BENEMÉRITA UNIVERSIDAD AUTÓNOMA DE PUEBLA

FACULTAD DE CIENCIAS BIOLÓGICAS

Geographic variation of the advertisement call of the frog *Eleutherodactylus nitidus* (Anura: Eleutherodactylidae) in central Mexico

Tesis que para obtener el título de

LICENCIADA EN BIOLOGÍA

PRESENTA:
ZELTZIN KARINA VÁZQUEZ HERNÁNDEZ

DIRECTOR: Dr. CARLOS ALBERTO HERNÁNDEZ JIMÉNEZ
CODIRECTOR: Dr. JOSÉ MANUEL SERRANO SERRANO

Enero de 2023

Acknowledgements

I express my gratitude to the Benemérita Universidad Autónoma de Puebla and La Facultad de Ciencias Biológicas for the academic quality provided, where I received incomparably good education through teachers and study programs.

To Dr. Carlos Alberto Hernández Jiménez, I am grateful for his undoubted support and follow-up on this study and his patience in dealing and answering any doubt. I thank him for encouraging me to take on challenges and overall to work on different projects. An invaluable assessor and person.

I thank to Dr. José Manuel Serrano Serrano for his priceless responsibility with the project, as well as his distinguished kindness and willingness to answer any question and inconvenient. I thank him for motivating me for future projects and for his priceless quality not only as an academic and assessor but as a person.

I am immensely grateful to my parents who have been the responsible ones that I got this far and unconditionally always supported me in every decision I have taken. To my brother, I thank him for always helping me and listening to me whenever I needed it, for his doubtless support and for being my role model in this world called science. Mom, Dad and Oscar, thank you so much for the patience, trust and overall for the unlimited love. I love you all.

To my grandparents Cruz, Pedro, Genoveva and Esteban, who have undoubtedly supported me in all my projects and have been a solid and important step in my education, not only academic and extracurricular activities but also in personal growth. Thank you for your immense love.

I am grateful to my boyfriend Gregor, who has believed in me and has unquestionable and unimaginable supported me. I am infinitely grateful to him for always being there, both in good and bad times, for motivating me and being the best partner I could have ever wished. Thank you so much for everything.

To my friends Vero and Carlos, who have offered me such a beautiful friendship, and overall a space to listen to me when certain circumstances are not going in the way I planned. Thank you so much.

Content

Acknowledgements II

Abstract 1

Keywords... 1

1. Introduction 1

2. Justification 3

3. Hypothesis 4

4. Objectives 4

General: 4

Particular: 4

5. Materials and methods 5

5.1. Study model 5

5.2. Study area 5

5.3. Call recordings 7

6. Analysis 7

6.1. Acoustic analysis 7

6.2. Statistical analysis 8

7. Results 9

8. Discussion 16

9. Conclusion 19

10. References 20

Appendix 1. Details of call acoustic intensity measurements 31

Appendix 2. Correlations between acoustic variables 32

Appendix 3. Average and standard error of acoustic variables 33

Abstract

Geographic variation in acoustic characteristics of anuran advertisement calls could be an important key for species divergence due to its importance for specie recognition within and among populations. In this study, we evaluate the geographic variation of 1676 advertisement calls of 66 individuals of *Eleutherodactylus nitidus* (Peters 1870) throughout six populations distributed in central Mexico. Additionally, we evaluate the influence of body size and environment on call variation. We compared six acoustic variables using ANOVA and linear discriminant analysis, as well as inter- and intra-population and intra-individual variation estimates to understand *E. nitidus* call variation. Advertisement calls were corrected for environmental and body size factors to rule out their influence on call variation. The results indicated that call duration, call intensity, dominant frequency, crests per call and highest crest differ between populations and such differences are independent of body size and environment. Additionally, Wilks' Lambda criterion applied to the linear discriminant analysis indicated that geographic call variation depends principally on call intensity. We concluded that, distance-separating populations of *E. nitidus* mainly determine differences between them due to divergence processes.

Keywords: Acoustic communication, acoustic divergence, bioacoustics, natural selection, urban anurans.

1. Introduction

Acoustic signals have evolved in diverse groups of animals, allowing different functions, relevant for courtship interactions, territory defense, predators warming and intraspecific interactions (Green and Marler 1979; Endler and Basolo 1998). In such scheme, geographic variation of vocal traits has been one of the interests from biologists and naturalists since its implications and consequences in evolutionary aspects, including social behavior and species divergence (Boughman 2002; Wilkins et al. 2013).

Anuran vocalizations have received considerable attention in behavioral ecology studies (Ryan 1988; Gerhardt 1994), since they play important roles for communication, principally in reproductive biology, as advertisement calls are generally produced by males to attract females during the reproductive season, allowing individual and species recognition (Castellano et al. 2002; Wells and Schwartz 2007). Thus, the use of these calls has helped to differentiate between taxonomic groups in anurans (e.g., Heyer et al. 1996; Tsuji-Nishikido 2012) and understand the divergence process of signals in species (e.g., Velásquez et al. 2013; Velásquez et al. 2014).

Different studies have established contrasting hypotheses to explain the evolution of advertisement calls as reproductive traits. First, those who propose the action of sexual selection through the intra-sexual struggle of signalers to attract the attention of receivers (Gerhardt 1982, 1994), and the choice of receivers following the attractive characteristics of advertisement calls (Gerhardt 1992; Chek et al. 2003; Pröhl et al. 2007). Secondly, acoustic signal variation is closely related to natural selection adaptations in locations with different ecological conditions, as well as geographic isolation (Gerhardt 1975; Baraquet et al. 2007), where factors such as temperature, humidity and type of habitat frequently affect the characteristics of advertisement calls (Brenowitz 1986; Rodríguez et al. 2010). For example, call duration of *Hylodes heyeri* and *Physalaemus cuvieri* decreases as temperature increase (Lignau and Bastos 2007; Gambale and Bastos 2014), while the dominant frequency in *Odontophrynus cordobae* increases with temperature (Grenat et al. 2013).

The influence of morphology on anuran advertisement calls variation has been largely documented in studies of mating choice (Gerhardt 1975; Rand 1985; McClelland et al. 1996). Since intraspecific morphological differences commonly occur between populations geographically distanced (Castellano et al. 2000; Laugen et al. 2002). Thereby, differences in body size could explain the distinctions between acoustic characteristics such as dominant frequency (e.g., Serrano et al. 2020). These kind of relationships with call variation are mainly determined by mass and size of the phonatory system (Wells and Schwartz 2007). Therefore, in frogs and toads, an inverse relationship between male size and dominant frequency has frequently been reported to vary geographically, where larger individuals show a lower dominant frequency (Gerhardt 1994; Bernal et al. 2005; Pröhl et al. 2007; Gingras et al. 2012).

In this study we compared advertisement calls of a widely distributed endemic frog in central Mexico (*Eleutherodactylus nitidus* Peters, 1870) from six different populations, with the purpose of distinguishing their intra and inter-population call variation. Calls were analyzed considering six acoustic variables to evaluate the differences between populations. Furthermore, we analyzed the influence of morphometric and environmental characteristics on advertisement calls variation between localities.

2. Justification

Acoustic communication plays a key role in species divergence, as it can be affected by different evolutionary forces (genetic drift, natural and sexual selection), as well as the influence of environmental and habitat properties facilitating call variation (Gerhardt 1991; Castellano and Giocoma 1998; Endler and Basolo 1998; Wollerman 1998; Boughman 2002; Gridi-Papp and Narins 2009; Wilkins et al. 2013). For example, acoustic differences between populations of *Hyla andersonii* differ because of environmental dissimilarities affecting sound attenuation properties (Warwick et al. 2015). However, populations of *Hyla cinerea* show greatest differences in advertisement calls related to distances separating them (Asquith et al. 1988), while call variation of *Hyla eximia* are not only related to geographic distance, but also to acoustic interference with other hylid frogs (Rodríguez-Tejeda et al. 2014).

In addition to the natural processes promoting the variation of acoustic signals in animals, the growth of human settlements and their extension to natural areas has produced acoustic signal changes (Hunter 2007; Kowarik 2011). Nevertheless, it has been reported that, some species of anurans keep unchanged acoustic signals despite being constantly under urban disturbances (e.g., Cunnington and Fahrig 2010; Kaiser et al. 2011).

Mexico is inhabited by 35 of the 205 known species of the genus *Eleutherodactylus*, where 29 are endemic to the country (AmphibiaWeb 2022; Frost 2022). However, advertisement calls have only been described for 32% of the total number of eleutherodactylids from Mexico (Reyes-Velasco et al. 2015;

Serrano 2016; Serrano and Penna 2018; Grünwald et al. 2021; Hernández-Austria et al. 2022). Additionally, some *Eleutherodactylus* species show a high tolerance to anthropic landscapes (Cruz-Elizalde et al. 2022). Accordingly, *E. nitidus* is an endemic anuran from Mexico persisting in areas with different levels of urbanization and anthropogenic pressures (Gómez-Benitez et al. 2021), which may be indirectly and gradually affecting the advertisement calls. Thus, our study would represent a solid base to know its call variation on larger scales, allowing us to answer basic aspects about its ecology and evolution, due to the importance of advertisement calls regarding the processes of speciation (Pröhl et al. 2006, 2007; Velásquez et al. 2013; Velásquez 2014).

