1 note). A similar pattern is detected for call duration (Table 3). This tendency could reflect a low-to-absent phylogenetic signal in these temporal call features (Escalona *et al*. 2019, Vargas-Salinas *et al*. 2024). Comparisons beyond those summarized in Table 2 are not possible at present because different call features have been recorded for the taxa. Call descriptions of *E. andina, E. audax,* and *E. callistomma* (Table 2) do not include data and analysis of potential effects of temperature and body size.

Our call description includes other features in addition to those described previously for

Table 2. Results of the principal component analysis summarizing variation in 26 advertisement call features for males of the glass frog *Espadarana prosoblepon* in a population located in central Andes of Colombia. Variables (call features) were assigned to a principal component if their loading was > |0.6| (highlighted in bold font). Note that a negative load value indicates that the variable (i.e., call feature) relates in a negative way with the corresponding PC. When applicable, temporal call features were measured in ms and spectral call features in kHz.

Call features	**Principal Components**			
	PC1	**PC2**	**PC3**	**PC4**
Initial high frequency Note 2	**0.96**	0.13	-0.05	-0.03
Initial high frequency Note 1	**0.94**	0.15	0.10	-0.13
Initial dominant frequency Note 2	**0.93**	0.24	-0.00	-0.09
Initial dominant frequency Note 1	**0.91**	0.22	0.17	-0.15
Low frequency of the whole call	**0.93**	0.31	0.07	-0.08
Middle high frequency Note 2	**0.90**	0.35	0.23	-0.07
Middle dominant frequency Note 2	**0.90**	0.40	0.06	-0.04
Initial low frequency Note 2	**0.89**	0.20	-0.01	-0.13
Dominant frequency of the whole call	**0.87**	0.10	0.12	0.04
Middle dominant frequency Note 1	**0.86**	0.40	0.14	0.07
Initial low frequency Note 1	**0.85**	0.24	0.31	-0.06
High frequency of the whole call	**0.85**	0.46	0.13	0.00
Middle high frequency Note 1	**0.85**	0.40	0.11	0.07
Middle low frequency Note 1	**0.80**	0.44	0.13	0.06
Middle low frequency Note 2	**0.78**	0.47	0.08	0.03
Final high frequency Note 2	**0.71**	0.47	0.08	0.00
Final low frequency Note 2	**0.69**	0.55	0.08	0.07
Final dominant frequency Note 2	**0.67**	0.63	0.06	-0.09
Final high frequency Note 1	0.52	**0.79**	0.13	-0.00
Final dominant frequency Note 1	0.52	**0.78**	0.14	-0.02
Final low frequency Note 1	0.44	**0.71**	0.33	0.06
Number of notes	-0.12	-0.19	**-0.94**	-0.15
Call duration	-0.10	-0.11	**-0.90**	0.41
Note emission rate	0.04	-0.03	0.41	**-0.88**
Interval between notes	-0.09	0.24	0.07	**0.85**
Duration of notes	-0.01	-0.40	0.09	**0.63**
Eigenvalue	**17.323**	**2.473**	**1.908**	**1.152**
Percentage of variance explained	**66.627**	**9.511**	**7.339**	**4.429**

