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THE EVOLUTION OF THE ADVERTISEMENT CALL IN GLASSFROGS (CENTROLENIDAE TAYLOR, 1951)

Porto Alegre
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Dissertação de Mestrado

THE EVOLUTION OF THE ADVERTISEMENT CALL IN GLASSFROGS (CENTROLENIDAE TAYLOR, 1951)

Moisés David Escalona Sulbarán
Orientador: Pedro Ivo Simões

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The evolution of the advertisement call in glassfrogs (Centrolenidae Taylor, 1951)

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DISSERTAÇÃO DE MESTRADO

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Dedico este trabajo a todos los venezolanos que murieron luchando por un "nuevo amanecer" en Venezuela durante los últimos años. Tengo la esperanza de que un día volvamos a tener una Venezuela linda, que pueda disfrutar junto a mi familia y ver a mis hijos crecer en su tierra. Estoy seguro de que muchos otros también mantienen la esperanza en ese sueño.

Dedico este trabalho a todos os venezuelanos que morreram lutando por um "novo amanhecer" na Venezuela nos últimos anos. Tenho a esperança de que um dia voltamos a ter uma Venezuela linda, que poda disfrutar junto a minha família e ver a meus filhos crescer na sua terra. Tenho a certeza de que muitos outros também mantem a esperança nesse sonho.

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RESUMO

Os anuros emitem cantos de anúncio com o objetivo de atrair parceiros e repelir concorrentes co-específicos. Eu investiguei a associação entre o comprimento do corpo, o sítio de vocalização, o cuidado parental e a variação em propriedades acústicas do canto (duração do canto, número de notas, freqüência de pico, largura de banda de freqüência e estrutura do canto) em pererecas-de-vidro—uma família de pererecas que habitam florestas neotropicais—controlando para efeitos de ancestralidade compartilhada. Eu também explorei o tempo e o modo de evolução dessas características e as comparei com as de três características morfológicas (comprimento do corpo, largura da cabeça e comprimento da tíbia). Eu gerei e compilei dados acústicos para 72 espécies de pererecas-de-vidro (46 % da riqueza descrita), incluindo representantes de todos os gêneros, e analisei os dados usando métodos comparativos filogenéticos. Descobri que quase todas as características acústicas avaliadas tinham sinal filogenético significativo, mas geralmente modesto. O pico de frequência dos cantos é significativamente associado ao comprimento do corpo, enquanto a estrutura dos cantos é significativamente associada ao sítio de vocalização e ao sexo do adulto que desempenha cuidado-parental. Meus resultados sustentam que, além das restrições filogenéticas, o tamanho do corpo está associado à evolução dos cantos de anúncio em Centrolenidae. A diversificação de características acústicas ao longo da filogenia de pererecas-de-vidro concorda com a radiação dos taxa na transição entre Mioceno e Plioceno. A disparidade estimada para características acústicas foi geralmente maior que a de características morfológicas, e o pico de disparidade de características acústicas geralmente ocorreu mais tarde na evolução do grupo, sugerindo um início historicamente recente da pronunciada divergência acústica entre espécies deste clado.

Palavras chaves: comunicação acustica; anfibios; método comparativo; Neotropico; disparidade fenotipica.

ABSTRACT

Anurans emit advertisement calls with the purpose of attracting mates and repelling conspecific competitors. I investigated the association between body size, calling site and parental care with call variables (call duration, number of notes, peak frequency, frequency bandwidth and call structure) in glassfrogs—a family of leaf-dwelling frogs which inhabit Neotropical forests—while controlling for effects of shared ancestry. I also explored the tempo and mode of evolution of these traits and compared them with those of three morphological traits (snout-vent length, and relative head width and tibia length). I generated and compiled acoustic data for 72 glassfrog species (46 % of described diversity), including representatives of all genera (n = 12), and analyzed the data using phylogenetic comparative methods. I found that almost all acoustic traits evaluated had significant, but generally modest phylogenetic signal. Peak frequency of calls is significantly associated with body-size, while call structure is significantly associated with calling site and with the sex of the caring parent. My results support that, besides phylogenetic constraints, body size is associated with the evolution of advertisement calls in glassfrogs. Diversification of acoustic traits along the phylogeny of glassfrogs is coincident with the radiation of extant taxa at the Miocene-Pliocene boundary. The estimated disparity of acoustic traits was larger than that of morphological traits and the peak in disparity of acoustic traits generally occurred later in the evolution of glassfrogs, suggesting a historically recent outset of the pronounced acoustic divergence in this clade.

Keywords: acoustic communication; amphibians; comparative method; Neotropics; phenotypic disparity.

1. INTRODUÇÃO GERAL

Os anuros emitem um tipo de sinal acústico com o objetivo de atrair fêmeas e repelir concorrentes co-específicos, o qual denomina-se de canto de anúncio (Wells, 2007). A variação deste tipo de sinal acústico é geralmente influenciada por pressões naturais, sexuais e deriva genética, determinando sua evolução (Wilkins *et al*. 2013). A evolução do canto do anúncio em um contexto comparativo (*i.e*., interespecífico) tem sido estudada desde meados do século passado (Crocoft & Ryan, 1995). Porém, até hoje, a evolução do canto de anúncio só foi investigada em quatro das 56 famílias de anuros atualmente reconhecidas (Erdtmann & Amézquita, 2009; Erdtmann 2012; Santos *et al.*, 2014; Goutte *et al*., 2016). Possivelmente, a falta de bancos de dados acústicos compreensivos para a maioria das famílias e a falta de filogenias estáveis e bem resolvidas foram os maiores impedimentos para testes de relações entre fatores ecológicos, anatômicos e variabilidade das propriedades acústicas dos cantos de anúncio em anuros.

Neste trabalho, eu estudei a evolução do canto de anúncio em pererecas-de-vidro (Centrolenidae), uma família de anuros neotropicais (Castroviejo-Fisher *et al*., 2014). Estas pererecas têm hábitos noturnos, territoriais, possuem cuidado parental e cantam a partir da superfície superior ou inferior de folhas na vegetação adjacente a ambientes lóticos (Señaris & Ayarzagüena, 2005; Kubicki, 2007; Cisneros-Heredia & McDiarmid, 2007; Guayasamin *et al*., 2009). Através da implementação de métodos comparativos filogenéticos (Felsenstein, 1985; Harvey & Pagel, 1991; Pagel, 1999; Paradis, 2014), eu avaliei o potencial papel que a ancestralidade compartilhada, além de fatores intrínsecos (comprimento do corpo, tipo de cuidado parental) e extrínsecos (sitio de vocalização) exerceram durante a evolução das características do canto do anúncio em Centrolenidae.

O produto de meu trabalho é apresentado no capítulo a seguir, como um manuscrito para artigo científico, formatado de acordo com as normas do periódico *Journal of Evolutionary Biology*.

2. CAPÍTULO 1. Manuscrito formatado para artigo

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The evolution of the advertisement call in Glassfrogs (Centrolenidae Taylor, 1951).

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Short running title: **Acoustic evolution in Glassfrogs**

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Abstract

Anurans emit advertisement calls with the purpose of attracting mates and repelling conspecific competitors. I investigated the association between body size, calling site and parental care with call variables (call duration, number of notes, peak frequency, frequency bandwidth and call structure) in glassfrogs—a family of leaf-dwelling frogs which inhabit Neotropical forests—while controlling for effects of shared ancestry. I also explored the tempo and mode of evolution of these traits and compared them with those of three morphological traits (snout-vent length, and relative head width and tibia length). I generated and compiled acoustic data for 72 glassfrog species (46 % of described diversity), including representatives of all genera (n = 12), and analyzed the data using phylogenetic comparative methods. I found that almost all acoustic traits evaluated had significant, but generally modest phylogenetic signal. Peak frequency of calls is significantly associated with body-size, while call structure is significantly associated with calling site and with the sex of the caring parent. My results support that, besides phylogenetic constraints, body size is associated with the evolution of advertisement calls in glassfrogs. Diversification of acoustic traits along the phylogeny of glassfrogs is coincident with the radiation of extant taxa at the Miocene-Pliocene boundary. The estimated disparity of acoustic traits was larger than that of morphological traits and the peak in disparity of acoustic traits generally occurred later in the evolution of glassfrogs, suggesting a historically recent outset of the pronounced acoustic divergence in this clade.

Keywords:

acoustic communication; amphibians; comparative method; Neotropics; phenotypic disparity

Introduction

Animal communication is an important behavioral feature that allows individuals to exchange information about their location, territory, condition, sex, size, and presence of potential predators (Duellman & Trueb, 1986; Barnard, 2004; Wells, 2007). There are several ways biological information can be transmitted, such as through visual, chemical, or acoustic signals. The latter are of crucial importance for anurans, considering their ubiquity among taxa and their role in long range communication, especially in visually-cluttered environments (Duellman & Trueb, 1986; Ryan, 1988).

In general, anurans have a simple vocal repertoire (Duellman & Trueb, 1986). Most breeding anuran males emit a kind of acoustic signal called advertisement call, which serves for attracting appropriate mates and repelling conspecific males (Wells, 1977). Such calls are genetically determined and highly stereotyped, playing an important role in species recognition and reproductive isolation (Duellman & Trueb, 1986; Wells, 2007; but see Köhler et al., 2017, pp. 39–42 for a recent review on intraspecific variation of advertisement calls). Because of their role in mate attraction, differentiation of these signals among populations may result in speciation in relatively short evolutionary time frames (Barnard, 2004; Vences & Wake, 2007).

Advertisement calls have time, frequency, and structural components (the first two are often referred to as temporal and spectral properties of the calls), which depend on multiple intrinsic and extrinsic factors. For example, one may expect that more closely related species should have more similar vocalizations due to the shared ancestry of the anatomy of sound-producing organs (Cocroft & Ryan 1995; Panhuis *et al.*, 2001). It is also known that some spectral properties depend on the size of the vocal apparatus. For example, the dominant frequency of vocalizations is usually negatively correlated with the body size of the emitter (Zweifel, 1968; Duellman & Trueb, 1986; Wells, 2007; Gingras *et al*., 2013a). Advertisement calls might also be adapted to the transmission environment, and call traits may be temporally and spectrally shaped in order to reduce distortion by attenuation and reverberation of sound signals by structural components of the species's native habitat (Morton, 1975; Kime *et al*., 2000; Ryan & Kime, 2003; Erdtmann & Lima, 2013). Hence, the causes of variation in acoustic signals among different frog species may include historical (i.e., phylogenetic), anatomical and ecological explanations.

Few studies have investigated the evolution of anuran advertisement calls in species-rich clades and at deep evolutionary time-scales. These kinds of studies rely on phylogenetic comparative methods (PCM), a number of analytical approaches that consider a set of species in a historical framework in order to test the evolutionary mechanisms involved in their diversification (Felsenstein, 1985; Harvey & Pagel, 1991; Pagel, 1999; Paradis, 2014). Although applicable in solving many evolutionary puzzles, obtaining meaningful results by applying these methods requires previous knowledge of the genealogical relationships among the species analyzed (i.e., a well-supported phylogeny) and a representative sample (i.e., in size and coverage) of the phenotypic traits of interest for the target taxon. These limitations have resulted in only a handful of empirical studies applying PCM to investigate the evolution of acoustic signals in anuran clades.

For instance, in a clade of poison-dart frogs (Dendrobatinae), pulse duration seems to be more associated with the phylogenetic relationships among species than the peak frequency of calls, which is more influenced by selective pressures acting on body size evolution (Erdtmann & Amézquita, 2009). When considering all clades of extant poison-dart frogs and their closest relatives (Superfamily Dendrobatoidea), the evolution of call traits is also apparently related to the occurrence of aposematic dorsal color patterns, with calls of brightly colored species diversifying at a faster rate than calls of cryptically colored species (Santos *et al.*, 2014). However, poison-dart frogs and their allies are primarily diurnal, and the evolutionary relationships between variation in call properties and morphological traits potentially evolving under natural selection (e.g., warning colors, body size) are not expected to be recovered across most of the extant anuran families, which comprise species that are vocally active during the night.