3. Hypothesis

In this study, we hypothesize that body size (snout-vent length and weight) of individuals will determine the variation of dominant frequency of advertisement calls. We expect that body size variation between populations determine the differences in acoustic characteristics of advertisement calls of *E. nitidus*, where greater differences in calls will be found respect to the greater body size variation between populations. Due to the relationships between acoustic and environmental characteristics previously registered in different anuran species, we expect that temperature, will also influence on acoustic properties of advertisement calls of *E. nitidus*.

4. Objectives

General: Investigate the geographic variation in the advertisement calls of *Eleutherodactylus nitidus* in six distant populations of central Mexico.

Particular:

1) Determine which acoustic variables give the greatest variability and differences in *E. nitidus* populations.

2) Identify whether there is a relationship between acoustic variables and morphometric characteristics, such as snout-vent length and weight.
3) Distinguish whether there is a relationship between acoustic characteristics and environmental variables, such as temperature and humidity.

5. Materials and methods

5.1. Study model

Eleutherodactylus nitidus was considered to inhabit in central Mexico from the south of Sinaloa through the Pacific to Oaxaca (García-Vázquez and Trujano-Ortega 2012). However, in a recent study supported by morphological, molecular and advertisement calls characteristics suggest that *E. nitidus* is currently a five-species complex, and the nominal species of *E. nitidus* is limited to the center of the country through states such as Puebla, Guerrero, Morelos, Estado de México and Oaxaca (Grünwald et al. 2021).

Eleutherodactylus nitidus is characterized by its small body size, reaching between 24.3 and 26.3 snout-vent length (SVL) in the adult stage, and presenting a yellowish lumboinguinal gland (Grünwald et al. 2021). This free toe frog is mostly found in rainy season inhabiting open areas such as secondary forest, open fields, oak and pine-oak deciduous forest, semi-desert areas and cities (Canseco and Gutiérrez-Mayén 2010; Grünwald et al. 2018; Gómez-Benitez et al. 2021). According to an incidental report (Palacios-Aguilar 2018), males of this specie can stablish shared nesting sites under rocks to take care about the eggs.

5.2. Study area

Six populations of *E. nitidus* covering a wide area of the known distribution of the specie (Figure 1) were studied. Four populations belong to Puebla state: Parque de las 7 Culturas, San Andrés Cholula (19° 3'25.51" N; 98°17'58.64" W), Tonantzintla, San Andrés Cholula (19°1'0.60" N; 98°19'6.69" W), Las Haras

Ciudad Ecológica, Puebla City (19°1'13.03" N; 98° 7'45.37" W) and Los Ahuehuetes in San Miguel Ayotla (18°41'37.11" N; 98°23'23.52" W). Two additional populations correspond to different states: Tepoztlán in Morelos (18°59'9.56" N; 99° 5'58.94" W) and Oaxaca de Juárez in Oaxaca (17° 4'15.32" N; 96°43'35.09" W). All study areas are located within urban land, except for Los Ahuehuetes and Las Haras Ciudad Ecológica, which are located on the edge of urban settlements.

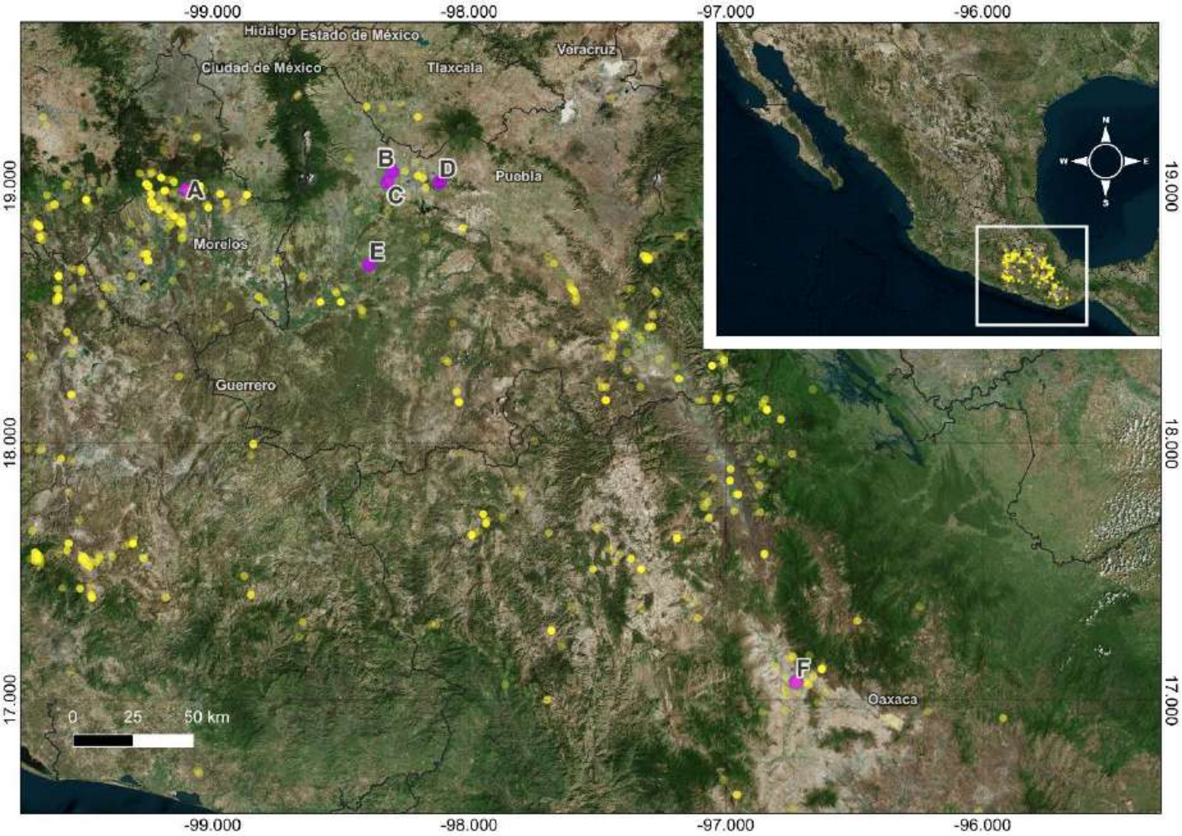


Figure 1. Sampling sites of the advertisement calls of *E. nitidus* in central Mexico. (A) Tepoztlán, Morelos; (B) Parque de las 7 Culturas, San Andrés Cholula, Puebla; (C) Tonantzintla, San Andrés Cholula, Puebla; (D) Las Haras Ciudad Ecológica, Puebla City, Puebla; (E) Los Ahuehuetes, San Miguel Ayotla, Puebla; (F) Oaxaca de Juárez, Oaxaca. The map was elaborated using QGis Desktop 3.22.2 and GBIF data of *E. nitidus* (yellow points) and localities sampled by this study (purple points).

5.3. Call recordings

Advertisement calls of *E. nitidus* were recorded from July to August 2015, and from July to September 2021, between 19:00 and 4:00 hrs. Two different unidirectional microphones were used: A Senheisser ME66 unidirectional condenser microphone (40 Hz to 20 Hz frequency response) and a Soundtrack Pro-Audio CON-300 condenser microphone (100 Hz to 16 kHz frequency response). These were separately connected to a recorder TASCAM DR-40 (96 kHz resolution). Call recordings were obtained in WAV format using a sampling rate of 24 kHz and recorded from 20 to 80 cm away from each individual for 5 to 10 min. In addition, temperature and humidity were registered with a portable weather station (Sper Scientific 850070), located in a nearly place where the individuals were calling.

Recorded individuals were captured to register SVL using a digital caliper with 0.03 mm precision, and weight by using a digital pocket scale (MH-500) with 0.01 g precision. After data collection, each individual was released at the exact place where it was found.

6. Analysis

6.1. Acoustic analysis

All advertisement calls recorded were analyzed using the software Raven Pro version 1.6, with a sampling frequency of 44100 Hz and 24-bit resolution. Spectral measurements were obtained from a Hann Window with a 512 sample size, an overlap of 50% and 256 points of resolution for the Fast Fourier Transformation (FFT). The following acoustic characteristics were measured in each advertisement call: 1) call duration of the single note call (s), 2) dominant frequency (Hz), 3) call rate (calls/min), 4) number of crests per call (counting of peaks of modulated amplitude oscillations), 5) highest crest (ordinal position from left to right of the highest crest) and 6) call intensity (measured as the standardized sound pressure level in full scale “SSPL FS”) (for more details on acoustic measurements, see Appendix 1).

6.2. Statistical analysis

Correlation matrices were performed to calculate relationships of acoustics with morphometric (SVL and weight) and environmental (temperature and humidity) variables. Afterwards, collinearity between acoustic, morphometric and environmental variables were analyzed and calculated by means of simple Pearson and Spearman correlations tests.