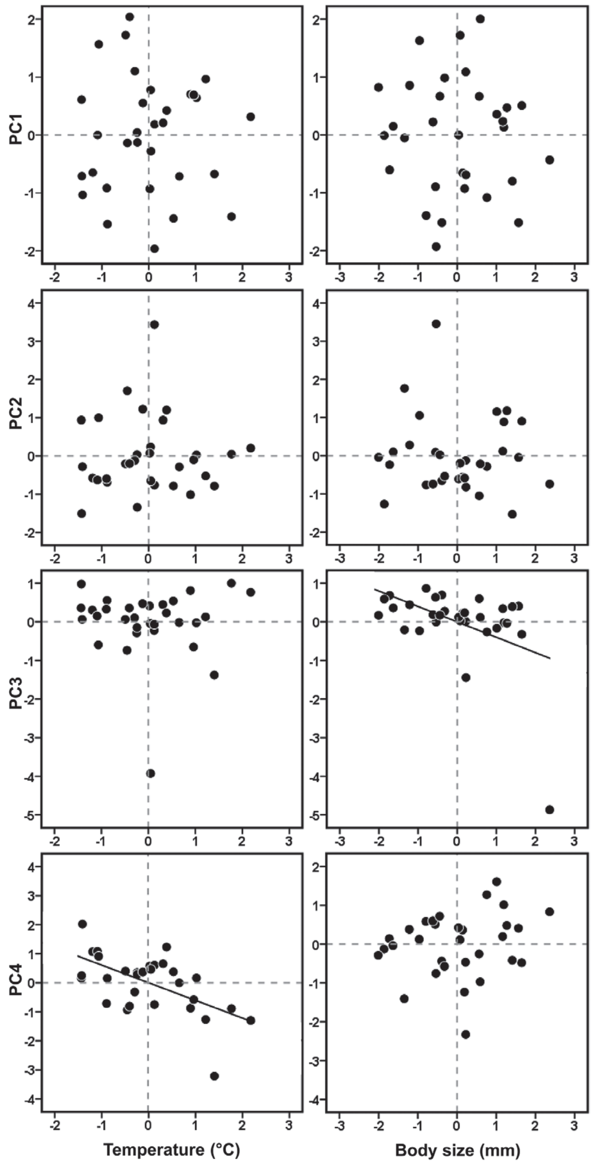

Figure 3. Partial regression plots showing the relationship between call features (summarized in four principal components, PCs) with temperature (first column) and body size (second column) of males of the glass frog *Espadarana prosoblepon*. Values in y-axis (left) and x-axis (bottom) are residuals of the multiple regression analysis. The regression line is shown only in those plots for which there was a significant relationship between variables at an alpha = 0.05. PC1 and PC2 summarize spectral call features while PC3 and PC4 summarize temporal call features. Details of call features included in each PC are indicated in Table 2. Note that the inverse relationship between body size and PC3 is highly influenced by an outlier corresponding to the largest recorded male (body size = 29.92 mm) with the higher average value of number of notes (3 notes) and call duration (427 ms). When the multiple regression analysis was run without this male, the relationship of PC3 with temperature disappeared (β = -0.021, t = -0.115, p = 0.910) and with body size remained as non-significant (β = -0.271, t = -1.466, p = 0.154).

Table 3. Summary of some advertisement call features (number of notes per call, note duration, call duration, dominant frequency) in glass frogs of the genus *Espadarana*. Data are presented as mean ± standard deviation and range. Sample size refers to number of males recorded/number of calls analyzed. * Values of this range correspond to the mean dominant frequency at the beginning of the note and the end of the note.

Species	Number of notes per call	Call duration (ms)	Note duration (ms)	Dominant frequency of the call (kHz)	Sample size	Source
E. andina	1	31.8 ± 5.5 (25.3–48.0)	31.8 ± 5.5 (25.3–48.0)	5.7 ± 0.1 (5.5–5.9)	4/24	Señaris and Ayarzaguena 2005
E. audax	1	38.0 ± 8.2 (26.0–53.0)	38.0 ± 8.2 (26.0–53.0)	6.1 ± 0.4 (5.4–6.7)	2/4	Guayasamin *et al.* 2020
E. callistomma	3–4	380.0 ± 66.0 (280–440)	30.0 ± 7.0 (14.0–40.0)	(5.3–5.8)	1/10	Guayasamin *et al.* 2020
E. prosoblepon	2–5	–	–	–	–	Freile *et al.* 2020
E. prosoblepon	2	212 ± 7 (207–223)	40 ± 8 (32–54)	(5.8–6.3) *	1/4	Guayasamin *et al.* 2020
E. prosoblepon	2.1±0.2 (2–4)	230.0 ± 40.0 (190–430)	41.1 ± 4.8 (31.0–48.7)	5.2 ± 0.2 (4.9–5.6)	31/220	This study

populations of *Espadarana prosoblepon* in Costa Rica (Jacobson 1985, Savage 2002, Kubicki 2007) and Ecuador (Freile *et al.* 2020, Guayasamin *et al.* 2020). Since the description by Jacobson (1985) is the most detailed and often cited, we compare our calls with results published by this author. General tendencies mentioned below agree with data shown in the other descriptions. The average dominant frequency of the call is slightly higher in the Costa Rica population (5.8 kHz, range 5.3–6.0 kHz; Jacobson 1985) than ours (5.2 kHz, range 4.9–5.6 kHz). This tendency is not attributed to interpopulation differences in body size because in any of these populations, a relationship existed between spectral features of the call and body size. The absence of an inverse relationship between male body size and call frequency in our data of *E. prosoblepon* was unexpected because in most anurans such traits relate in an inverse way (Vargas-Salinas and Amézquita 2014, Tonini *et al*. 2020). Some exceptions to this tendency occur, typically in species associated with noisy habitats (Tonini *et al*. 2020, Maria *et al*. 2023) such as those in which glass frogs call and breed (i.e., streams). Regarding glass frogs, a weak negative relationship has been found between body size of males and dominant call frequency in studies of multiple species (Escalona *et al*. 2019, Mendoza-Henao *et al*. 2023, Vargas-Salinas *et al*. 2024). It is possible that at the intraspecific level, such an inverse relationship could be even weaker or absent in some species, in part because of a low variability in body size of males within a given population.