Among true frogs (Ranidae), variation in advertisement call traits (i.e., dominant frequency, frequency bandwidth, frequency modulation and note duration) is probably constrained by acoustic specificities of noisy torrent habitats, in addition to associations between some of these traits with interspecific variation in body size (Goutte *et al.*, 2016, 2017). Jointly, these studies encompassed three of the 56 currently recognized anuran families and less than 10 % of the currently recognized anuran species diversity (Frost, 2018). Certainly, the paucity of empirical studies hampers our understanding of patterns in the evolution of advertisement calls in anurans.

Glassfrogs (Centrolenidae) are charismatic Neotropical anurans characterized by having partially or completely transparent ventral skin (Guayasamin *et al.*, 2009; Castroviejo-Fisher *et al.*, 2014). They constitute a clade of 155 species, distributed in humid forests from Mexico to Bolivia, along the Andes and across the Amazon, with vicariant species distributed in Tobago Island and in the Brazilian Atlantic Forest (Castroviejo-Fisher *et al.*, 2014; Frost, 2018).

The phylogenetic relationships among glassfrog species are relatively well explored and stable (Fig. 1), with the most complete phylogeny to date including more than 73 % of the described species diversity (Delia *et al.*, 2017). Castroviejo-Fisher *et al.* (2014) inferred that

Centrolenidae started to diversify in the Miocene in South America, with 11 independent dispersals into Central America. Within South America, a mixture pattern of vicariant and dispersal events among biogeographic areas explains the current species distribution, with the Northern Andes as the main dispersal source. The diversification of glassfrogs followed a constantly increasing diversity model and it was further suggested that the success of some centrolenid clades in spreading across Neotropical rain forests might be related to the presence of altitudinal generalist species (Castroviejo-Fisher *et al*., 2014).

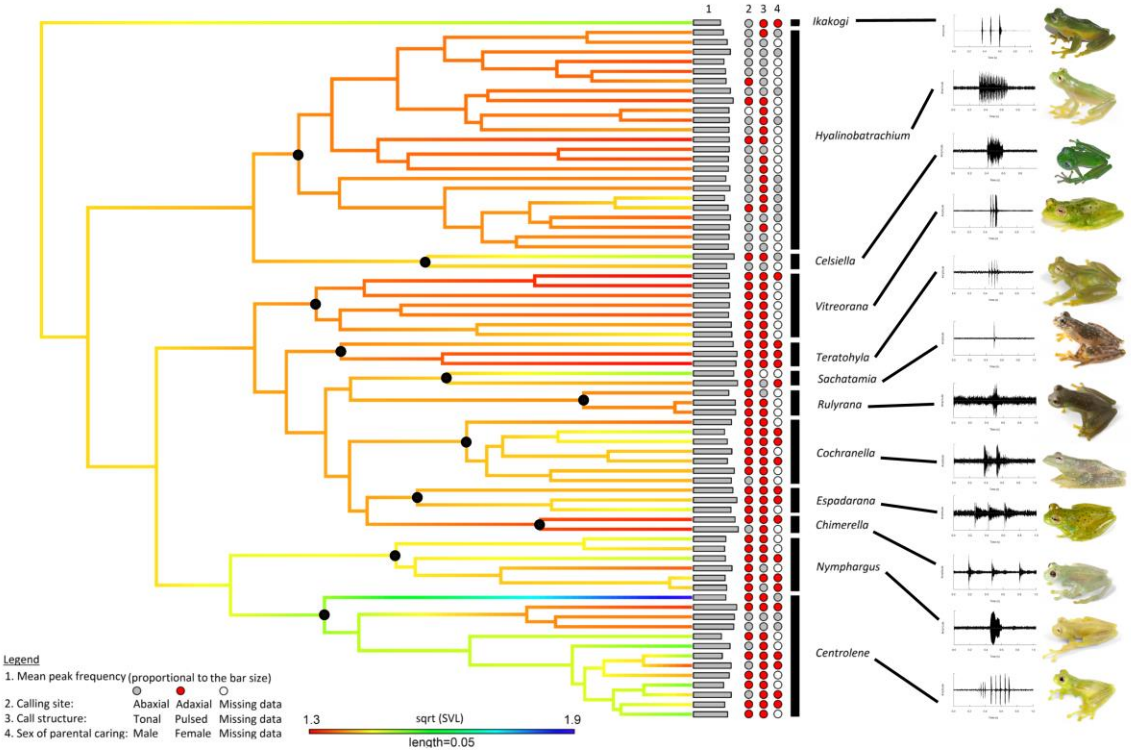

Figure 1. Maximum clade credibility tree of Centrolenidae modified from Delia *et al.* (2017). Only the species included in this study (n = 72) are represented as terminals. Black dots on nodes represent the ancestral node for Centrolenidae genera. Internal branch colours represent ML reconstructions of body size ($\log_{10}$ of snout-vent length in mm), while terminal branch colours represent the average body size of each species. Mean peak frequency, calling site, call structure and sex of parental caring is shown at tips. Graphs and photographs represent call waveforms (*x*-axis = 1 ms) and dorsolateral views of representative species in Centrolenidae (from top to bottom): *Ikakogi tayrona*; *Hyalinobatrachium chirripoi*; *Celsiella vozmedianoi*; *Vitreorana ritae*; *Teratohyla spinosa*; *Sachatamia albomaculata*; *Rulyrana flavopunctata*; *Cochranella mache*; *Espadarana*

prosoblepon; *Chimerella mariaelenae*; *Nymphargus griffithsi*; and *Centrolene ballux*. Photos: Santiago R. Ron (Anfibios del Ecuador; http://www.bioweb.bio/faunaweb/amphibiaweb/), except *I. tayrona* (Marco Rada), and *C. vozmedianoi* (Juan Guayasamin).

Glassfrogs have arboreal and nocturnal habits, being found near lotic environments. Males of most species vocalize while perched on vegetation overhanging streams, except some *Centrolene*, *Rulyrana* and *Sachatamia* species (roughly 10 spp.) that call from rocks near water (Guayasamin *et al*., 2009). When calling from vegetation, males assume a calling position on the upper (adaxial) or lower (abaxial) surface of a leaf, and calling position is generally fixed within each glassfrog species (Cisneros-Heredia & McDiarmid, 2007; Guayasamin *et al*., 2009). Male glassfrogs are territorial and, during reproduction, egg clutches are deposited by females near the male's calling site. Eggs are attended by one or both parents, for a period of time that can vary from a few hours to the hatching of tadpoles, depending on the species (Delia *et al*., 2017). Calling and attending egg clutches are both energetically costly in anurans (Prestwich, 1994; Townsend, 1986; Crump, 1996) and the amount of energy necessary to produce advertisement calls in anurans often relates to temporal traits such as the number of notes or pulses emitted at a given time interval (Gerhardt & Huber, 2002). However, no associations were ever investigated between call traits and investment in parental care by male anurans.

The combination of a widely distributed, species-rich clade, with a well-resolved phylogeny, and a relatively good knowledge of the diversity of advertisement calls—to date, the calls of 48 % of glassfrog species have been quantitatively described—makes centrolenids an ideal model to study the relative effect that historical and ecological factors might have had on the evolution of acoustic signals. Using PCM, I herein evaluate the power of historical (phylogeny), intrinsic (body size, exclusively male parental care) and extrinsic (calling site) factors to explain the evolution of advertisement call traits. Based on current knowledge on advertisement call evolution in anurans, I devise five predictions on how call traits and intrinsic and extrinsic explanatory variables potentially relate to each other.

First, variation in spectral traits of calls is generally associated with body size because larger frogs have a larger sound-emitting apparatus than smaller frogs (Duellman & Trueb, 1986). Hence, I predict that peak frequency of calls will be higher in smaller-sized glassfrog species, independently of their shared ancestry.

Second, calling position (adaxial or abaxial leaf surfaces and streamside rocks) is conserved within species of glassfrogs, and several nocturnal anurans are known to vocalize from positions that enhance the transmission of information contained in acoustic signals (Hödl, 1977; Kime *et al*., 2000; Muñoz & Penna, 2016). Thus, I predict that similar calling positions led to the evolution of similar advertisement calls (in temporal, spectral, and structural traits) because of akin selective pressures on signal transmission efficiency.

Third, glassfrog species with males providing parental care invest more time brooding than species with maternal care (Delia *et al*., 2017). I predict that species with males providing parental care should emit simpler (i.e., of one note and tonal) and shorter calls than species with maternal care, as an energy trade-off between calling and brooding.

Fourth, the historical biogeography of centrolenids coupled with large transformations of tropical America environments and landscapes (Castroviejo-Fisher *et al*., 2014; Hoorn & Wesselingh, 2010) suggests that the intensity of environmental selection may have varied though their evolutionary history, either by dispersal into new regions or by modifications of already inhabited areas. Hence, I expect acoustic diversification in glassfrogs was not constant through the group’s diversification, and I predict that major shifts in the disparity of acoustic traits may be coincident with dispersal events into new ecological regions (e.g., Central America, after the formation of the isthmus of Panama, and the Amazonian plains, after the establishment of the Amazon River drainage) and/or major geological events that transformed the landscape.

Fifth, the tempo and mode of evolution of acoustic traits in glassfrogs differ from that of non-sexually selected (i.e., ecology-related) traits because traits directly involved in mate choice are often under directional selection, while ecology-related traits are more likely to be experiencing stabilizing selection (Ridley, 2004; Gonzalez-Voyer & Kolm, 2011). I predict that acoustic traits will show a different tempo and mode of evolution than ecology-related traits (i.e., head width, length of limbs).

Materials and methods

Taxon sampling and acoustic data

I analysed a total of 830 advertisement calls from 67 species, plus a putative undescribed species (*Hyalinobatrachium* aff. *bergeri*), compiled from audio recordings gathered from personal and museum collections (origin of recordings, authorship, and other relevant information are detailed in Dataset S1). This covered 48 % of the species richness in Centrolenidae, including representatives of all genera. I analysed calls of as many individuals as possible for each species (mean = 3 individuals; S.D. = 2.5; range = 1–12 individuals) to obtain a good estimate of the central tendency statistics for call properties of each species. I analysed five calls per individual and used the average value for each call property measured among all individuals of each species in all analyses.

I measured temporal and spectral call traits from oscillograms and power spectra, respectively. I estimated power spectra using a Fast Fourier Transform (FFT) analysis with a Blackman window of 5 ms of size, 80 % of overlap in time grid and a DFT size of 1024 samples in frequency grid. I carried out all bioacoustic analyses using RavenPro v. 1.4 (Bioacoustics Research Program, 2011).

Acoustic trait definition and terminology followed Köhler *et al*. (2017). I measured four acoustic traits: call duration (CD), measured as the time elapsed between the beginning and the end of the call as viewed in the oscillogram; number of notes (NN), measured as the number of tonal notes or pulse groups that are temporally separated from other notes or pulse groups by large silent intervals; peak frequency (PF), measured as the frequency emitted with the highest energy; and bandwidth (BW), which is the difference between the upper and lower frequency bounds of the notes, as measured 20 dB below the peak frequency. The - 20 dB threshold was adopted in order to avoid considering frequencies originated from background noise in the analyses. Additionally, I categorized the call structure (CS) as pulsed (when at least one note presented sequential 100 % amplitude modulation between pulses) or tonal (when no sequential amplitude modulation was present within any note that formed the advertisement call).

Other call variables commonly used in bioacoustics (e.g., number of harmonics in a note, duration of individual notes in a call and duration of the silent intervals between notes) were not considered because of the impossibility of verifying the homology of different call elements (individual notes or intervals) among all species. The display of note harmonics on audiospectrograms or power spectra are also strongly affected by low signal-to-noise ratio. Hence, these elements were more or less detectable as a result of the intensity of background environmental noise captured in each sound recording.

I compiled data on the same call traits (except for BW) from the literature for 10 glassfrog species for which I could not access original recordings (Dataset S2). Hence, call traits were obtained for a total 78 glassfrog terminals (species plus the putative undescribed species). Nevertheless, only 72 of those terminals are represented in the phylogenetic tree considered in the PCM analyses (see below).