To discard whether population differences in acoustic variables were mainly influenced by body size variation and environment, acoustic variables were analyzed by correcting for SVL and temperature (following Platz and Forester 1988) for those variables at which significant relationships were detected among acoustic and morphometric and environmental variables (see Table 2 in Results). Normality criteria for the data was verified for all the variables using Bartlett test (Crawley 2013). Once body size and temperature corrections were made, differences between populations along their geographical variation were determined through analysis of variance (ANOVA) for each acoustic, morphometric and environmental variable. When we found significant differences, a Tukey test was applied to detect between-group differences.

Additionally, a linear discriminant analysis (LDA) was performed, to identify separated groups of acoustic populations. To build the LDA model, we used those acoustic variables that were uncorrelated with each other (Naes and Mevik 2001; see Appendix 2). Finally, a Wilks' Lambda criterion (Alrawashdeh and Radwan 2017) was used to determine which acoustic variables better assign differences between populations.

According to Gerhardt (1991), call variability is considered a way to classify its dispersion into the continuous rank between high or low variability, also known as the sexual selective dispersion between dynamic (highly variable low selective) or static (highly selective low variance). To evaluate such selective dispersion of each acoustic variable, a coefficient of variation (CV = [SD/Mean] x 100) was performed at three levels: inter-population (CVp), intra-population (CVip) and intra-individual (CVi). The CVp for each variable was obtained from the mean and standard deviation values from all the individuals independent to the six populations; subsequently, every CVip was calculated from the means and the standard deviation from individuals of each population, and finally the CVi derived from the mean value of the CVip from all the individuals (Pröhl et al. 2007; Velásquez et al. 2013; Serrano et al. 2020). To describe the individual variation of the advertisement calls at the population, the relation (CVip/CVi) for each variable was evaluated. In concordance to Aubin et al. (2007), call individuality is represented numerically as a higher value than one, so that, intra-individual variability is more representative in comparison to inter-individual variability. We used the software R Studio (R Studio Team 2020) for all the statistical analysis and the following packages: 1) PerformanceAnalytics (Peterson et al. 2020) for the building of correlation matrices, 2) MASS (James et al. 2013) for LDA, 3) klaR (Roever et al. 2022) for Wilks' Lambda statistical analyses, 4) ggplot2 (Wickham 2016; Wickham et al. 2022) for building boxplots for ANOVA and the LDA graphic and 5) seewave (Sueur et al. 2008) to visualize representative advertisement calls of *E. nitidus* from each population through spectrograms and oscillograms (Figure 2, respectively).

7. Results

A total of 1676 calls from 66 male individuals (previously determined during field work) spread in six populations in central Mexico were analyzed (see representative examples in Figure 2). The mean values (average and standard error) of acoustic variables are listed in Table 3 (see Appendix 3). The correlations between acoustic and temporal variables indicated positive and negative values (Table 2): dominant frequency correlated negatively with SVL and weight, while call intensity correlated positively with SVL and temperature, finally, call duration correlated positively with weight and negatively with temperature.

All the acoustic characteristics showed differences between populations (Figure 3), except for the call rate ($F = 0.681$, $p = 0.639$). Acoustic variables who did not correlated with body size showed significant differences between populations such as call duration ($F = 15.16$, $p < 0.001$), crests per call ($F = 8.83$, $p < 0.001$)

and highest crest ($F = 12.91$, $p < 0.001$). In the acoustic variables where body size and temperature showed significant relationships, such populations differences kept afterwards the dataset was re-analyzed by SVL correction (Platz and Forester 1988) in dominant frequency ($F = 4.048$, $p < 0.001$) and call intensity ($F = 13.09$, $p < 0.001$). Similarly, call duration ($F = 17.73$, $p < 0.001$) and call intensity ($F = 13.66$, $p < 0.001$) maintained differences between populations regardless to temperature correction was performed.

Morphometric variables differed between populations, as well as environmental variables (Figure 4). The four characteristics recorded, SVL ($F = 11.88$, $p < 0.001$), weight ($F = 14.28$, $p < 0.001$), temperature ($F = 17.72$, $p < 0.001$) and humidity ($F = 3.357$, $p < 0.01$) showed a significant variation between populations.

The LDA showed that acoustic variables can distinguish differences between populations. All the four acoustic variables considered for the analysis contributed significantly to the overall model, explaining the population distinctiveness (Table 4). The variable call intensity showed the higher score for discriminating populations in the differential model of the Wilks' Lambda test. The first discriminant function of the LDA explained most of the variation displayed among populations (LD1: 63%) allowing to discern the most distant population of Oaxaca (Figure 5), while the population of Tepoztlán and Puebla (Cholula, Las Haras, Los Ahuehuetes and Tonantzintla; Figure 5) showed a discrimination between each other in the second discriminant function (LD2: 26%).

The variability of acoustic characteristics (Table 5) showed that the highest variation at the intra-population (CVip) and inter-population (CVp) level corresponded to the highest crest and crest per call, while the lowest corresponded to dominant frequency, call duration and call intensity. To classify the variables, we considered a threshold of CV higher and lower than 8, where the static variables correspond to a CV value < 8, while those with CV > 8 were classified as dynamic. Thus, call duration, dominant frequency and call intensity were considered as statics at intra-population and intra-individual, whereas crests per call and the highest crest corresponded as dynamic variables. Eventually, all acoustic variables indicated potential for an individual distinction with a CVip/CVi > 1 for most of the populations (Table 5).

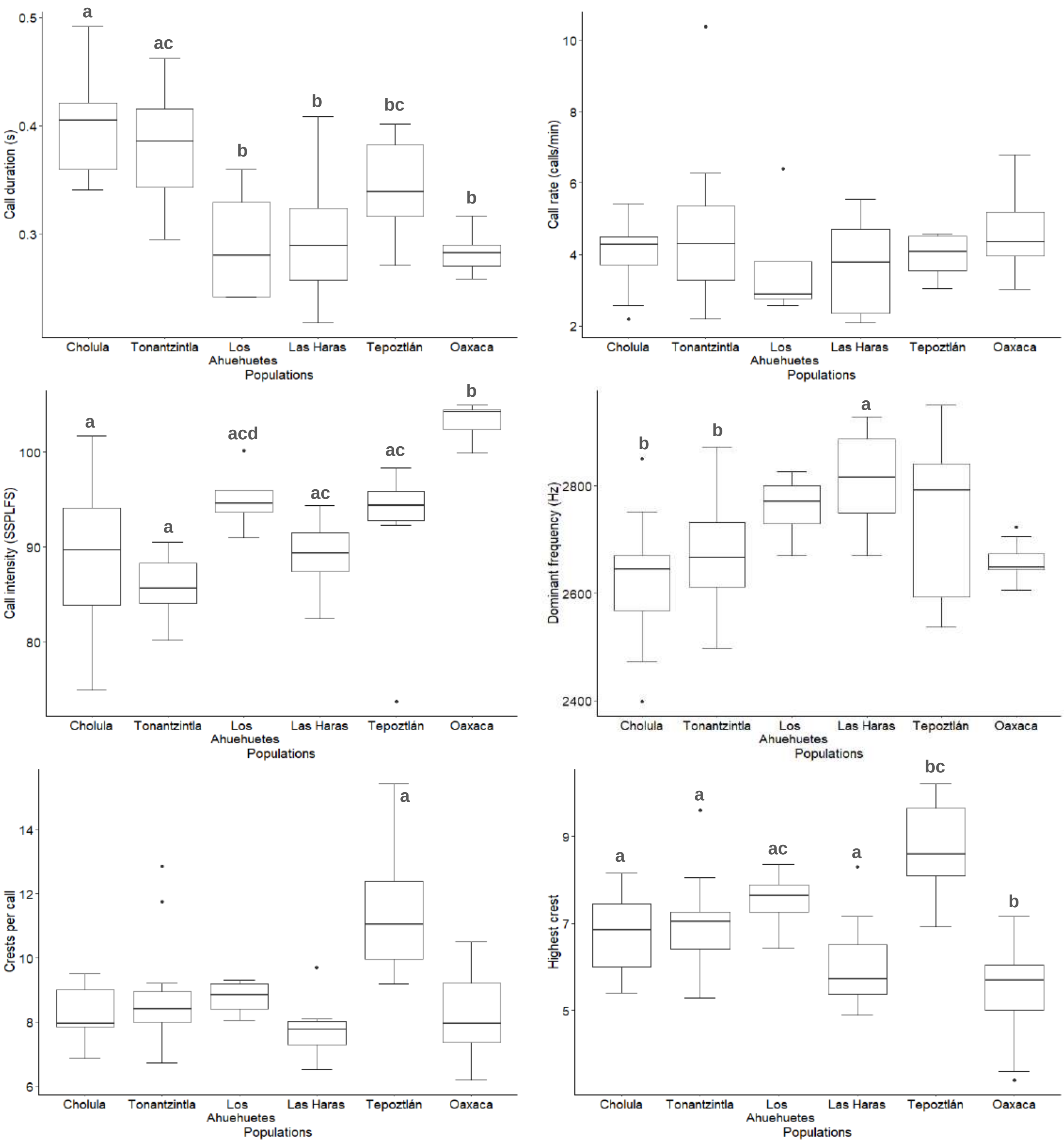


Figure 3. Comparison of acoustic characteristics of the advertisement calls of *Eleutherodactylus nitidus* from six different populations among central Mexico. Different combinations of letters (a, b, c) indicate statistical differences between populations' in the post hoc tests.