Note duration might show a contrary tendency than dominant frequency; that is, it was slightly shorter in the Costa Rica population (40 ms, range 25–50 ms) than in our population (41.9 ms, range 32.7–57.7 ms). This comparison should be taken as preliminary since PC3 (that includes the feature “note duration”) was inversely correlated to temperature in our study (but see Figure 3), and Jacobson (1985) did not test this potential effect. The number of notes per call apparently do not differ between populations; Jacobson (1985) recorded up to five notes per call while we found up to four notes per call, but in the field, we were able to hear calls with five notes. A more detailed comparison of the advertisement call between populations of *E. prosoblepon* is not feasible to date, because 1) no data is available for Costa Rica populations regarding call features such as duration of interval between notes, dominant frequency per note, and minimum and maximum frequency per note (Jacobson 1985, Savage 2002, Kubicki 2007), and 2) the best description for an Ecuadorian population is based on only one male (Guayasamin *et al.* 2020).

The available information for Costa Rica populations (Jacobson 1985, Savage 2002, Kubicky 2007) and other populations from Costa Rica, Panamá (Appendix I) and Ecuador (Table 2) suggests little differentiation in advertisement call features between populations of *Espadarana prosoblepon* examined here. More recordings and data are necessary for a robust conclusion. In these studies, it will be necessary to test potential effects of temperature and body size on call features.

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Appendix I. *Summary advertisement call features for two males belonging to populations of* Espadarana prosoblepon *in Bocas del Toro, Panamá (code Fonozoo.com = ID: 19092, FZ-SOUND-CODE: 7616) and Las Cruces biological station, Costa Rica (code Fonozoo.com = ID: 22957, FZ-SOUND-CODE: 12939). Body size of the recorded male was not given in either case. When applicable, temporal call features are expressed in ms and spectral call features in kHz.*

Attributes	Bocas del Toro, Panama	Las Cruces, Costa Rica
Temperature (°C)	16.6	–
Number of calls	1	1
Call duration	270.7	395.6
Number of notes	2	3
Duration Note 1	31.6	34.2
Duration Note 2	35.9	29.5
Duration Note 3	–	33.5
Interval between Note 1 and 2	203.1	130.1
Interval between Note 2 and 3	–	167
Dominant frequency Note 1	6.4	6.6
Dominant frequency Note 2	6.2	6.6
Dominant frequency Note 3	–	6.5
Low frequency Note 1	5.5	6
High frequency Note 1	7.2	7.5
Low frequency Note2	5.4	6
High frequency Note 2	7.0	7.3
Low frequency Note 3	–	6
High frequency Note 3	–	7.1
Initial dominant frequency Note 1	6.4	6.7
Initial dominant frequency Note 2	6.0	6.7
Initial dominant frequency Note 3	–	6.6
Initial low frequency Note 1	5.7	6.0
Initial high frequency Note 1	7.2	7.4
Initial low frequency Note 2	5.4	6.0
Initial high frequency Note 2	7.0	7.3
Initial low frequency Note 3	–	5.4
Initial high frequency Note 3	–	7.1
Middle dominant frequency Note 1	6.4	6.7
Middle dominant frequency Note 2	6.4	6.7
Middle dominant frequency Note 3	–	6.6
Middle low frequency Note 1	5.5	6
Middle high frequency Note 1	7.2	7.6
Middle low frequency Note 2	5.4	6
Middle high frequency Note 2	7.1	7.4

Appendix I. *Continued.*

Attributes	Bocas del Toro, Panama	Las Cruces, Costa Rica
Middle low frequency Note 3	–	5.9
Middle high frequency Note 3	–	7.3
Final dominant frequency Note 1	6.4	6.9
Final dominant frequency Note 2	6.4	6.6
Final dominant frequency Note 3	–	6.6
Final low frequency Note 1	5.2	6.1
Final high frequency Note 1	7.2	7.6
Final low frequency Note 2	5.4	6.2
Final high frequency Note 2	7.1	7.2
Final low frequency Note 3	–	6.1
Final high frequency Note 3	–	7