In one species, *Centrolene lynchi*, three different calls were found among recordings, associated to three different localities in Pichincha department, Ecuador: (1) “3.5 km NE Mindo, 1541 m a.s.l.” (voucher: KU 164710; sound record: Fonozoo 194-8176); (2) “Reserva Las Gralarias, Lucy’s creek” (voucher: unknown; sound records: LS00018, 00020, 00029, 00031, and ASL013; source: Ana Salgado); (3) “Tandayapa, 1.0 km SW of on Mindo road, 1640 m a.s.l.” (voucher: RWM 12178; sound record: 201486_44k_C_lynchi; source: Macaulay Library). The three localities are approximately at the same distance (~ 37 km) from *Centrolene lynchi*’s type locality in Pichincha (00°21' S, 78°54' W; 1140 m a.s.l.), Ecuador, where no sound recordings were available. For this reason, I kept only calls corresponding to specimens included in the molecular phylogeny, which were obtained from Reserva Las Gralarias (Castroviejo-Fisher *et al*., 2014).

Morphological, behavioral and ecological data

I obtained morphometric data for 67 glassfrog species from the literature, and for 11 species from fellow researchers (Dataset S3). I selected three morphometric traits of biological relevance in anurans, which were shared among all references, to compare their evolution with acoustic traits: i) snout-vent length (SVL), the most common descriptor of body size in anuran bioacoustics studies (Köhler *et al*., 2017), ii) head width (HW), which is related to feeding habits, as it limits maximum prey size (Toft, 1980, 1981; Emerson, 1985), and iii) tibia length (TL), a hind-limb's measurement, which is related to jumping performance (Emerson, 1978, 1991; Zug, 1978). I considered the average values of SVL and the average values of the ratios HW/SVL and TL/SVL among males of each species for all analyses. Ratios were used instead of the raw values of HW and TL in order to rule out their collinearity with the SVL.

I compiled the predominant calling site of each species from the literature (72 species) and complemented it with field observations provided by fellow researchers (Dataset S3). I categorized calling site as abaxial leaf surface or adaxial leaf surface/on rocks, because there was no reason to assume that call traits would be differently affected by different substrates considering the frog's calling position above them (as opposed to a calling frog positioned on the underside of a leaf forming a dome, which could potentially reverberate or absorb differentially certain spectral bands of a sound signal). Parental care (i.e., prolonged attendance) data was obtained from Delia *et al.* (2017) (Dataset S3), based on field observations of 39 species, distributed in 11 genera. Thirty-three of these species were represented in the acoustic dataset described above (Dataset S3).

Phylogeny

To quantify the evolutionary relationships among glassfrog species, I used the most recent and complete molecular ultrametric phylogeny of Centrolenidae (Delia *et al*., 2017). This phylogeny includes 111 of the 155 currently named species, plus 10 putative undescribed species, and includes representatives of all currently recognized genera. This accounts for 72 % of glassfrog species diversity. The phylogeny was based on DNA sequences of up to three mitochondrial and seven nuclear genes (totaling 6645 bp), and was inferred from a Bayesian analysis with the dataset partitioned by gene using a GTR + I + Γ nucleotide substitution model, and dated with the relaxed-clock method (Delia *et al*., 2017). The topology used in all my analyses corresponds to the maximum clade credibility tree of 10,001 trees sampled from the posterior distribution, with mean branch lengths (Dryad; doi:10.5061/dryad.16vc0). The topology is completely resolved and only 13 % of nodes have a posterior probability < 0.90). For the purpose of the PCM, I trimmed the original tree by removing the species for which I had no phenotypic data (Fig. 1), using the "drop.tip" function as implemented in the package APE (Paradis *et al*., 2004) in R (R Core Team, 2017).

Phylogenetic comparative analysis

I used PCM to estimate phylogenetic signal of acoustic traits, to measure correlated evolution between acoustic traits and other variables (i.e., morphology, calling site and parental care), and to infer the tempo of phenotypic evolution of acoustic and morphometric variables.

I coded categorical variables (e.g., call structure, calling site) as numeric discrete variables. Continuous traits were log or square root-transformed (Table 1) in order to fulfill requirements of the statistical methods (i.e., normality of the residuals) (Freckleton, 2009) and the assumptions of the evolutionary model (Felsenstein, 1985). For all phylogenetic linear models, I visually assessed distribution of residuals and tested the correlations between the absolute values of the phylogeny-corrected residuals and their predicted values, in order to assess homogeneity of variance (Freckleton, 2009).

I estimated the value of the evolutionary parameter λ, a measure of the phylogenetic signal, for each acoustic trait separately. This parameter indicates the extent to which closely related species tend to resemble each other in relation to a given trait (Pagel, 1999). A value of $\lambda = 1$ indicates high phylogenetic signal in a trait (i.e., phylogenetically closer species are more similar to each other), whereas a value of $\lambda = 0$ indicates no phylogenetic signal (i.e., trait similarity among species is independent of shared ancestry; Freckleton *et al.*, 2002). The value of λ was estimated through a maximum-likelihood approach assuming a constant-rate Brownian motion model (BM) of evolution (Symonds & Blomberg, 2014), which considers that traits undergo a random process of successive and independent small changes. The net change of the trait is drawn from a normal distribution with mean at zero and variance proportional to the time elapsed (Felsenstein, 1985). Thus, the magnitude of evolutionary change of a phenotypic trait along a branch will be proportional to the branch length (Symonds & Blomberg, 2014). The λ parameter was estimated using the “phylosig” function as implemented in the package PHYTOOLS (Revell, 2012) in R (R Core Team, 2017).

I estimated the phylogenetic signal of binary traits using the D parameter (Fritz & Purvis, 2010). A value de $D = 0$ indicates high phylogenetic signal in a trait (i.e., phylogenetically closer species are more similar to each other), whereas a value of $D = 1$ indicates no phylogenetic signal (i.e., trait similarity among species is independent of shared ancestry; Fritz & Purvis, 2010). The D parameter was estimated using the “phylo.d” function as implemented in the package CAPER (Orme *et al.*, 2013) in R.

In order to address each question of correlated evolution between continuous acoustic traits and other phenotypic or environmental variables, I performed tests using phylogenetic generalized least squares models (PGLS). PGLS is a generalization of Felsenstein's (1985)'s phylogenetic independent contrasts, which incorporates phylogenetic relationships among species into the error structure of the regression formula, considering a model of phenotypic evolution that best fits the observed data (Grafen, 1989). More specifically, the expected covariance structure of the residuals from the regression equation is defined as the amount of shared evolutionary history between the species—the phylogenetic covariance matrix (Symonds & Blomberg, 2014). The λ parameter is a scaling parameter of the phylogeny in the PGLS model. This is a multiplier of the off-diagonal elements of the expected variance-covariance matrix and indicates whether the shared evolutionary histories as specified by the phylogeny produce the patterns of similarity observed in the residuals (Symonds & Blomberg, 2014). I used PGLS models considering the λ's transformation because their interpretation is less complex than that of alternative transformation parameters. When λ is not statistically different from one, a Brownian model of phenotypic evolution is assumed. The value of λ was estimated by maximum likelihood. Analyses were carried out using the "pgls" function as implemented in the package CAPER (Orme *et al.*, 2013) in R.

I assessed associations between binary acoustic traits and phenotypic variables using phylogenetic logistic regressions (Ives & Garland, 2010). The alpha (α) parameter estimates the level of phylogenetic correlation in the regression (Ives & Garland, 2010). Analyses using phylogenetic logistic regressions were carried out using the "phyloglm" function as implemented in the package PHYLOLM (Ho & Ane, 2014) in R.

I studied the tempo of evolution of acoustic (CD, NN, PF, BW) and morphometric (SVL, HW/SVL, TL/SVL) traits by estimating their phenotypic disparity index (DI). I considered the DI *sensu lato* from the Harmon *et al*. (2003)'s morphological disparity index (MDI), such as considered previously for other categories of phenotypic data (Gonzalez-Voyer & Kolm, 2011; Gonzalez-Voyer *et al*., 2013). The DI is an estimate of the temporal dynamic of phenotypic evolution that describes how phenotypic disparity is partitioned along the phylogeny. DI values > 0 suggest that most of the phenotypic disparity is distributed within clades; negative DI values suggest that disparity is distributed among clades, implicating an early divergence of the trait; DI values near 0 indicate accumulation of phenotypic disparity among and within clades that is consistent with the Brownian motion model. Disparity-through-time (DTT) plots represent the time course of phenotypic diversification using average pairwise Euclidean distances between species (Harmon *et al*., 2003). These plots are meant to visually represent the average relative disparity of each subclade, which is estimated from dividing the average disparity of all subclades whose ancestral lineages were present at that time by the average disparity of the clade as a whole, and repeating this procedure at each node moving up from the root to the tip of the phylogeny (Harmon *et al*., 2003).

The DI was calculated as the sum of the areas between the curve describing the disparity of the trait and the curve describing the disparity under the null hypothesis of Brownian motion (Harmon *et al*., 2003). I estimated disparity for only the first 4/5 of the phylogeny to avoid artifacts due to incomplete species sampling and overestimation of disparity between closely related species (Harmon *et al*., 2003; Gonzalez-Voyer & Kolm, 2011). I calculated the DI and produced the DTT plots using the "dtt" function as implemented in package GEIGER (Harmon *et al*., 2007) in R. The values of the time-scale generated by default by the DTT plot were replaced with the adjusted first 4/5's of the glassfrog's radiation chronogram estimated by Castroviejo-Fisher *et al*. (2014).

Results

The final dataset contained 72 glassfrog species. Fourty-three species (60 %) vocalized from the adaxial surface of leaves, whereas 28 species (39 %) vocalized from abaxial surface of leaves. One species (*Centrolene geckoideum*) vocalized from rocks. Seventeen species had tonal advertisement calls, while 54 species had pulsed calls. In one species for which call parameters were obtained from the literature, *Sachatamia ilex*, data on call structure was not available; hence this terminal was coded as missing data for this trait. The mode of NN was 1 note (range: 1−13). Mean call duration (CD) estimated among glassfrogs was 0.274 ms (range: 0.006−1.443 ms), mean peak frequency (PF) was 4880.1 Hz (range: 2713.2−7407.4 Hz), mean frequency bandwidth (BW) was 1226.3 Hz (range: 810.4−2476.1 Hz). The average SVL among glassfrogs was 23.8 mm (range: 18.4−75.2 mm), the mean HW/SVL ratio was 0.36 (range: 0.32−0.41) and the mean TL/SVL was 0.56 (range: 0.50−0.67). Thirteen species were described as having the male taking part in parental care, whereas in 20 species parental care is conducted exclusively by the female (Dataset S3).

Phylogenetic signal

Estimates of the evolutionary parameter λ indicated that two acoustic traits (PF and BW) had pronounced phylogenetic signal (λ = 0.72 and 0.73, respectively). The value of λ for

NN was marginally significant but weak ($\lambda = 0.21$), while the value of λ for CD was not statistically significant (Table 1). The values of D for call structure and calling site indicated that both have a significant phylogenetic signal (Table 2), although modest for call structure (D = 0.43). Hence, closely related glassfrog species tend to call while perched in similar places (over leaves/rocks or under the surface of leaves) and to emit calls with similar structure (e.g., tonal or pulsed), with more similar peak frequencies and bandwidths.

Table 1. Maximum likelihood estimates of lambda (λ) and disparity index (DI) for acoustic and morphometric variables for Centrolenidae. CD = call duration; NN = number of notes; PF = peak frequency; BW = bandwidth frequency; SVL = snout-vent length; HW = head width; TL = tibia length. Asterisks indicate significant correlations (*).

Trait	N	λ	Loglik	*P*	DI	*P*
$\log_{10}$ (CD)	72	0.45	- 43.96	0.11	0.26	0.97
Sqrt (NN)	72	0.21	- 65.42	0.04*	0.16	0.96
Sqrt (PF)	72	0.72	- 239.76	1.56×10^{-5}*	0.03	0.79
Sqrt (BW)	64	0.73	- 173.22	1.02×10^{-7}*	0.27	0.97
$\log_{10}$ (SVL)	72	0.94	87.29	1.06×10^{-5}*	-9.9×10^{-4}	0.76
Log (HW/SVL)	72	0.77	114.61	4.08×10^{-6}*	0.004	0.75
Log (TL/SVL)	72	0.28	115.50	0.004*	0.23	0.81

Two morphometric traits evaluated, SVL and HW/SVL, had strong phylogenetic signal ($\lambda = 0.94$ and 0.77, respectively), whereas TL had a weak but significant phylogenetic signal (Table 1). This indicates that glassfrog species sharing a more recent common ancestor are generally more similar in body size (Fig. 1) and head width than species with a more distant common ancestor.