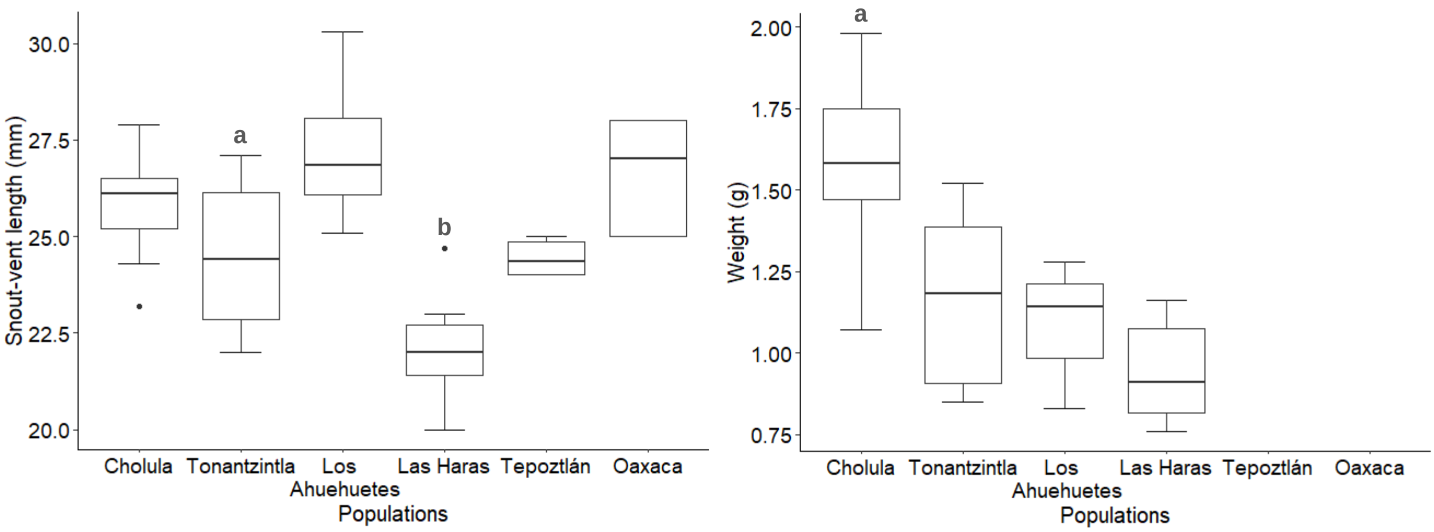


Figure 4. Comparison of morphometric characteristics (snout-vent length and weight) of *Eleutherodactylus nitidus* from six different populations among central Mexico. Different combinations of letters (a, b) indicate statistical differences between populations' in the post hoc tests.

Table 2. Pearson correlation between acoustic and temporal variables from advertisement calls of *Eleutherodactylus nitidus* (Significance levels = * indicates p < 0.05, ** indicates p < 0.01).

Variable	SVL	Weight	Temperature	Humidity
Call duration (s)	0.09	0.42**	-0.49**	0
Dominant frequency (Hz)	-0.39**	-0.61**	0.21	-0.1
Crests per call	-0.03	0.08	0.08	0.28
Highest crest	-0.22	0.07	-0.09	0.33
Call intensity (SSPLFS)	0.32*	-0.01	0.49**	0.12
Call rate (calls/min)	0.1	0.19	-0.04	0.04

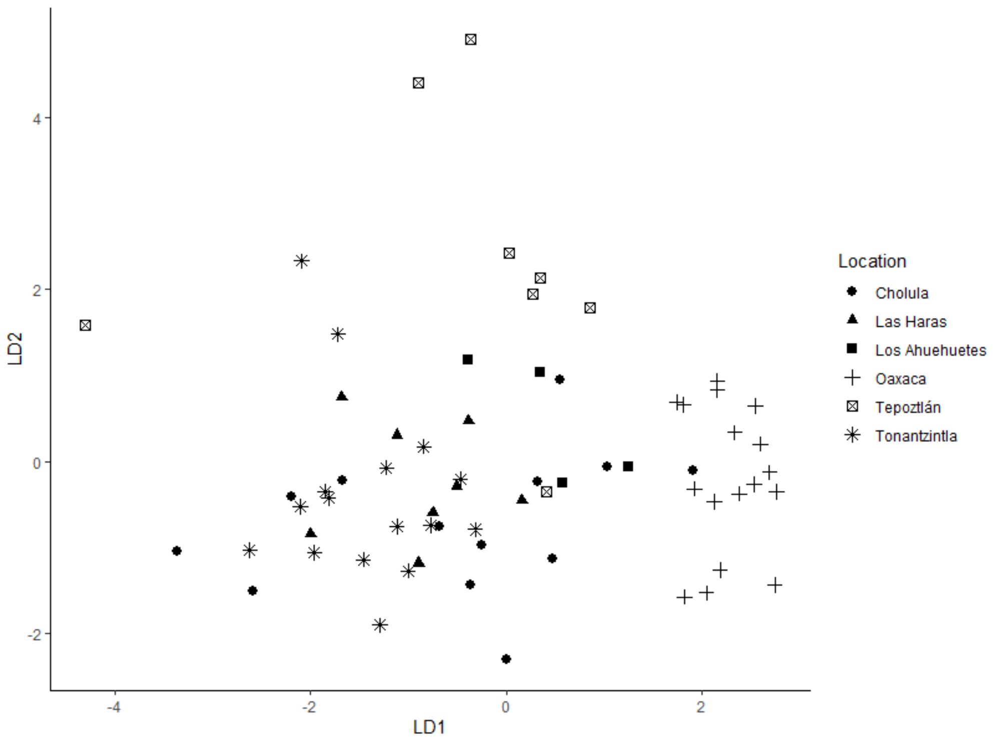
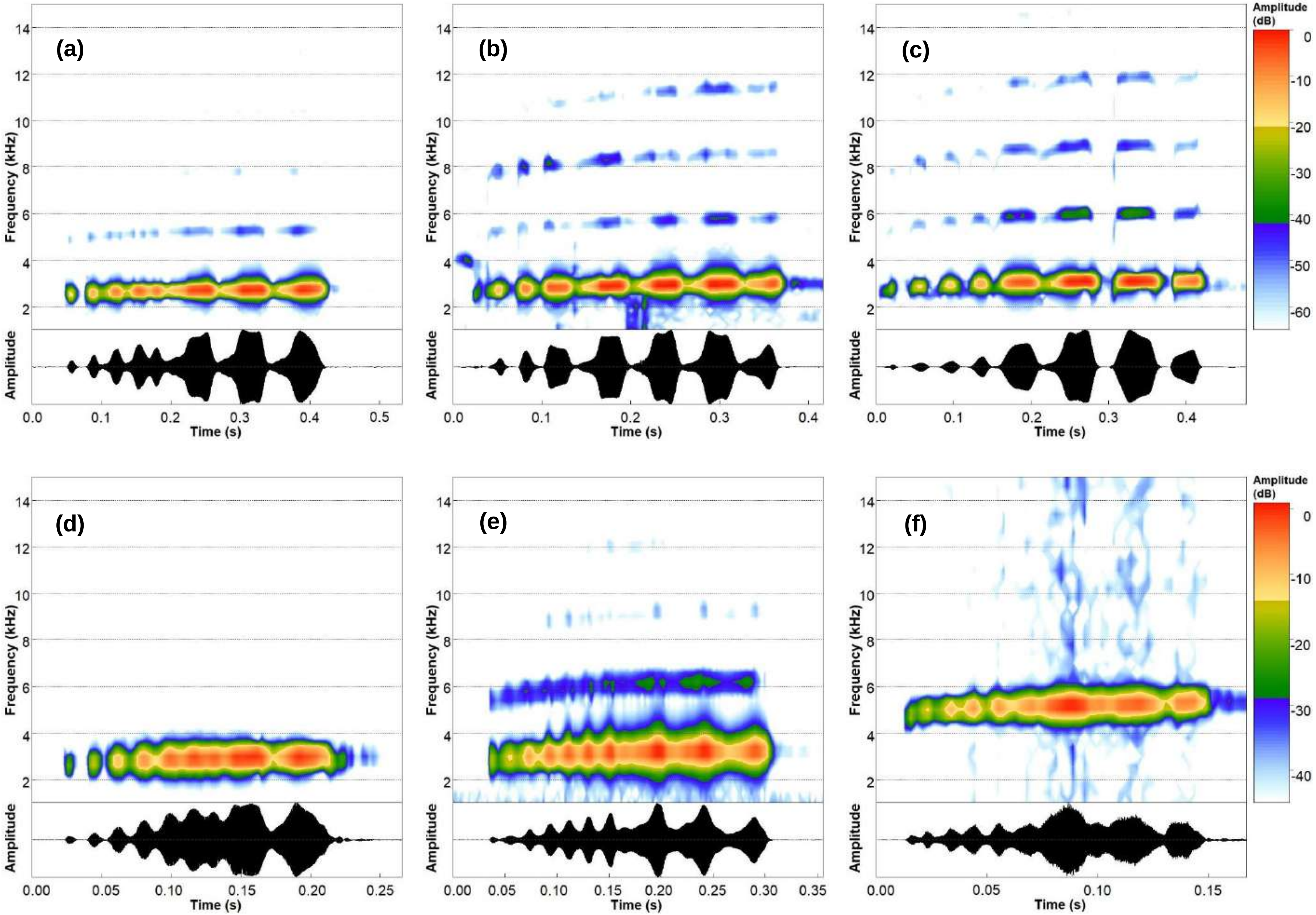


Figure 5. Discriminant plot showing up populations' separation by linear discriminant variables LD1 and LD2.

Table 4. Results from the linear discriminant analysis of advertisement calls properties. Significance levels = * indicates $p < 0.05$, ** $p < 0.01$.

Variables	LD1	LD2	Wilks' Lambda	F overall	F differential
Dominant frequency (Hz)	-0.001312893	0.00404179	0.1350161	11.401254**	4.305193**
Crests per call	-0.077329033	0.78476936	0.1851257	15.625131**	8.497615**
Call rate (calls/min)	-0.061504802	-0.28261638	0.1215937	8.432076**	1.258416**
Call intensity (SSPLFS)	0.208812805	0.02544549	0.3184415	25.683535**	25.683535**
Portion of trace	0.6385	0.2673			



(a)
(b)
(c)
(d)
(e)
(f)
Frequency (kHz)
Amplitude
Time (s)
Amplitude (dB)

Figure 2. Representative advertisement calls of *Eleutherodactylus nitidus* from six different populations of central Mexico: (a) Parque de las 7 Culturas, San Andrés Cholula; (b) Tonantzintla, San Andrés Cholula; (c) Las Haras Ciudad Ecológica, Puebla City; (d) Los Ahuehuetes, San Miguel Ayotla; (e) Tepoztlán, Morelos; (f) Oaxaca de Juárez, Oaxaca. These calls had from 7 to 10 crests (8, 7, 8, 9, 10 and 9, respectively). Air temperature: 14.4°C, 15.7°C, 14.9°C, 23°C, 21°C and 22°C, respectively. Sample rate for all recordings: 44.1 kHz.

Table 5. Coefficients of variation (CV) for advertisement calls at the inter-population (CVp), intra-population (CVip) and intra-individual (CVi) levels in six populations of *Eleutherodactylus nitidus* spread around Central Mexico. Abbreviations: CH (Cholula), TO (Tonantzintla), HA (Las Haras), AH (Los Ahuehuetes), TE (Tepoztlán) and OA (Oaxaca).

Acoustic variables	Inter-population (CVp)	Intra-population (CVip)						Intra-individual (CVi)						CVip/Cvi					
		CH	TO	AH	HA	TE	OA	CH	TO	AH	HA	TE	OA	CH	TO	AH	HA	TE	OA
Call duration (s)	4.21	5.12	3.76	4.02	3.68	3.74	4.35	5.09	3.74	4.03	3.73	3.61	4.35	1.004	1.004	0.999	0.986	1.036	1.000
Dominant frequency (Hz)	1.24	2.54	1.05	0.93	0.66	0.87	0.99	2.69	1.03	0.92	0.66	0.89	1.00	0.944	1.013	1.012	0.995	0.979	0.994
Crests per call	9.77	9.12	8.41	7.80	11.6	10.2	11	8.85	8.25	7.68	11.9	9.83	10.8	1.03	1.02	1.015	0.97	1.042	1.018
Highest crest	13.6	11.6	12.6	11.9	12.4	14.2	17.3	11.3	12.2	12.1	12.99	14.7	18.07	1.017	1.034	0.976	0.956	0.968	0.955
Call intensity (SSPLFS)	2.34	1.93	2.31	4.5	2.5	2.3	1.91	2.01	2.33	4.5	2.52	2.3	1.91	0.96	0.989	1.001	0.991	1.002	1.000

8. Discussion

Our study shows that advertisement calls of *E. nitidus* have a wide geographic variation that apparently depends mainly on geographic distance but not on the deterministic influence of body size and environmental factors. The correlation analysis displayed significant relationships between temperature, call duration and call intensity of *E. nitidus*. However, our results suggest that such environmental influence on temporal characteristics of advertisement calls does not explain the geographic differentiation of *E. nitidus* calls among populations, as statistical differences remain after temperature correction. Additionally, a similar situation occurred with the influence of body size on acoustic characteristics. The correlation analysis between morphometric and acoustic characteristics of *E. nitidus* displayed negative and positive relationships of body size with dominant frequency and call intensity, respectively, but such differences remained on these acoustic characteristics after body size correction.

Differences between environmental factors usually have a great importance on the divergence of anuran advertisement calls, since these components influence the outcome of different conditions for sound propagation and degradation (Velásquez 2014). In the case of *E. nitidus*, our study has been able to rule out that geographic variation is behind the divergence of advertisement calls, but this does not restrict the possibility that within each population such factors maintain a relevance on the performance of acoustic signals.

Some acoustic characteristics of advertisement calls are usually related to vocal apparatus and consequently affect call properties (Gingras et al. 2012). Therefore, several studies have frequently supported an inverse relationship between SVL and dominant frequency in distinct anuran species (e.g., McClelland et al. 1996; Giasson and Haddad 2006; O'Neill and Beard 2011; Gingras et al. 2012; Gambale et al. 2014; Galvis et al. 2018; Arriaga-Jaramillo et al. 2021; Augusto-Alves et al. 2021; James et al. 2022). On the contrary, in most studies, it has not been found any significant relationship between call characteristics and weight when it was measured. Nevertheless, some few cases of weight influence on call characteristics have been found in number of calls, inter-note intervals, fundamental and dominant frequency (e.g., Friedl and Klump

2002; Wang et al. 2012; Serrano et al. 2020). Thus, our results agree with previous studies, where dominant frequency tends to be lower respect to larger sizes, additionally to display a relationship with call intensity. Moreover, weight is considered an important morphometric characteristic influencing calls of *E. nitidus*, due to the displayed relationships with acoustic characteristics.

Except for call rate, ANOVA analysis revealed significant differences between populations of *E. nitidus* within all non-corrected and corrected comparisons by body size and temperature. In other species, call duration and dominant frequency have been important characteristics for species delimitation (e.g., Márquez and Bosch 1995; Padial et al. 2008; Nuñez et al. 2012). However, for other Terrana frogs, distinct acoustic characteristics supporting a difference between populations have been identified; for example, distinctions between populations of *Eleutherodactylus glamyrus* are attributed to call duration, call rate and rise time (Rodríguez et al. 2010), while call duration and inter-note interval are attributed to distinguish the populations of *Craugastor fitzingeri* (Höbel 2005). The differences observed between acoustic characteristics in this study (call duration, dominant frequency, call intensity, crests per call and highest crest) could reflect a process of divergence or isolation between populations of *E. nitidus*. Therefore, our results could be important for future studies for species of the genus in Mexico with purposes of classification between species, since eleutherodactylids species display difficulties for its own identification, due to a high phenotypic variability and few morphologic differences within populations and among species (Crawford and Smith 2005; Grünwald et al. 2021).

The results of LDA showed that distinctiveness of *E. nitidus* populations are involved acoustic characteristics like dominant frequency, crests per call, call rate and call intensity. Our results agree with previous studies where the distinctions of populations are in part determined by dominant frequency, call rate and call duration (Bee et al. 2010; Serrano et al. 2020). Despite LDA was obtained using a no-corrected data by SVL and temperature, it is evident that most of distinctive characters among *E. nitidus* populations are not mainly influenced by body size and temperature. Eventually, our results of CV indicate that, variation among populations of *E. nitidus* depend principally on call duration, crests per call and call intensity, while LDA indicates that, distinctions are determined by different acoustic characteristics (including crests per call and call intensity) between populations. Furthermore, the coefficients of variance estimations indicate that for *E. nitidus*, call duration, dominant frequency and call intensity are considered as static variables at intra-population and intra-individual, whereas crests per call and the highest crest corresponded as dynamic. The CVip/CVi results displayed values higher than 1 in most of the populations, indicating that all acoustic variables present potential for an individual distinction.

According to our results, crests per call and the highest crest in the advertisement calls of *E. nitidus* are variables which are possibly related to abiotic environmental differences between populations of central Mexico. The populations of *E. nitidus* from Tepoztlán and Oaxaca are distributed mostly in open areas in contrast to populations in Puebla who inhabit in urban settlements (Canseco and Gutiérrez-Mayén 2010; Grünwald et al. 2018; Gómez-Benitez et al. 2021). In previous studies (overall in birds), the influence of environment on sound propagation has been reported (Wiley 2009; Liptai et al. 2015), where the structural characteristics of environments could positively influence and improve such propagation (e.g., Bowman 1979; Gish and Morton 1981; Velásquez et al. 2018). Therefore, we suggest that differences between crests per call and the highest crest of the advertisement calls of *E. nitidus* in populations of Puebla, Tepoztlán and Oaxaca could represent an acoustic adaptation for call propagation to closed or open areas with distinct environmental conditions.