Table 2. Estimates of the parameter D for binary variables assessed for Centrolenidae. Call structure was coded as tonal (0) or pulsed (1). Calling site was coded as "upper side of leaves or rocks" (0) or "underside of leaves" (1).

Trait	N	D	P_{Random}	P_{Brownian}
Call structure	71	0.43	0.012	0.155
Calling site	72	0.04	0	0.478

Environmental, behavioural, and morphological correlates of call traits

PGLS analysis revealed a significant correlation ($P < 0.001$; $\beta = - 36.255$; $R^2 = 0.155$) between SVL and PF (Table 3). Consequently, body size explained a proportion of the observed variation of PF in glassfrogs, when accounting for phylogenetic non-independence of species, suggesting that SVL and PF might have evolved concomitantly.

Table 3. Phylogenetic generalized least squares model of the relationship between peak frequency and snout-vent length (SVL) for Centrolenidae. Asterisks (*) indicate significant correlations.

	N	β ± SE	*t* value	*P* value
Sqrt (Peak frequency)	72			
Lambda value = 0.76				
$R^2 = 0.155$				
Intercept		117.476 ± 14.359	8.818	8.47×10^{-12}
Log10 (SVL)		- 36.255 ± 10.116	- 3.584	6.21×10^{-4}*

I only found an association between calling site and call structure ($P = 0.02$; $b_1 = -1.458$), indicating that similar calling sites evolved alongside with call structure: glassfrogs that call from the underside of leaves tended to emit tonal calls (Tables 4 and 5). Calls of species with male brooding were generally characterized by pulsed notes (Table 4). I found no additional associations between acoustic variables and male investment in parental care (Table 6).

Table 4. Phylogenetic logistic regressions of associations between call structure and calling site (underside of leaves vs. upper side of leaves or rocks), and between call structure and male attendance during brooding of egg clutch for Centrolenidae. Asterisks (*) indicate significant correlations.

	N	*b* ± SE	*z* value	*P* value
Call structure	70			
Alpha value = 26.57				
Intercept		1.787 ± 0.552	3.237	0.001
Calling site		- 1.458 ± 0.617	- 2.363	0.018*
Call structure	29			
Alpha value = 80.59				
Intercept		1.951 ± 0.695	2.805	0.005
Male brooding		- 1.784 ± 0.921	- 1.934	0.053*

Table 5. Phylogenetic generalized least squares models of associations between advertisement call traits and calling site (underside of leaves vs. upper side of leaves or rocks) for Centrolenidae. Asterisks (*) indicate significant correlations.

	N	β ± SE	*t* value	*P* value
Log 10 (Call duration)	71			
Lambda value = 0.33				
$R^2 = 0.007$				
Intercept		- 0.842 ± 0.145	- 5.826	1.71×10^{-7}
Calling site		0.092 ± 0.130	0.710	0.480
Sqrt (Number of notes)	71			
Lambda value = 0.23				
$R^2 = 0.007$				
Intercept		1.330 ± 0.170	7.847	4.15×10^{-11}
Calling site		0.116 ± 0.173	0.668	0.507
Sqrt (Peak frequency)	71			
Lambda value = 0.746				
$R^2 = 0.016$				
Intercept		66.300 ± 3.351	19.788	$< 2 \times 10^{-16}$
Calling site		1.992 ± 1.889	1.055	0.295
Sqrt (Bandwidth frequency)	63			
Lambda value = 0.703				
$R^2 = 0.002$				
Intercept		34.107 ± 1.738	19.621	$< 2 \times 10^{-16}$
Calling site		- 0.358 ± 1.110	- 0.322	0.748

Tempo and mode of evolution of call and morphological traits

The analyses of DI suggested that CD, NN and BW differ from two morphometric traits (i.e., SVL and HW) but not TL in terms of their tempo and mode of evolution. Similarly, PF mirrows the pattern of evolution disparity of SVL and HW. Acoustic traits had higher DI's when compared to morphometric traits (0.03–0.27 vs. - 9.9 x 10^{-4}–0.004, respectively; Table 1), except TL, which had a DI = 0.23, close to the maximum DI estimated for acoustic traits. Peak frequency had the lowest value of DI among acoustic traits. Considering the disparity-through-time analysis, disparity of all phenotypic traits peaked (with different magnitudes) at the first third of the centrolenid radiation (around 15 MYA) and then decreased (Fig. 2). However, all acoustic traits except PF increased their relative DI towards the present, around 10–8 MYA—approximately at the mid time of the glassfrog's radiation—, deviating from DI values predicted under a BM model (Fig. 2 A–D). A similar pattern is also observed in TL. This indicates that: i) for most acoustic traits assessed and TL, a greater proportion of the disparity was concentrated within subclades; and ii) that acoustic divergence did not follow a purely Brownian model (BM) and, instead, had a more recent divergence, if compared to morphometric traits (i.e., SVL and HW)—which present a model of evolution better approximated by a BM model.

Table 6. Phylogenetic generalized least squares models of the relationship between advertisement call traits with male attendance during brooding of egg clutch for Centrolenidae. Asterisks (*) indicate significant correlations.

	N	β ± SE	*t* value	*P* value
Log 10 (Call duration)	29			
Lambda value = 0				
R^2 = 0.002				
Intercept		- 0.649 ± 0.116	- 5.595	6.20 x 10^{-6}
Male brooding		- 0.050 ± 0.198	- 0.254	0.801
Sqrt (Number of notes)	29			
Lambda value = 0				
R^2 = 0.084				
Intercept		1.583 ± 0.137	11.591	5.46 x 10^{-12}

Male brooding	- 0.367 ± 0.232	- 1.578	0.126

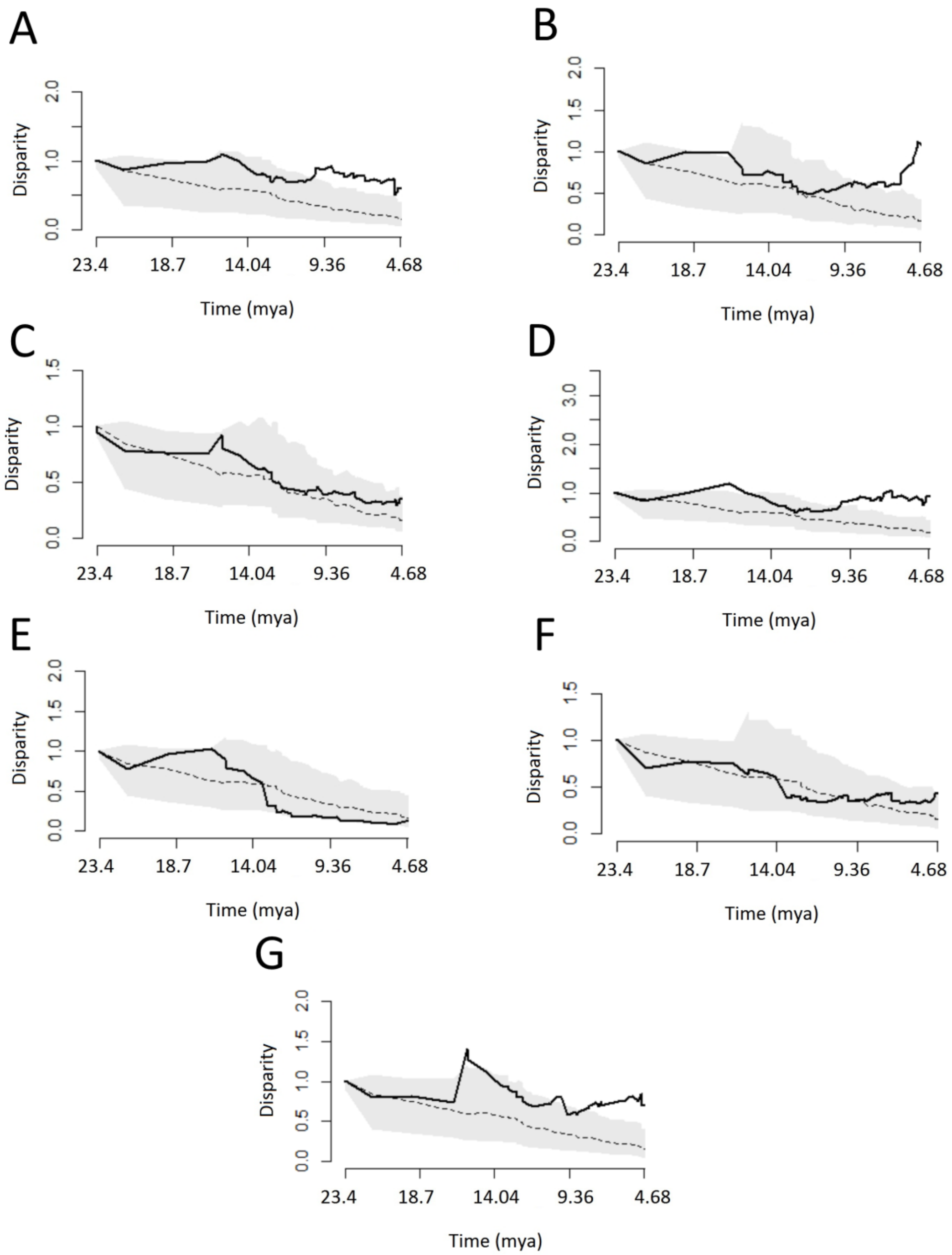

Figure 2. Disparity-through-time (DTT) plots for acoustic and morphometric traits of Centrolenidae. Vertical axis represents average subclade disparity (an estimate of relative

disparity among species within a sub-clade compared with total clade disparity). A) Call duration; B) Number of notes; C) Peak frequency; D) Frequency bandwidth; E) Snout-to-vent-length; F) Head width; and G) Tibia length. The time-scale was estimated from the chronogram of Castroviejo-Fisher *et al*. (2014). The solid line represents the relative disparity through the time estimated from the dataset, the dashed line represent a null model (i.e., Brownian model), and the gray zone represents the 95 % confidence intervals estimated from the simulations under Brownian motion.

Discussion

I investigated associations of shared ancestry, body size, calling site and parental care with structural, temporal and spectral properties of advertisement calls in glassfrogs. I found that common ancestry is associated with interspecific variation of all acoustic traits, as they all, but CD, exhibit statistically significant phylogenetic signal (Tables 1, 2). Body size is negatively correlated with the peak frequency of calls, whereas calling site and male parental care are associated with call structure, broadly defined as tonal or pulsed calls (Tables 3, 4). Additional environmental factors deemed to be important selective forces in other families of frogs, such as masking interference with background noise (Vargas-Salinas & Amézquita, 2014; Goutte *et al.*, 2016, 2017) are discussed below.

I also uncovered that all studied phenotypic variables share a disparity peak around 15 MYA, which is either above the 95 % confidence interval or on its limit (Fig. 2). However, three acoustic (CD, NN and BW) and one morphometric (TL/SVL) variables show a significant increase of phenotypic disparity towards the present, around 10–8 MYA. This later result indicates that the disparity of these four phenotypic variables is disproportionately partitioned within subclades.

Phylogenetic signal in acoustic traits

The idea that behavioral characters, such as anuran vocalizations, carry phylogenetic information has a relatively old origin in evolutionary biology. For example, Blair (1958) suggested that, in frogs, “Groups of species within a genus […] show basic similarities in call structure, which, with other evidence, are indicative of evolutionary relationship”. This notion constitutes the basis for using components of sounds emitted by animals as characters to study phylogenetic relationships. However, difficult issues arise when one tries to code characters with a clear continuous nature (e.g., call duration) and establish homology among non-transitive characters (Cocroft & Ryan, 1995; Goicoechea *et al.*, 2010; Wheeler, 2012). A somehow different approach, which I took herein, consists on statistically testing how well observed variation in acoustic parameters can be explained by common ancestry (phylogenetic signal), given a phylogenetic hypothesis.

Although behavioral traits are generally deemed more labile than morphological, ecological, and physiological traits in vertebrates (Blomberg *et al*., 2003; but see Wenzel, 1992), my results indicate that all evaluated acoustic traits but one have significant phylogenetic signal. These findings agree with previous studies in other anuran groups that reported phylogenetic signal in most, if not all, acoustic traits assessed (Cocroft & Ryan, 1995; Wollenberg *et al*., 2007; Erdtmann & Amézquita, 2009; Goicoechea *et al.*, 2010; Gingras *et al*., 2013b; but see Cannatella *et al*., 1998; Tobias *et al*., 2011). Although the similarity of acoustic traits among close relatives had been previously suggested for centrolenids (Mc Diarmid, *in* Cocroft & Ryan, 1995; Weng et al., 2012), this is the first quantitative study. I also found that the strength of the phylogenetic signal varied among acoustic traits, with NN having a lower phylogenetic signal than spectral traits. My results indicate that CD is not phylogenetically constraint, which combined with new results indicating that it is a variable with low intraspecific variability among anurans (Khöler et al., 2017), may indicate its potential for species identification purposes. Phylogenetic signal was also weaker among acoustic traits when compared to that of morphological traits (Table 1), which may indicate that the evolution of traits under sexual selection is less constraint than other characters.

Correlated evolution of peak frequency and body size

It is a well-known principle in bioacoustics that the size of the structure producing a sound is inversely correlated with the peak frequency of the sound (Wilkins *et al*., 2013). In general, allometric relationships allow us to link body size with the size of sound producing structures, such as the vocal cords of anurans (Wells, 2007). My results show a significant negative correlation between SVL and PF when considering the phylogeny, which is consistent with numerous prior findings for anurans (Zweifel, 1968; Erdtmann & Amézquita, 2009; Gingras *et al*., 2013a; Vargas-Salinas & Amézquita, 2014; Goutte *et al*., 2017). However, the variation explained by the PGLS analysis is relatively small (R^2 = 0.155) if compared to body size-peak frequency relationships found in other anurans (Zweifel, 1968; Erdtmann & Amézquita, 2009; Gingras *et al*., 2013a; Vargas-Salinas & Amézquita, 2014; Röhr *et al*., 2016).

The weak correlation between SVL and PF in glassfrogs could be a result of the limited body size variation in this clade, if compared to those observed in other anuran groups in which the evolution of call parameters has been previously investigated (Erdtmann & Amézquita, 2009; Gingras *et al*., 2013a; 2013b; Goutte *et al*., 2016; Röhr *et al*., 2016). Body size variation in male glassfrogs is largely restricted and skewed towards small sizes (male SVL interquartile range: 20.82–24.86 mm), with a single outlier—the giant *Centrolene geckoideum* (male SVL range: 70.20–80.70 mm; Guayasamín *et al*., 2018).

The restricted variance in body size may be explained by a developmental constraint (Gould, 1980; Smith *et al*., 1985; Stearns, 1986). Testing this hypothesis would require detailed developmental studies and such data are not available. Nevertheless, mapping body size evolution on the phylogeny reveals several independent evolutionary transitions between relatively large and small species (Fig. 1), which would not be expected if a common developmental constraint limited the distribution of body size among species of glassfrogs. In any case, several species emit calls at frequencies higher than those expected by the PGLS model—which is particularly noteworthy for *Centrolene geckoideum* but not restricted to this species (Fig. S1)—and a historical constraint may not be the only factor at play behind the observed pattern. Glassfrogs' body size could be limited by their arboreal, leaf-dwelling habit, with leaf surfaces acting as calling perches, oviposition sites and background camouflage (Señaris & Ayarzagüena, 2005; Kubicki, 2007). Hence, body sizes should be small enough so leaves can provide support and/or reduce predation risk (a topic further discussed in the following section).

Besides developmental constrains or substrate selective preasures, the high-pitched calls of some relatively large species could be an adaptation to outcompete the masking interference of torrent noise. This could also contribute to the weak correlation between SVL and PF. Previous studies have suggested that anurans inhabiting lotic environments tend to emit calls with higher frequencies as a result of acoustic adaptation to reduce interference with the constant noise produced by waterfalls and rapids (Narins *et al*., 2004; Feng *et al*., 2006; Boonman & Kurniati, 2011; Goutte *et al*., 2016; Röhr *et al*., 2016; Goutte *et al*., 2017). Background noise produced by running water typically occupies the first 2.5 kHz of the sound spectrum (Hödl & Amézquita, 2001; Ellinger & Hödl, 2003; Narins *et al*., 2004), and PF of all glassfrog species analysed here were above 2.5 kHz (around 75 % of the species investigated had peak frequencies ≥ 3.5 kHz). In other words, to be heard in a noisy stream a glassfrog needs to emit a relatively high pitch call and a simple solution to that challenge is to be small. This implies that there should be a correlation between frequency band of background noise at calling sites and peak frequency of glassfrogs. As background noise data was missing for all calls analyzed, I tested associations between potential torrent noise environments and PF using the terrain ruggedness index (TRI) in a 90 m radius around each recording site as a proxy to quantify the potential noise of torrent environments. At this very coarse scale, I found no correlation between interspecific variation in PF and variation in the TRI. A detailed description of data acquisition and analyses involving TRI is presented in Appendix 1.

As some noise frequencies of torrents are more emphasized than others depending on physical properties of the lotic water bodies and their margins (Narins *et al*., 2004), future studies could benefit from collecting data on the intensity and bandwidth of the background noise (Schwartz & Bee, 2013; Goutte *et al*., 2016, 2017). Additionally, as the background noise naturally attenuates along its transmission path as a result of spherical propagation, glassfrog species using different vertical strata of the vegetation overhanging streams should be more or less prone to have their calls masked by noise produced by running water (Señaris & Ayarzagüena, 2005; Kubicki, 2007). Thus, data about the height above the ground occupied by recorded males, as well as standardized measurements of call amplitude along the background noise gradient (Schwartz & Bee, 2013), should be considered in future investigations.

Advertisement call and calling site

Glassfrogs use very similar habitats, but dissimilar calling sites (Guayasamin *et al.*, 2009). Several anuran species vocalize from positions that enhance the integrity of information contained in acoustic signals along the transmission path (Hödl, 1977; Bailey & Roberts, 1981; Penna & Solis, 1996; Lardner & bin Lakim, 2002; Muñoz & Penna, 2016; Schwartz *et al.*, 2016). Thus, I predicted that similar calling positions should led to convergent evolution in temporal, spectral and structural traits of advertisement calls because of similar selective pressures on signal transmission efficiency. However, the acoustic structure of calls (i.e., whether calls contained pulsed or only tonal notes) was the only acoustic trait associated with the type of calling site, which I discuss from the point of view of the perceived ecological costs associated with the calling site.

Males that signal from the abaxial surface of leaves may emit tonal calls to minimize the energetic budget, since pulsed calls potentially require more contractions of muscles associated with sound production and hanging upside-down may be energetically more demanding than standing on a flat surface. Alternatively, calling site could drive the acoustic evolution indirectly by selection on body size, which in turn would partially explain the variation in PF (as discussed previously). The ability to adhere to abaxial leaf surfaces or to stand on leaves while not bending them may be limited by body mass which, in anurans, is strongly correlated with the SVL (Santini *et al.*, 2018). However, I found no significant differences in SVL between species that use different calling sites (PHYLANOVA: F = 1.43; *P* = 0.63; n = 72), ruling out this alternative hypothesis.

Calling site has a high and significant phylogenetic signal (D = 0.04; $P_{\text{Brownian}} = 0.478$). Nevertheless, since it is not associated with non-structural acoustic traits, neither with body size, differences in calling sites among glassfrogs could be related to predation risk (Travis *et al*., 1985; Richards & Bull, 1990). Bats have been long recognized as sound-oriented predators, eavesdropping during flight and discriminating edible anuran prey from their acoustic signals (Tuttle & Ryan, 1981). A study of populations of *H. fleischmanni* found an association between the presence of a frog-eating bat (*Trachops cirrhosus*) and a tendency of males to call from the lower surface of leaves (Delia *et al*., 2010). Thus, species that call from the abaxial leaf surfaces may benefit from using such calling sites as shelters from acoustically-oriented predators, especially those targeting for sound signals emitted at specific frequency bandwidths.

Future work would greatly benefit from measuring the size and the resistance of leaves used as calling perches by glassfrogs as well as from analyses of potential vibrational responses of these leaves—for example, by means of laser doppler vibrometry (LDV)—and from experimental approaches investigating the role of bats and nocturnal birds of prey as glassfrog predators.

Evolution of parental care and advertisement call

Parental care by males may impose trade-offs with acoustic signaling during reproduction, since both activities are energetically demanding (Townsend, 1986; Prestwich, 1994; Crump, 1996). Anuran males that allocate energy to brooding have shown a decrease in the emission of advertisement call, with a corresponding decrease in mate attraction (Townsend, 1986). I predicted that glassfrog species with males providing parental care would emit simpler and shorter calls than species with exclusive maternal care. However, the association is only significant for call structure (tonal vs. pulsed notes), suggesting that species in which males are involved in parental care generally produce calls that demand a smaller number of muscle contractions.

Glassfrog species characterized by paternal care normally invest more time brooding than species with exclusive maternal care (Delia *et al*., 2017). Hence, the average time invested by the male in brooding in each species could be a better (continuous) predictor of associations between acoustic traits and reproductive behavior in glassfrogs. Unfortunately, behavioral data of this type is currently available for fewer than ten glassfrog species (Delia *et al*., 2017). Also, the average call rate or note emission, or estimates of calling activity along a particular time span, could be better descriptors of energy spent in calling by male glassfrogs than call structure or call duration. Such rates could not be considered in this study due to limitations of the sound recordings, which were generally too short to allow their estimation.

Tempo and mode of phenotypic evolution

Evolutionary radiations, defined as the increase of taxa in a clade, have been a research theme of great interest in evolutionary biology for more than a century (review in Simões *et al*., 2016). Typically, studies have approached evolutionary radiations by analyzing morphological traits (Simões *et al*., 2016), whith relatively fewer studies having explored the temporal pattern of an evolutionary radiation by assessing acoustic traits (e.g., Gonzalez-Voyer *et al*., 2013). Here, I investigated the tempo and mode of acoustic evolution in glassfrogs through analyses of trait disparity (DI and DTT, Harmon *et al*., 2003).

Several studies have found a rapid and early increase of morphological disparity in some animal clades (Foote, 1997). I found that the disparity of most acoustic traits (CD, NN and FW) is partitioned within subclades, hinting at a late diversification along the glassfrog radiation. Values of mean relative disparity near one indicate that subclades have independently evolved to occupy similar regions of phenotypic space (Harmon *et al*., 2003). Thus, glassfrog subclades could have evolved independently to occupy similar regions of the acoustic space.

When considering the historical biogeography of Centrolenidae, a constantly increasing diversity model explains best its species diversification, thus rejecting a diversity-dependent cladogenesis model (Castroviejo-Fisher *et al*., 2014). However, it also suggests that environmental selection varied along the evolutionary history of this group, in association with dispersal events to different biogeographic regions and elevational specialization (Castroviejo-Fisher *et al*., 2014). I found that the disparity of most acoustic traits is mainly partitioned within subclades, with an increase in disparity around 10–8 MYA—when values of observed disparities raised above the null models' 95% confidence intervals (Fig. 2). Time estimates for the onset of increasing disparity of such acoustic traits coincide with 1) major changes within biogeographic areas already inhabited by ancestral glassfrogs such as the main orogeny of the Andes and the formation of the modern Amazon basin (Hoorn & Wesselingh, 2010); 2) the dispersal into new biogeographic areas such the large radiation of Cochranellini and the colonozation of the MRCA of *Teratohyla* of the Amazonian lowlands and of *Celsiella* of the highlands.

The uplifting of the Andes and the formation of the modern Amazon basin changed the tropical South American landscape dramatically and may have triggered changes in the way ancestral glassfrogs vocalized. Thus, the dynamic of acoustic disparity in glassfrogs may be explained by differentiation associated to adaptation to new landscapes derived by geological changes. During this period of time, some clades of glassfrogs dispersed into new biogeographic areas and/or expanded their elevational distributions. If vegetation structure varies along elevational gradients and biogeographic regions, then the specialization to certain elevations and the dispersion to new biogeographic areas may have also played a key role in the observed increase in disparity in acoustic traits. Along their evolution, glassfrog species had a small rate of change in their elevational range distribution, and closely related extant species usually inhabit the same biogeographic area (Castroviejo-Fisher *et al*., 2014). Associations between temporal and spectral properties of sound signals and the cluttering of the transmission environment by vegetation (the Acoustic Adaptation Hypothesis sensu Morton, 1975) have been widely reported in birds (Gonzalez-Voyer *et al.*, 2013; reviews in Morinay *et al.*, 2013; Wilkins *et al*., 2013), but less so in anurans (see Ey & Fisher, 2009; Erdtmann & Lima, 2013). A necessary prediction to this model would be a correlation between elevational distribution of taxa and the density of streamside forests. This hypothesis still needs investigation, as data on streamside forest structure is lacking for the vast majority of sites (if not all) where glassfrog species were recorded. The occupation of vacant acoustic spaces after dispersal into new biogeographic regions, such as those following the diversification Cochranellini 15–10 MYA (Castroviejo-Fisher *et al*., 2014), may be also behind the observed recent boost in acoustic disparity (Fig. 2).