Geographic variation between species can be influenced by different factors such as natural and sexual selection, and genetic drift (Wilczynski and Ryan 1999; Bernal et al. 2005; Velásquez 2014). Acoustic signals could work as indicators of genetic distance between populations (Velásquez et al. 2013), due to the genetic structure could facilitate faster changes in the structure of the signal, and therefore its divergence (Wilkins et al. 2013). Thus, the inconsistency between the geographical variation of the advertisement calls of *E. nitidus*, independent of the relationship between acoustic and temporal characters such as SVL and temperature, could be explained through an evolutionary scenario, where genetic drift plays a fundamental role. Additionally, as *E. nitidus* is a species with wide distribution in central Mexico, it is possible that populations in Tepoztlán and Oaxaca are located in contact areas with populations of *Eleutherodactylus* *maurus* and *Eleutherodactylus pipilans*, respectively (Lemos-Espinal and Smith 2020; Mata-Silva et al. 2021). Therefore, the divergence of these populations is not only attach to distance or genetic drift, but also probably to the interference of other eleutherodactylids, where *E. nitidus* has a pressure to differentiate its advertisement calls among calls of different closely related species.

9. Conclusion

Our results show that temperature rather than body size has a relevant influence on the acoustic characteristics of advertisement calls of *E. nitidus*. Our study suggests that, whether a considerable distance separating populations, there will be more discrepancy between calls due to divergence processes, for example, the closest populations within Puebla show less differences than populations of Tepoztlán and Oaxaca. Despite the resilience of *E. nitidus* to highly urbanized habitats, genetic drift and interaction with other species may be gradually contributing to the observed variation in acoustic characteristics. Additionally, the results displayed important characters that allow to visualize the differences between populations according to the acoustic characteristics, where call duration, crests per call and call intensity are important variables which allow a greater discrepancy for the distinction between populations. However, although interspecific acoustic dissimilarities within populations, individuals remain as a unit and not as different species. Our results represent a contribution to studies of the genus *Eleutherodactylus* and the first acoustic study addressed on *E. nitidus*, in addition to providing evidence to facilitate its identification through its advertisement calls. Despite the variation in advertisement calls among populations of *E. nitidus* in this study, we suggest that this evidence will be reinforced with molecular studies, as well as evaluating the influence of environment on advertisement calls mainly to sound propagation and species recognition through acoustic characteristics, to corroborate its acoustic and morphological differences and assess the importance of geographic variation between populations.

10. References

Alrawashdeh MJ, Radwan T. 2017. Wilk's lambda based on robust method. AIP Conf Proc. 1842(1): 030032.

AmphibiaWeb. 2022- . University of California, Berkeley, CA (USA). [updated 2022 Jan; accessed 2022 Apr 19]. https://amphibiaweb.org/

Arriaga-Jaramillo FG, Cuellar-Valencia OM, García-Gómez I, Ceballos-Castro I, Bolívar-García W, Velásquez-Trujillo DA, Ortiz-Baez AS, Ospina-Sarria JJ. 2021. Acoustic segregation of five sympatric and syntopic species of genus *Pristimantis* (Anura: Strabomantidae) from Western Colombia. Stud Neotrop Fauna Environ. 1–8.

Asquith A, Altig R, Zimba P. 1988. Geographic variation in the mating call of the green treefrog *Hyla cinerea*. Am Midl Nat. 119(1): 101–110.

Aubin T, Mathevon N, Staszewski V, Boulinier T. 2007. Acoustic communication in the Kittiwake *Rissa tridactyla*: potential cues for sexual and individual signatures in long calls. Polar Biol. 30: 1027-1033.

Augusto-Alves G, Dena S, Toledo LF. 2021. Acoustic allometry, background stream noise and its relationship with large-bodied and voiceless rheophilic frogs. Zool Anz. 295: 156-162.

Baraquet M, Salas NE, Di Tada IE. 2007. Variación geográfica en el canto de advertencia de *Hypsiboas pulchellus* (Anura, Hylidae) en Argentina [Geographic variation in the advertisement call of *Hypsiboas pulchellus* (Anura, Hylidae) in Argentina]. Rev Esp Herp. 21: 107-118. Spanish.

Bee MA, Cook JM, Love EK, O'Bryan LR, Pettitt BA, Schrode K, Vélez A. 2010. Assessing acoustic signal variability and the potential for sexual selection and social recognition in boreal chorus frogs (*Pseudacris maculata*). Ethology. 116(6): 564–576.

Bernal XE, Guarnizo C, Lüddecke H. 2005. Geographic variation in advertisement call and genetic structure of *Colostethus palmatus* (Anura, Dendrobatidae) from the Colombian Andes. Herpetologica. 61(4): 395-408.

Boughman JW. 2002. How sensory drive can promote speciation. Trends Ecol Evol. 17(12): 571-577.

Bowman RI. 1979. Adaptive morphology of song dialects in Darwin's finches. J Ornithol. 120: 353-389.

Brenowitz EA. 1986. Environmental influences on acoustic and electric animal communication. Brain Behav Evol. 28(1-3): 32-42.

Canseco L, Gutiérrez-Mayén MG. 2010. Anfibios y reptiles del Valle de Tehuacán-Cuicatlán. 1st ed. México (CDMX): Comisión Nacional para el Conocimiento y Uso de la Biodiversidad.

Castellano S, Cuatto B, Rinella R, Rosso A, Giacoma C. 2002. The advertisement call of the European treefrogs (*Hyla arborea*): a multilevel study of variation. Ethology. 108(1): 75-89.

Castellano S, Giocoma C. 1998. Stabilizing and directional female choice for male calls in the European green toad. Anim Behav. 56(2): 275-287.

Castellano S, Giacoma C, Dujsebayeva T. 2000. Morphometric and advertisement call geographic variation in polyploid green toads. Biol J Linn. Soc. 70(2): 341-360.

Chek AA, Bogart JP, Lougheed SC. 2003. Mating signal partitioning in multi-species assemblages: a null model test using frogs. Ecol Lett. 6(3): 235-247.

Crawford AJ, Smith EN. 2005. Cenozoic biogeography and evolution in direct-developing frogs of Central America (Leptodactylidae: *Eleutherodactylus*) as inferred from a phylogenetic analysis of nuclear and mitochondrial genes. Mol Phylogenet Evol. 35(3): 536–555.

Crawley MJ. 2013. The R Book. 2nd ed. UK: John Wiley & Sons, Ltd.

Cruz-Elizalde R, Pineda-López R, Ramírez-Bautista A, Berriozabal-Islas C. 2022. Diversity and composition of anuran communities in transformed landscapes in central Mexico. Community Ecol. 23: 103-114.

Cunnington GM, Fahrig L. 2010. Plasticity in the vocalizations of anurans in response to traffic noise. Acta Oecol. 36(5): 463-470.

Endler JA, Basolo AL. 1998. Sensory ecology, receiver, biases and sexual selection. Trends Ecol Evol. 13(10): 415-420.

Friedl TWP, Klump GM. 2002. The vocal behaviour of male European treefrogs (*Hyla arborea*): implications for inter- and intrasexual selection. Behaviour. 139(1), 113–136.

Frost DR. 2022- . Amphibian Species of the World: An Online Reference. Version 6.1. New York (USA): American Museum of Natural History. [updated 2022 Jan; accessed 2022 Feb 03]. Electronic Database accessible at https://amphibiansoftheworld.amnh.org/index.php.

Galvis PA, Zaffaroni-Caorsi V, Sánchez-Pacheco SJ, Rada M. 2018. The advertisement calls of three *Eleutherodactylus* species from Hispaniola (Anura: Eleutherodactylidae). Bioacoustics. 27(1): 1–12.

Gambale PG, Bastos RP. 2014. Vocal repertoire and bioacoustic analyses in *Physalaemus cuvieri* (Anura, Leptodactylidae) from southern Brazil. Herpetol J. 24(1): 31-39.

Gambale PG, Signorelli L, Bastos RP. 2014. Individual variation in the advertisement calls of a Neotropical treefrog (*Scinax constrictus*). Amphib-reptil. 35(3): 271-281.

García-Vázquez UO, Trujano-Ortega M. 2012. Nuevos registros de *Eleutherodactylus nitidus* (Anura: Eleutherodactylidae) en Tlaxcala y centro de Puebla, México [New records of *Eleutherodactylus nitidus* (Anura: Eleutherodactylidae) in Tlaxcala and central Puebla, Mexico]. Rev Mex Biodivers. 83(3): 856-858. Spanish.

GBIF.org. 2022- . GBIF Occurrence Download. [accessed 2022 Mar 18]. https://doi.org/10.15468/dl.z8gtkm.

Gerhardt HC. 1975. Sound pressure levels and radiation patterns of the vocalizations of some North American frogs and toads. J Comp Physiol. 102: 1-12.

Gerhardt HC. 1982. Sound pattern recognition in some North American treefrogs (Anura: Hylidae): implications for mate choice. Am Zool. 22(3): 581-595.

Gerhardt HC. 1991. Female mate choice in treefrogs: static and dynamic acoustic criteria. Anim Behav. 42(4): 615-635.

Gerhardt HC. 1992. Multiple messages in acoustic signals. Seminars in Neuroscience. 4(6): 391-400.

Gerhardt HC. 1994. The evolution of vocalization in frogs and toads. Annu Rev Ecol Syst. 25: 293-324.

Giasson LOM, Haddad CFB. 2006. Social interactions in *Hypsiboas albomarginatus* (Anura: Hylidae) and the significance of acoustic and visual signals. J Herpetol. 40(2): 171–180.

Gingras B, Boeckle M, Herbst CT, Fitch WT. 2012. Call acoustics reflect body size across four clades of anurans. J Zool. 289(2): 143-150.

Gish SL, Morton ES. 1981. Structural adaptations to local habitat acoustics in Carolina Wren songs. Z Tierpsychol. 56(1): 74-84.

Gómez-Benitez A, Gribbins KM, Granados-González G, Canseco-Márquez L, Hernández-Gallegos O. 2021. *Eleutherodactylus nitidus*. Habitat and nesting. Herpetol Rev. 52(2): 370-371.

Green S, Marler P. 1979. The Analysis of Animal Communication. In: Marler P, Vandenbergh JG, editors. Social Behavior and Communication. Boston (MA): Springer; p. 73-158.

Grenat PR, Valetti JA, Martino AL. 2013. Intra-specific variation in advertisement call of *Odontophrynus cordobae* (Anura, Cycloramphidae): a multilevel and multifactor analysis. Amphib-reptil. 34(4): 471-482.

Gridi-Papp M, Narins PM. 2009. Environmental influences in the evolution of tetrapod hearing sensitivity and middle ear tuning. Integr Comp Biol. 49(6): 702-716.

Grünwald CI, Reyes-Velasco J, Franz-Chávez H, Morales-Flores KI, Ahumada-Carrillo IT, Jones JM, Boissinot S. 2018. Six new species of *Eleutherodactylus* (Anura: Eleutherodactylidae: subgenus *Syrrhophus*) from Mexico, with a discussion of their systematic relationships and their validity of related species. Mesoam Herpetol. 5(1): 6-83.

Grünwald CI, Reyes-Velasco J, Franz-Chávez H, Morales-Flores KI, Ahumada-Carrillo IT, Rodríguez CM, Jones JM. 2021. Two new species of *Eleutherodactylus* (Anura: Eleutherodactylidae) from Southern Mexico, with comments on the taxonomy of related species and their advertisement calls. Amphib Reptile Conserv. 15(1) [Taxonomy section]: 1-35. e272.

Hernández-Austria R, García-Vázquez UO, Grünwald CI, Parra-Olea G. 2022. Molecular phylogeny of the subgenus *Syrrhophus* (Amphibia: Anura: Eleutherodactylidae), with the description of a new species from Eastern Mexico. Syst Biodivers. 20(1): 1-20.

Heyer WR, García-Lopez JM, Cardoso AJ. 1996. Advertisement call variation in the *Leptodactylus mystaceus* species complex (Amphibia: Leptodactylidae) with a description of a new sibling species. Amphib-reptil. 17(1): 7-31.

Höbel G. 2005. On the acoustic communication system of *Eleutherodactylus fitzingeri* (Anura: Leptodactylidae). Herpetol Rev. 36(3), 242-244.

Hunter P. 2007. The human impact on biological diversity. How species adapt to urban challenges sheds light on evolution and provides clues about conservation. EMBO Rep. 8(4): 316-318.

James G, Witten D, Hastie T, Tibshirani R. 2013. An introduction to statistical learning: with applications in R. New York (NY): Springer.

James LS, Tylor RC, Hunter KL, Ryan MJ. 2022. Evolutionary and allometric insights into anuran auditory sensitivity and morphology. Brain Behav Evol 97(3-4): 140-150.

Kaiser K, Scofield DG, Alloush M, Jones RM, Marczak S, Martineau K, Oliva MA, Narins PM. (2011). When sounds collide: the effect of anthropogenic noise on a breeding assemblage of frogs in Belize, Central America. Behaviour, 148(2): 215–232.

Kowarik I. 2011. Novel urban ecosystems, biodiversity, and conservation. Environ Pollut. 159(8-9): 1974-1983.

Laugen AT, Laurila A, Merilä J. 2002. Maternal and genetic contributions to geographical variation in *Rana temporaria* larval life-history traits. Biol J Linn Soc. 76(1): 61-70.

Lemos-Espinal JA, Smith GR. 2020. A conservation checklist of the herpetofauna of Morelos, with comparisons with adjoining states. Zookeys, 941: 121-144.

Lingnau R, Bastos RP. 2007. Vocalizations of the Brazilian torrent frog *Hylodes heyeri* (Anura: Hylodidae): repertoire and influence of air temperature on advertisement call variation. J Nat Hist. 41(17-20): 1227–1235.

Liptai P, Badida M, Lukáčová K. 2015. Influence of atmospheric conditions on sound propagation - mathematical modeling. Óbuda University e-Bulletin. 5(1): 127-134.

Lisa Yang Center for Conservation Bioacoustics. 2022. Raven Pro: Interactive Sound Analysis Software. Ithaca, NY: The Cornell Lab of Ornithology [Computer software]. Version 1.6, [accessed 2021 Jul 11]. https://ravensoundsoftware.com/.

Márquez R, Bosch J. 1995. Advertisement calls of the midwife toads *Alytes* (Amphibia, Anura, Discoglossidae) in continental Spain. J Zoolog Syst Evol Res. 33(3-4):185–192.

Mata-Silva V, García-Padilla E, Rocha A, Desantis DL, Johnson JD, Ramírez-Bautista A, Wilson LD. 2021. A Reexamination of the Herpetofauna of Oaxaca, Mexico: composition update, physiographic distribution, and conservation commentary. Zootaxa, 4996(2): 201-252.

Naes T, Mevik BH. 2001. Understanding the collinearity problem in regression and discriminant analysis. J Chemometr. 15:413-426.

Nuñez JJ, Rabanal FE, Formas JR. 2012. Description of a new species of *Eupsophus* (Amphibia: Neobatrachia) from the Valdivian Coastal range, Southern Chile: an integrative taxonomic approach. Zootaxa. 3305(1:9): 53-68.

McClelland BE, Wilczynski W, Ryan MJ. 1996. Correlations between call characteristics and morphology in male cricket frogs (*Acris crepitans*). J Exp Biol. 199(9): 1907-1919.

O'Neill EM, Beard KH. 2011. Clinal variation in calls of native and introduced populations of *Eleutherodactylus coqui*. Copeia. 2011(1): 18–28.

Padial JM, Köhler J, Muñoz A, De la Riva I. 2008. Assessing the taxonomic status of tropical frogs through bioacoustics: geographical variation in the advertisement calls in the *Eleutherodactylus discoidalis* species group (Anura). Zool J Linn Soc. 152(2): 353-365.

Palacios-Aguilar R. 2018. Notas sobre el sitio de anidamiento de la rana *Eleutherodactylus nitidus* (anura, Eleutherodactylidae) en Guerrero, México [Notes on the nesting site of the frog *Eleutherodactylus nitidus* (Anura, Eleutherodactylidae) in Guerrero, Mexico]. Rev Latinoamer Herp. 1(2): 51-53. Spanish.

Peterson BG, Carl P, Boudt K, Bennett R, Ulrich J, Zivot E, Cornilly D, Hung E, Lestel M, Balkissoon K, et al. 2020. PerformanceAnalytics: Econometric tools for performance and risk analysis [R package]. Version 2.0.4. [accessed 2022 Sep 21] https://CRAN.R-project.org/package=PerformanceAnalytics.

Platz JE, Forester DC. 1988. Geographic variation in mating call among the four subspecies of the chorus frog: *Pseudacris triseriata* (Wied). Copeia. 1988(4):1062–1066.

Pröhl H, Hagemann S, Karsch J, Höbel G. 2007. Geographic variation in male sexual signals in strawberry poison frogs (*Dendrobates pumilio*). Ethology. 113(9): 825-837.

Pröhl H, Koshy RA, Mueller U, Rand AS, Ryan MJ. 2006. Geographic variation of genetic and behavioral traits in northern and southern Túngara frogs. Evolution. 60(8): 1669-1679.

QGIS.org, 2022. QGIS Geographic Information System. QGIS Association [Computer program]. Version 3.16.14, [accessed 2022 Aug 09]. http://www.qgis.org/.

Rand AS. 1985. Tradeoffs in the evolution of frog calls. Proceedings: Animal Sciences. 94: 623-637.