Early studies investigating morphological traits supported a rapid and early proliferation of morphological diversity in some animal clades (Foote, 1997). However, my results suggest that the mode of evolution for SVL, HW and PF in glassfrogs was probably constant along time, in agreement to results found in some Neotropical vertebrates (e.g., in

Anolis and Phrynosomatine lizards - Harmon *et al*., 2003). More specifically, the evolution of SVL and HW agree with the BM null model, which suggests that diversification in these traits could be explained by fluctuations in selection regimes (O'Meara *et al*., 2006; Revell *et al*., 2008), rendering high values of phylogenetic signal (Revell *et al*., 2008), as effectively observed in the corresponding estimates of λ (Table 1). In contrast, the TL differentiation mirrows the pattern observed in the acoustic variables CD, NN and BW, which occurred more recently in the radiation of the group, hinting at a recent change of selection regime on locomotion, potentially triggered by past environmental changes or by dispersals into new areas.

The temporal pattern of evolution can differ between traits of dissimilar nature due to evolutionary contingency or due to different selection regimes acting upon the evolution of each trait. Traits directly involved in mate choice are often under directional selection, whereas traits that are not involved in mate choice could be under stabilizing, disruptive or directional selection (Ridley, 2004; Coyne & Orr, 2004; Gonzalez-Voyer & Kolm, 2011). Examples derived from studies with Tanganyikan cichlids (Gonzalez-Voyer & Kolm, 2011) found that disparity in ecological characters (e.g., diet, habitat) was concentrated early in the species radiation, whereas disparity in sexual characters (e.g., mating system, sexual size dimorphism) remained high throughout the evolutionary history of this group. In centrolenids, call traits seem to have diversified within a limited phenotypic space in early stages of the evolutionary history of glassfrogs, such that distant clades independently colonized similar regions of the acoustic space. Explanations for differences in the tempo of evolution of sexual traits among different vertebrate groups could stem from ecological opportunities being available at different historical periods, what has been invoked to explain the tempo and mode of evolution in some morphological traits (Harmon *et al*., 2003). Otherwise, distinct pattern of disparity found among exclusively sexual and other traits may reflect differences in ecological, physiological or genetic constraints.

Conclusions

Although this study is the most comprehensive investigation on anuran call evolution at the family level considering the available coverage of species and acoustic data sampling, one cannot help but notice the need for more data as outlined in the Discussion to test some competing hypotheses. It is not only the amount of data which is important but its quality. The vast majority of analyzed recordings follow quality standards for taxonomic studies (Khöler *et al*., 2017) and are of limited use for evolutionary ecology studies. Nonetheless, my results allow the appreciation of a general scenario, providing a theoretical background for these tests. In this model variation in PF and CS have key relationships with body size, calling position, and parental care. I speculate that variation in PF has been influenced by adaptation to torrent noise and limitation in body size range, mediated by predation risk and other ecological pressures which selected for small animals. Regarding CS, I postulate that the energetic costs associated with dangling upside-down on leaves and with parental care by males limit their ability to produce complex, amplitude modulated calls. I hypothesize that the tempo of acoustic evolution in glassfrogs could be mainly explained by differentiation associated with the occupation of new habitats (vacant acoustic spaces). Finally, future work could also focus on the role of selection by females in shaping advertisement call evolution in glassfrogs, as experimental data on this key factor is scarce.

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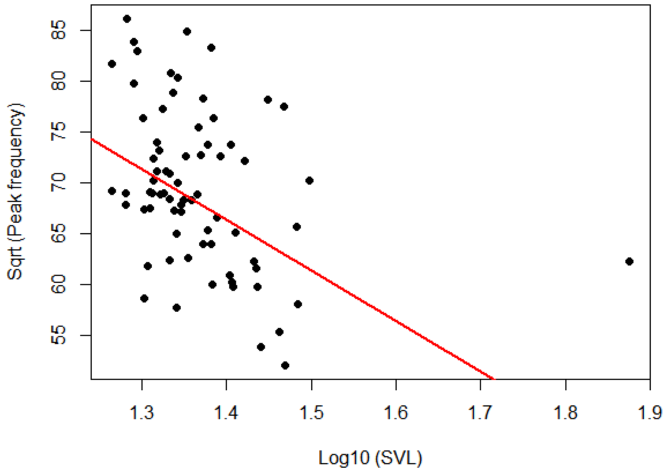
Supplementary materials

Figure S1. Relationships between square-root of peak frequency and $\log_{10}$ of snout-vent length (SVL) for Centrolenidae (n = 72). Each dot represents the average value for each species; the red line represents the fitted PGLS model. See Table 1 for details of the PGLS model.



Appendix 1

The evolution of the advertisement call in Glassfrogs (Centrolenidae Taylor, 1951)

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Association between the terrain ruggedness index and call traits

Most centrolenids habit in the Andes (Hutter et al., 2013), associated to lotic environments (Señaris & Ayarzagüena, 2005). When running on sloped terrain, streams can turn into torrents or waterfalls, producing background noise with sound energy normally peaking on the lower frequency spectrum— mainly around 2.5 kH (Hödl & Amézquita, 2001; Ellinger & Hödl, 2003; Narins et al., 2004). Some anurans that inhabit lotic environments utter calls with higher frequency, thus potentially reducing the masking interference of background noise (Narins et al., 2004; Feng et al., 2006; Boonman & Kurniati, 2011; Goutte et al., 2016, 2017), and calls with shorter notes and narrower frequency bandwidth (Dubois & Martens, 1984; Goutte et al., 2017). I tested the prediction that the advertisement calls of glassfrogs inhabiting areas with greater topographical variation are shorter, with higher dominant frequencies, and narrower frequency bandwidths.

Methods

I considered the Terrain Ruggedness Index (TRI) as a proxy of the occurrence of potentially noisy streamside environments. The TRI is an objective quantitative measure of the topographic heterogeneity that calculates the sum change in elevation between a grid cell and its eight neighbour grid cells in a digital elevation model (Riley et al., 1999). The presence of waterfalls and fast-flowing streams is expected to be more associated with rugged terrains (high TRI values) than with flat landscapes (low TRI values). Unfortunately, testing associations between TRI and amplitude of the vocalizations (as measured in dB or any descriptor of sound pressure) is hindered by differences in recording procedures and sound equipment among authors of the recordings.

The mean TRI per species was calculated based on the individual values obtained for the localities where the audio recordings were conducted. Geographic coordinates of recording localities were provided by the authors of the recordings or obtained from the literature. In some cases, the localities were georeferenced in Google Earth 7.1.8.3036 (Google Inc. 2017) (Dataset S2). I retrieved the TRI values using the Raster Terrain Analysis plugin as implemented in QGIS v 2.18.7 (Quantum GIS Development Team, 2016) with the available digital elevation model of the NASA Shuttle Radar Topographic Mission (SRTM) with 90 m resolution (Jarvis et al., 2008). The TRI values for recordings with imprecise localities (Dataset S2) were determined as the mean of the TRIs of localities reported for those species in the country from where recordings proceeded (Dataset S3).

Results

The potential presence of noisy streamside environments (as assessed by the TRI) was not associated with interspecific variation in acoustic traits (Table A1). The analysis considering peak frequency of calls as the dependent variable retrieved a marginally significant model, although the variance explained was modest, at best ($P = 0.05$; $\beta = -0.775$; $R^2 = 0.053$). These results are against my predictions, which suggest that topographic characteristics of the breeding habitat of glassfrogs and call traits (call duration, peak frequency, and bandwidth frequency) apparently did not evolve in adaptation to torrent noise.

Table A1. Phylogenetic generalized least squares models of associations between call traits of glassfrogs and the terrain ruggedness index (TRI), which is here used as a proxy to the potential existence of noisy streamside environments, for Centrolenidae. Asterisks (*) indicate significant correlations.

	N	*β* ± SE	*t* value	*P* value
Log 10 (Call duration)	72			
Lambda value = 0.14				
$R^2 = 0.004$				
Intercept		- 0.674 ± 0.216	- 3.111	0.003
TRI		- 0.013 ± 0.025	- 0.495	0.622
Sqrt (Peak frequency)	72			
Lambda value = 0.23				
$R^2 = 0.053$				
Intercept		73.136 ± 3.553	20.586	$< 2 \times 10^{-16}$
TRI		- 0.775 ± 0.394	- 1.966	0.053
Sqrt (Bandwidth frequency)	64			
Lambda value = 0.82				

$R^2 = 0.026$			
Intercept	31.880 ± 2.428	13.129	< 2 x 10^{-16}
TRI	- 0.246 ± 0.193	1.278	0.206

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Dataset S1. Sound recordings analyzed per species, with the corresponding authorship, locality, and coordinates with its source.

Species	**Code (track_name)**	**Autorship**	**Locality**	**Lat**	**Long**	**Coordinates source**
Celsiella vozmedianoi	2_V4b_04	Santiago Castroviejo-Fisher	Venezuela: Sucre: Cerro El Humo	10.69964	-62.61669	Santiago Castroviejo-Fisher
Centrolene altitudinale	9_V1_04	Santiago Castroviejo-Fisher	Venezuela: Mérida: Río Albarregas (2090-2140 m)	8.63080	-71.16075	Georreferenced from Google Earth
Centrolene ballux	Fonozoo 186-7942	W. E. Duellman	Ecuador: Pichincha: 14 km W Chiriboga	-0.23137	-78.89448	Georreferenced from Google Earth
Centrolene buckleyi	Fonozoo 185-7909	W. E. Duellman	Ecuador: Loja: Abra de Zamora, 13 km E Loja	-3.99340	-79.14379	Coordinates from AmphibiaWebEcuador (QCAZ 11330) (http://zoologia.puce.edu.ec/Vertebrados/anfibios/Mapa.aspx?Id=1493)
Centrolene buckleyi	Fonozoo 185-7910	W. E. Duellman	Ecuador: Pichincha: 14 km W Chiriboga,	-0.23137	-78.89448	Georreferenced from Google Earth
Centrolene buckleyi	Fonozoo 185-7911	W. E. Duellman	Ecuador: Loja: Abra de Zamora, 13 km E Loja	-3.99340	-79.14379	Coordinates from AmphibiaWebEcuador (QCAZ 11330) (http://zoologia.puce.edu.ec/Vertebrados/anfibios/Mapa.aspx?Id=1493)
Centrolene condor	002Centrolene condor16032007	Ana Almendariz	Ecuador: Zamora Chinchipe: Paquisha, Paquisha Alto, 2270 m	-3.89605	-78.47668	Almendariz & Batallas 2012
Centrolene daidaleum	Centrolene daidaleum 1ra Qda Las Antenas 12-07-2008	Fernando Rojas-Runjaic	Venezuela: Zulia: Cerro Las Antenas, cuenca del río Lajas, 1430 m	10.34345	-72.56141	Rojas-Runjaic et al. 2012
Centrolene daidaleum	Centrolene daidaleum 1ra Qda Las Antenas 13-07-2008	Fernando Rojas-Runjaic	Venezuela: Zulia: Cerro Las Antenas, cuenca del río Lajas, 1430 m	10.34345	-72.56141	Rojas-Runjaic et al. 2012
Centrolene daidaleum	Centrolene daidaleum Las Antenas 03-2008	Fernando Rojas-Runjaic	Venezuela: Zulia: Cerro Las Antenas, cuenca del río Lajas, 1430 m	10.34345	-72.56141	Rojas-Runjaic et al. 2012
Nymphargus grandisone	Fonozoo 167	Morley Read	Ecuador: ?	?	?	Coordinates from records in GBIF
Nymphargus grandisone	Fonozoo 8124	W. E. Duellman	Ecuador: Pichincha: 4 km N Dos Rios, 1140 m	-0.30526	-78.88081	Coordinates from AmphibiaWebEcuador (QCAZA49925) (http://zoologia.puce.edu.ec/vertebrados/anfibios/Mapa.aspx?Id=1215)
Nymphargus grandisone	Fonozoo 8175	W. E. Duellman	Ecuador: Pichincha: 4 km N Dos Rios, 1140 m	-0.30526	-78.88081	Coordinates from AmphibiaWebEcuador (QCAZA49925) (http://zoologia.puce.edu.ec/vertebrados/anfibios/Mapa.aspx?Id=1215)
Nymphargus grandisone	LS100030_Ng	Ana Salgado	Ecuador: Pichincha: Reserva Las Gralarias, Lucy's creek	0.00400	78.73800	Ana Salgado

Dataset S1. Continued.