Reyes-Velasco J, Ahumada-Carrillo I, Burkhardt TR, Devitt TJ. 2015. Two new species of *Eleutherodactylus* (subgenus *Syrrhophus*) from western Mexico. Zootaxa. 3914(3): 1-17.

Rodríguez A, De la Nuez D, Alonso R. 2010. Intraspecific variation in the advertisement call of the cloud-forest frog *Eleutherodactylus glamyrus* (Anura: Eleutherodactylidae). J Herpetol. 44(3): 457-466.

Rodríguez-Tejeda RE, Méndez-Cárdenas MG, Islas-Villanueva V, Macías-Garcia C. 2014. Geographic variation in the advertisement calls of *Hyla eximia* and its possible explanations. PeerJ 2:e420. 1-29.

Roever C, Raabe N, Luebke K, Ligges U, Szepannek G, Zentgraf M, Meyer D. 2022. klaR: Classification and visualization [R package]. Version 1.7-1. [accessed 2022 Sep 21]. https://CRAN.R-project.org/package=klaR.

RStudio Team. 2015. RStudio: Integrated Development Environment for R. RStudio, Inc., Boston. [Computer program]. Version 4.1.2, [accessed 2022 Aug 20]. http://www.rstudio.com/

Ryan MJ. 1988. Constraints and patterns in the evolution of anuran acoustic communication. In: Fritzsch B, Ryan MJ, Wilczynski W, Hetherington TE, Walkowiak W, editors. The evolution of the amphibian auditory system. New York(NY): Willey-Interscience; p. 637-677.

Serrano JM. 2016. El canto de anuncio de la rana endémica del Pedregal de la ciudad de México [The advertisement call of the endemic frog from the Pedregal in Mexico City]. Rev Mex Biodivers. 87(2): 535-539. Spanish.

Serrano JM, Penna M. 2018. Sexual monomorphism in the advertisement calls of a Neotropical frog. Biol J Linn Soc. 123(2): 388-401.

Serrano JM, Penna M, Soto-Azat C. 2020. Individual and population variation of linear and non-linear components of the advertisement call of Darwin's frog (*Rhinoderma darwinii*). Bioacoustics. 29(5): 572-589.

Sueur J, Aubin T, Simonis C. 2008. Seewave, a free modular tool for sound analysis and synthesis. Bioacoustics. 18(2): 213–226.

Tsuji-Nishikido BM, Kaefer IL, de Freitas FC, Menin M, Lima AP. 2012. Significant but not diagnostic: differentiation through morphology and calls in the Amazonian frogs *Allobates nidicola* and *A. masniger*. Herpetol J. 22(2): 105-114.

Velásquez NA. 2014. Geographic variation in acoustic communication in anurans and its neuroethological implications. J Physiol Paris. 108(2-3): 167-173.

Velásquez NA, Opazo D, Díaz J, Penna M. 2014. Divergence of acoustic signals in a widely distributed frog: relevance of inter-male interactions. PLoS One. 9(1) e87732: 1-9.

Velásquez NA, Marambio J, Brunetti E, Méndez MA, Vásquez RA, Penna M. 2013. Bioacoustic and genetic divergence in a frog with a wide geographical distribution. Biol J Linn Soc. 110(1): 142-155.

Velásquez NA, Moreno-Gómez FN, Brunetti E, Penna M. 2018. The acoustic adaptation hypothesis in a widely distributed South American frog: Southernmost signals propagate better. Sci Rep. 8(6990): 1-12.

Wang J, Cui J, Shi H, Brauth SE, Tang Y. 2012. Effects of body size and environmental factors on the acoustic structure and temporal rhythm of calls in *Rhacophorus dennysi*. Asian Herpetol Res. 3(3): 205-212.

Warwick AR, Travis J, Lemmon EM. 2015. Geographic variation in the Pine Barrens treefrog (*Hyla andersonii*): concordance of genetic, morphometric and acoustic signal data. Mol Ecol. 24(13): 3281–3298.

Wells KD, Schwartz JJ. 2007. The Behavioral Ecology of Anuran Communication. In: Narins PM, Feng AS, Fay RR, Popper AN, editors. Hearing and Sound Communication in Amphibians. New York(NY): Springer; p. 44-86.

Wickham H. 2016. ggplot2: Elegant graphics for data analysis. 2nd ed. New York (NY): Springer.

Wickham H, Chang W, Henry L, Pedersen TL, Takahashi K, Wilke C, Woo K, Yutani H, Dunnington D, RStudio. 2022. ggplot2: Create elegant data visualisations using the grammar of graphics [R package] Version 3.3.6. [accessed 2022 Sep 21] https://CRAN.R-project.org/package=ggplot2.

Wilczynski W, Ryan MJ. 1999. Geographic variation in animal communication systems. In: Foster SA, Endler JA, editors. Geographic variation in behavior: perspectives on evolutionary mechanisms. [place unknown]: Oxford University Press; p. 234-261.

Wiley RH. 2009. Signal transmission in natural environments. New Encycl Neurosci. 8: 827-832.

Wilkins MR, Seddon N, Safran RJ. 2013. Evolutionary divergence in acoustic signals: causes and consequences. Trends Ecol Evol. 28(3): 156-166.

Wollerman L. 1998. Stabilizing and directional preferences of female *Hyla ebraccata* for calls differing in static properties. Anim Behav. 55(6): 1619-1630.

Appendix 1. Details of call acoustic intensity measurements

We refer to the peaks of the amplitude modulations in oscillograms as crest (Figure 2). In addition to counting the number of these crests, we registered the amplitude of the most prominent crests by clicking on this to measure its RMS (root mean square) intensity level. We named such crest as the highest crest.

To measure call intensity, we calculated their standardized sound pressure level (SSPL) using the full-scale peak power density (PPD) of Raven software. For this purpose, the SPL of a standard recording was calibrated using a tone of 1 s duration and a frequency of 1000 Hz. This tone was launched on a Logitech speaker at 50 cm and using the same gain level of microphones in the recorders as in the field recordings. The SPL was registered for each microphone and used as reference to perform different subtractions from the PPD values for Sennheiser and Soundtrack Pro-Audio microphones (I and II, respectively).

$$SSPL\ FS = 93.9 - \left(-12.36 - (PPD)\right) \quad \text{(I)}$$

$$SSPL\ FS = 94.1 - \left(-20.8 - (PPD)\right) \quad \text{(II)}$$

The call rate was obtained from the time subtraction of the last call (LC) emitted in the recording respect to the first call (FC), consequently, the residue was divided by 60 in order to get the time value in minutes, as follows:

$$Call\ rate = \left[number\ of\ calls / \left[\frac{FC - LC}{60}\right]\right]$$

Appendix 2. Correlations between acoustic variables

Table 1. Pearson and Spearman correlation between acoustic variables from advertisement calls of *Eleutherodactylus nitidus* (Significance levels = * indicates $p < 0.05$, ** indicates $p < 0.01$). Abbreviations: Superscript "a" (indicates spearman correlation between variables highest crest and crests per call).

Variable	Call duration	Dominant frequency	Crests per call	Highest crest[a]	Call intensity	Call rate
Call duration (s)	-	-0.49**	0.26*	0.31*	-0.60**	0.03
Dominant frequency (Hz)	-0.49**	-	-0.12	-0.08	0.16	-0.08
Crests per call[a]	0.26*	-0.12	-	0.67**	-0.1	0.19
Highest crest	0.31*	-0.08	0.67**	-	-0.35**	0.15
Call intensity (SSPLFS)	-0.60**	0.16	-0.1	-0.35**	-	0.1
Call rate (calls/min)	0.03	-0.08	0.19	0.15	0.1	-

Appendix 3. Average and standard error of acoustic variables

Table 3. Acoustic variables of advertisement calls of *Eleutherodactylus nitidus* from Central Mexico. Average ± standard error.

Variables	Populations					
	Cholula (n= 13)	Tonantzintla (n= 16)	Los Ahuehuetes (n= 4)	Las Haras (n= 8)	Tepoztlán (n= 8)	Oaxaca (n= 17)
Call duration (s)	0.40 ± 0.01	0.38 ± 0.01	0.29 ± 0.03	0.30 ± 0.02	0.34 ± 0.01	0.28 ± 0.004
Dominant frequency (Hz)	2622.44 ± 34.35	2667.22 ± 27.77	2759.44 ± 33.81	2814.63 ± 33.67	2735.22 ± 56.56	2707.98 ± 17.35
Crests per call	8.27 ± 0.21	8.74 ± 0.39	8.76 ± 0.29	7.78 ± 0.33	11.50 ± 0.73	8.15 ± 0.29
Highest crest	6.76 ± 0.26	7.03 ± 0.24	7.51 ± 0.40	6.09 ± 0.40	8.75 ± 0.40	5.50 ± 0.25
Call intensity (SSPLFS)	89.67 ± 2.09	85.99 ± 0.75	95.26 ± 1.83	88.81 ± 1.35	91.87 ± 2.73	103.66 ± 0.37
Call rate (calls/min)	4.06 ± 0.26	4.52 ± 0.50	3.68 ± 0.91	3.67 ± 0.49	3.95 ± 0.23	4.40 ± 0.23