Species	Code (track_name)	Autorship	Locality	Lat	Long	Coordinates source
Centrolene heloderma	Fonozoo 158-7135	W. E. Duellman	Ecuador: Pichincha: 14 km W Chiriboga	-0.23137	-78.89448	Georreferenced from Google Earth
Centrolene lynchi	ALS013	Ana Salgado	Ecuador: Pichincha: Reserva Las Gralarias, 1800-2050 m	-0.01697	-78.73334	Maldonado 2012
Centrolene lynchi	LS100018	Ana Salgado	Ecuador: Pichincha: Reserva Las Gralarias, 1800-2050 m	-0.01697	-78.73334	Maldonado 2012
Centrolene lynchi	LS100020	Ana Salgado	Ecuador: Pichincha: Reserva Las Gralarias, 1800-2050 m	-0.01697	-78.73334	Maldonado 2012
Centrolene lynchi	LS100029	Ana Salgado	Ecuador: Pichincha: Reserva Las Gralarias, 1800-2050 m	-0.01697	-78.73334	Maldonado 2012
Centrolene lynchi	Centrolene_lynchi (QCAZ_Ana Salgado)	Ana Salgado	Ecuador: Pichincha: Reserva Las Gralarias, 1800-2050 m	-0.01697	-78.73334	Maldonado 2012
Centrolene peristictum	ALS002	Ana Salgado	Ecuador: Pichincha: Reserva Las Gralarias, 1800-2050 m	-0.01697	-78.73334	Maldonado 2012
Centrolene peristictum	ALS014	Ana Salgado	Ecuador: Pichincha: Reserva Las Gralarias, 1800-2050 m	-0.01697	-78.73334	Maldonado 2012
Centrolene peristictum	ALS015	Ana Salgado	Ecuador: Pichincha: Reserva Las Gralarias, 1800-2050 m	-0.01697	-78.73334	Maldonado 2012
Centrolene peristictum	ALS017X	Ana Salgado	Ecuador: Pichincha: Reserva Las Gralarias, 1800-2050 m	-0.01697	-78.73334	Maldonado 2012
Centrolene peristictum	ALS019	Ana Salgado	Ecuador: Pichincha: Reserva Las Gralarias, 1800-2050 m	-0.01697	-78.73334	Maldonado 2012

Dataset S1. Continued.

Species	**Code (track_name)**	**Autorship**	**Locality**	**Lat**	**Long**	**Coordinates source**
Centrolene peristictum	ALS020	Ana Salgado	Ecuador: Pichincha: Reserva Las Gralarias, 1800-2050 m	-0.01697	-78.73334	Maldonado 2012
Centrolene sabini	Coc28m21-99(1)	Alessandro Catenazzi	Peru: Cusco: near Esperanza, upper Manu National Park, 2805 m	-13.17717	-71.60894	Catenazzi et al. 2009
Centrolene sabini	Coc28m21-99(2)	Alessandro Catenazzi	Peru: Cusco: near Esperanza, upper Manu National Park, 2805 m	-13.17717	-71.60894	Catenazzi et al. 2009
Centrolene sabini	Coc28m21-99(3)	Alessandro Catenazzi	Peru: Cusco: near Esperanza, upper Manu National Park, 2805 m	-13.17717	-71.60894	Catenazzi et al. 2009
Centrolene sabini	Coc28m21-99(5)	Alessandro Catenazzi	Peru: Cusco: near Esperanza, upper Manu National Park, 2805 m	-13.17717	-71.60894	Catenazzi et al. 2009
Centrolene savagei	Macho4Yotoco	Natalia Díaz	Colombia: Valle del Cauca: Reserva Forestal Bosque de Yotoco, 1200-1600 m	3.87197	-76.42173	Georreferenced from Google Earth
Centrolene savagei	MZ000004	Santiago Castroviejo-Fisher	Colombia: Quindío: Vereda El Mendillal, Reserva de Bremen	4.66850	-75.61650	Marco Rada
Centrolene savagei	savagei CATAZ034	Fernando Vargas-Salinas	Colombia: Valle del Cauca: Reserva Forestal Bosque de Yotoco, 1200-1600 m	3.87197	-76.42173	Georreferenced from Google Earth
Chimerella corleone	Chimerella corleone San Jose 1	Evan Twomey	Peru: San Martín: top of a large waterfall, stream near the village of San Jose in the Cainarachi valley near Tarapoto	-6.41739	-76.28926	Twomey et al. 2014
Cochranella erminae		Evan Twomey	Peru: Amazonas: Campanilla	-8.32380	-76.70995	Evan Twomey
Cochranella erminae		Evan Twomey	Peru: Amazonas: Muyo, 629 m	-5.29833	-78.40055	Evan Twomey

Dataset S1. Continued.

Species	Code (track_name)	Autorship	Locality	Lat	Long	Coordinates source
Cochranella euknemos	Fonozoo 21_66959	Roberto Ibañez D., A, Stanley Rand, Michael J. Ryan y Cesar A. Jaramillo A.	Panama: ?	?	?	Coordinates from records in GBIF
Cochranella euknemos	MZ000023	Jesse Delia	Panama: ?	?	?	Coordinates from records in GBIF
Cochranella euknemos	MZ000024	Jesse Delia	Panama: ?	?	?	Coordinates from records in GBIF
Cochranella euknemos	MZ000025	Jesse Delia	Panama: ?	?	?	Coordinates from records in GBIF
Cochranella granulosa	Fonozoo 21_66958	Roberto Ibañez D., A, Stanley Rand, Michael J. Ryan y Cesar A. Jaramillo A.	Panama: ?	?	?	Coordinates from records in GBIF
Cochranella granulosa	Fonozoo 232-8908	Adrián García-Rodríguez	Costa Rica: Limón: Cahuita	9.71817	-82.84241	Georreferenced from Google Earth
Cochranella guayasamini	Cochranella sp2_20C_7-04_S6.41689_W76.29353	Evan Twomey	Peru: San Martin: San Jose, Cainarachi valley	-6.42074	-76.29124	Evan Twomey
Cochranella guayasamini	MZ0000002	Evan Twomey	Peru: San Martin: San Jose, Cainarachi valley	-6.42074	-76.29124	Evan Twomey
Cochranella mache	final	Mauricio Ortega-Andrade	Ecuador: Esmeraldas: Rompe-fuente rivulet, Bilsa Biogical Station, 800 m	0.35917	-79.70056	Ortega-Andrade et al. 2013
Cochranella nola	Fonozoo 96-5136	José M. Padial	Bolivia: Santa Cruz: Camino a Bella Vista-Florida	-18.18903	-63.71467	Fonozoo
Cochranella nola	Fonozoo 108-5584	Jörn Köhler	Bolivia: Santa Cruz: Quebrada El Fuerte, 1600 masl, semi-deciduos forest remanent of the Río Piray Valley, Samapaita region, Florida Province	-18.17337	-63.81406	Georreferenced from Google Earth

Dataset S1. Continued.

Species	**Code (track_name)**	**Autorship**	**Locality**	**Lat**	**Long**	**Coordinates source**
Cochranella resplendens	Cochranella resplendens San Jose edit (Jesse Delia)	Jesse Delia	Peru: San Martin: San Jose, Cainarachi valley	-6.42233	-76.28837	Evan Twomey
Cochranella saxiscandens	Ahuashiyacu_1	Evan Twomey	Peru: San Martín: Ahuashiyacu waterfalls, 797 m	-6.45547	-76.30898	Twomey et al. 2014
Cochranella saxiscandens	Ahuashiyacu_2	Evan Twomey	Peru: San Martín: Ahuashiyacu waterfalls, 797 m	-6.45547	-76.30898	Twomey et al. 2014
Cochranella saxiscandens	Ahuashiyacu_3	Evan Twomey	Peru: San Martín: Ahuashiyacu waterfalls, 797 m	-6.45547	-76.30898	Twomey et al. 2014
Cochranella saxiscandens	Ahuashiyacu_4	Evan Twomey	Peru: San Martín: Ahuashiyacu waterfalls, 797 m	-6.45547	-76.30898	Twomey et al. 2014
Cochranella saxiscandens	Ahuashiyacu_5	Evan Twomey	Peru: San Martín: Ahuashiyacu waterfalls, 797 m	-6.45547	-76.30898	Twomey et al. 2014
Cochranella saxiscandens	Ahuashiyacu_6	Evan Twomey	Peru: San Martín: Ahuashiyacu waterfalls, 797 m	-6.45547	-76.30898	Twomey et al. 2014
Cochranella saxiscandens	Ahuashiyacu_7	Evan Twomey	Peru: San Martín: Ahuashiyacu waterfalls, 797 m	-6.45547	-76.30898	Twomey et al. 2014
Cochranella saxiscandens	Ahuashiyacu_8	Evan Twomey	Peru: San Martín: Ahuashiyacu waterfalls, 797 m	-6.45547	-76.30898	Twomey et al. 2014

Dataset S1. Continued.

Species	**Code (track_name)**	**Autorship**	**Locality**	**Lat**	**Long**	**Coordinates source**
Cochranella saxiscandens	Rulyrana tangarana 21.5C (7 km E Pacayzapa) 1	Evan Twomey	Peru: San Martín: 7 km E Pacayzapa, 1047 m	-6.28135	-76.73274	Twomey et al. 2014
Cochranella spinosa	Fonozoo 21_66957	Roberto Ibañez D., A, Stanley Rand, Michael J. Ryan y Cesar A. Jaramillo A.	Panama: ?	?	?	Coordinates from records in GBIF
Espadarana andina	1_V2_04	Santiago Castroviejo-Fisher	Venezuela: Mérida: Quebrada La Azulita (980-1060 m)	8.71418	-71.43902	Georreferenced from Google Earth
Espadarana andina	Espadarana andina Ipika IX-2008	Fernando Rojas-Runjaic	Venezuela: Zulia: headwaters of the Cascada Kusare, near Ipika, Río Tokuko basin, PN Sierra de Perijá, Municipio Machiques, 845 m	10.72019	-72.48794	Rojas-Runjaic et al. 2012
Espadarana audax	Fonozoo 198-8379	José M. Padial	Peru: San Martin: Lamas, Quebrada 3 km de Roque	-6.42431	-76.50836	Georreferenced from Google Earth
Espadarana prosoblepon	Fonozoo 21	Roberto Ibañez D., A, Stanley Rand, Michael J. Ryan y Cesar A. Jaramillo A.	Panama: ?	?	?	Coordinates from records in GBIF
Hyalinobatrachium aff. *bergeri*	HAF00015	Santiago Castroviejo-Fisher	?	?	?	?
Hyalinobatrachium aff. *bergeri*	Fonozoo 196-8238	José M. Padial	Peru: Cusco: Ouispicanchis, entre San Miguel y Marcapata	-13.47419	-70.89620	J. Padial
Hyalinobatrachium aff. *bergeri*	Fonozoo 196-8237	José M. Padial	Peru: Cusco: Ouispicanchis, entre San Miguel y Marcapata	-13.47419	-70.89620	J. Padial

Dataset S1. Continued.

Species	Code (track_name)	Autorship	Locality	Lat	Long	Coordinates source
Hyalinobatrachium anachoretus	Hyalinobatrachium anachoretus Alto Mayo 1	Evan Twomey	Peru: Amazonas (?), Alto Mayo near Puente Nieva	-5.66890	-77.76683	Evan Twomey
Hyalinobatrachium anachoretus	Hyalinobatrachium anachoretus Alto Mayo 2	Evan Twomey	Peru: Amazonas (?), Alto Mayo near Puente Nieva	-5.66890	-77.76683	Evan Twomey
Hyalinobatrachium bergeri	Fonozoo 185-7907	W. E. Duellman	Peru: Ayacucho: Tutumbaro, 1840 m	-12.72364	-73.94801	Georreferenced from Google Earth
Hyalinobatrachium bergeri	Fonozoo 185-7915	W. E. Duellman	Peru: Ayacucho: Tutumbaro, 1840 m	-12.72364	-73.94801	Georreferenced from Google Earth
Hyalinobatrachium bergeri	Fonozoo 196-8249	José M. Padial	Peru: Cusco: Ouispicanchis, A 5 km de San Lorenzo hacia Quince Mil	-13.19672	-70.55578	Fonozoo
Hyalinobatrachium bergeri	Fonozoo 96-5137	José M. Padial	Bolivia: La Paz: Sadiri-Arroyo Yariapo, P. N. Madidi	-14.19028	-67.93611	Fonozoo
Hyalinobatrachium bergeri	Fonozoo 96-5138	José M. Padial	Bolivia: La Paz, Puesto guardaparques-Arroyo Huabudahaida, P. N. Madidi	-14.17012	-67.91890	José M. Padial
Hyalinobatrachium bergeri	Fonozoo 97-5187	José M. Padial	Bolivia: La Paz: Arroyo Pico Plancha, Senda entre San José y Apolo	-14.27214	-68.09336	Fonozoo
Hyalinobatrachium bergeri	bergeri_15000	José M. Padial	Peru: Cusco: Ouispicanchis, 6.1 km de Puente Fortaleza hacia Quince Mil	-13.18603	-70.58060	José M. Padial
Hyalinobatrachium bergeri	bergeri_marcapata-1	José M. Padial	Peru: Cusco: Ouispicanchis, entre San Miguel y Marcapata	-13.47419	-70.89620	José M. Padial
Hyalinobatrachium bergeri	bergeri_marcapata-2	José M. Padial	Peru: Cusco: Ouispicanchis, entre San Miguel y Marcapata	-13.47419	-70.89620	José M. Padial

Dataset S1. Continued.

Species	Code (track_name)	Autorship	Locality	Lat	Long	Coordinates source
Hyalinobatrachium cappellei	12_V3_05	Santiago Castroviejo-Fisher	Venezuela: Bolívar: primera quebrada em el primer campamento antes del Auyán-tepui	5.68485	-62.52562	Santiago Castroviejo-Fisher
Hyalinobatrachium cappellei	2_V4_05	Santiago Castroviejo-Fisher	Venezuela: Bolívar: Quebrada um poco más abajo del campamento El Oso, Auyán-tepui	5.78730	-62.53659	Santiago Castroviejo-Fisher
Hyalinobatrachium cappellei	7_V2_05	Santiago Castroviejo-Fisher	Venezuela: Bolívar: Km.127 Crta. El Dorado - Sª Elena	5.96700	-61.39412	Santiago Castroviejo-Fisher
Hyalinobatrachium cappellei	8_V4a_04	Santiago Castroviejo-Fisher	Venezuela: Bolívar: Quebrada Jaspe, San Ignacio de Yuruaní, Parque Nacional Canaima	4.91738	-61.09344	Georreferenced from Google Earth
Hyalinobatrachium cappellei	Hyalinobatrachium_cappellei_(crurifasciatum)_AMNH REEL 256 (track 6)	Charles W. Myers, Maureen A. Donnelly & John W. Daly	Venezuela: Amazonas: Sierra Tapirapecó, north base Pico Tamacuari: 1160-1200 m	1.23623	-64.75167	AMNH (corrected to agree with the description of the locality)
Hyalinobatrachium cappellei	Hyalinobatrachium_cappellei_(eccentricum)_AMNH REEL 264 (track 1)	Charles W. Myers	Venezuela: Amazonas: Cerro Yutajé, 1700 m	5.75293	-66.14633	AMNH (corrected to agree with the description of the locality)
Hyalinobatrachium carlesvilai	Fonozoo 200_76055	I. De la Riva	Bolivia: Santa Cruz: Río Cheyo, Parque Nacional Amboró	-17.58330	-63.65000	http://mapasamerica.dices.net/bolivia/mapa.php?nombre=Rio-Cheyo&id=15073
Hyalinobatrachium carlesvilai	carlesvilai_peru_tambopata_copy1	José M. Padial	Peru: Cusco: Unión, Valle de Kosñipata	-13.05606	-71.54676	José M. Padial
Hyalinobatrachium carlesvilai	carlesvilai_peru_tambopata_copy2	José M. Padial	Peru: Cusco: Unión, Valle de Kosñipata	-13.05606	-71.54676	José M. Padial
Hyalinobatrachium chirripoi	chirripoi-1.wav	Evan Twomey	Costa Rica: Limón: Río Banano, 80 m	9.89984	-83.15000	Google Earth

Dataset S1. Continued.

Species	Code (track_name)	Autorship	Locality	Lat	Long	Coordinates source
Hyalinobatrachium colymbiphyllum	FNJV 11742	Adão Cardoso	Panama: Gamboa: Rio Frijoles	9.13830	-79.77360	http://pa.geoview.info/rio_frijoles,3709457
Hyalinobatrachium colymbiphyllum	Fonozoo 66963	Roberto Ibañez D., A, Stanley Rand, Michael J. Ryan y Cesar A. Jaramillo A.	Panama: ?	?	?	Coordinates from records in GBIF
Hyalinobatrachium colymbiphyllum	Fonozoo 159-7143	W. E. Duellman	Panama: Dairén: Tacarcuna	8.16574	-77.29520	Georreferenced from Google Earth
Hyalinobatrachium duranti	1_V3_05	Santiago Castroviejo-Fisher	Venezuela: Mérida: Quebrada El Chorotal, entre La Azulita y Mérida (1820-1840 m)	8.66472	-71.38140	http://mapasamerica.dices.net/venezuela/mapa.php?nombre=El-Chorotal&id=56131
Hyalinobatrachium duranti	10_V1_04	Santiago Castroviejo-Fisher	Venezuela: Mérida: Quebrada del Loro, La Mucuy, Parque Nacional Sierra Nevada	8.63152	-71.04045	Georreferenced from Google Earth
Hyalinobatrachium duranti	11_V1_04	Santiago Castroviejo-Fisher	Venezuela: Mérida: Quebrada del Loro, La Mucuy, Parque Nacional Sierra Nevada	8.63152	-71.04045	Georreferenced from Google Earth
Hyalinobatrachium duranti	8_V1_04	Santiago Castroviejo-Fisher	Venezuela: Mérida: Río Albarregas (2090-2140 m)	8.63080	-71.16075	Georreferenced from Google Earth
Hyalinobatrachium fleischmanni	Fonozoo 151-6944	W. E. Duellman	Costa Rica: Cartago: Tapantí	9.75323	-83.78382	Georreferenced from Google Earth
Hyalinobatrachium fleischmanni	Fonozoo 151-6945	W. E. Duellman	Costa Rica: Cartago: Tapantí	9.75323	-83.78382	Georreferenced from Google Earth
Hyalinobatrachium fleischmanni	Fonozoo 151-6954	W. E. Duellman	Costa Rica: Cartago: Tapantí	9.75323	-83.78382	Georreferenced from Google Earth
Hyalinobatrachium fleischmanni	Fonozoo 158-7138	W. E. Duellman	Costa Rica: Cartago: Tapantí	9.75323	-83.78382	Georreferenced from Google Earth

Dataset S1. Continued.

Species	Code (track_name)	Autorship	Locality	Lat	Long	Coordinates source
Hyalinobatrachium fleischmanni	Fonozoo 158-7139	W. E. Duellman	Costa Rica: Cartago: Tapantí	9.75323	-83.78382	Georreferenced from Google Earth
Hyalinobatrachium fleischmanni	Fonozoo 158-7142	W. E. Duellman	Nicaragua: Matagalpa: Hacienda La Cumplida	12.99981	-85.84904	http://lacumplida.com/contacto/
Hyalinobatrachium fleischmanni	Fonozoo 21_66962	Roberto Ibañez D., A, Stanley Rand, Michael J. Ryan y Cesar A. Jaramillo A.	Panama: ?	?	?	Coordinates from records in GBIF
Hyalinobatrachium fleischmanni	Hyalinobatrachium_fleischmanni_AMNH REEL 125 (track 6)	Richard G. Zweifel	Panama: Canal Zone: Barro Colorado	9.15579	-79.84663	Georreferenced from Google Earth
Hyalinobatrachium fleischmanni	Hyalinobatrachium_fleischmanni_AMNH REEL 125 (track 7)	Richard G. Zweifel	Panama: Canal Zone: Barro Colorado	9.15579	-79.84663	Georreferenced from Google Earth
Hyalinobatrachium fragile	12_V2_05	Santiago Castroviejo-Fisher	Venezuela: Cojedes: stream that intersects the road Manrique-La Sierra, near San Carlos, 564 m	9.86222	-68.54944	Wen *et al*. (2012)
Hyalinobatrachium iaspidiense	H. nouraguensis-01	Michel Blanc	Guyana: Mabura Hill Forest Reserve, Maiko creek, 60 m	5.15536	-58.69973	Castroviejo-Fisher *et al*. (2011)
Hyalinobatrachium iaspidiense	H. nouraguensis-02	Michel Blanc	Guyana: Mabura Hill Forest Reserve, Maiko creek, 60 m	5.15536	-58.69973	Castroviejo-Fisher *et al*. (2011)
Hyalinobatrachium iaspidiense	H. nouraguensis-03	Michel Blanc	Guyana: Mabura Hill Forest Reserve, Maiko creek, 60 m	5.15536	-58.69973	Castroviejo-Fisher *et al*. (2011)
Hyalinobatrachium iaspidiense	H. nouraguensis-04	Michel Blanc	Guyana: Mabura Hill Forest Reserve, Maiko creek, 60 m	5.15536	-58.69973	Castroviejo-Fisher *et al*. (2011)
Hyalinobatrachium kawense	H. kawense AG 241 Rivière de Kaw 06-02-05	Michel Blanc	French Guiana: Rivière de Kaw 1–10 m	4.60917	-52.05694	Castroviejo-Fisher *et al*. (2011)

Dataset S1. Continued.

Species	**Code (track_name)**	**Autorship**	**Locality**	**Lat**	**Long**	**Coordinates source**
Hyalinobatrachium kawense	H. kawense présence femelle Rivière de Kaw 16-04-05	Michel Blanc	French Guiana: Rivière de Kaw 1–10 m	4.60917	-52.05694	Castroviejo-Fisher *et al*. (2011)
Hyalinobatrachium mondolfii	20_V1_05	Santiago Castroviejo-Fisher	Venezuela: Caño Acoima, campamento Amigos de Doñana (localidad tipo)	8.45306	-61.58520	Señaris & Ayarzaguena 2001 (corrected to adjust the specifications of the locality)
Hyalinobatrachium mondolfii	H. mondolfii AG 320 Crique Gabrielle 28-04-07	Michel Blanc	French Guiana: Crique Gabrielle, 2 m	4.68333	-52.30000	Castroviejo-Fisher *et al*. (2011)
Hyalinobatrachium mondolfii	mondolfii-Guyana_kok	Philippe Kok	Guyana: Mabura Hill Forest Reserve, Maiko creek, 60 m	5.15536	-58.69971	Castroviejo-Fisher *et al*. (2011)
Hyalinobatrachium mondolfii	MZ000012	Santiago Castroviejo-Fisher	Colombia: Amazonas: Biological station Zafire, 14.4 km SW from Tanimboca	-4.00304	-69.89500	Santiago Castroviejo-Fisher
Hyalinobatrachium orientale	Fonozoo 340_76716	Morley Read	Trinidad & Tobago: Tobago: Main Ridge Forest Reserve	11.28089	-60.61938	Georreferenced from Google Earth
Hyalinobatrachium orientale	19_V1_05	Santiago Castroviejo-Fisher	Venezuela: Monagas: Cueva El Guácharo	10.17417	-63.55167	Santiago Castroviejo-Fisher
Hyalinobatrachium orocostale	11_V3_04	Santiago Castroviejo-Fisher	Venezuela: Guárico: Cerro Platillón, Hacienda Picachitos (1200 m)	9.85639	-67.50253	Santiago Castroviejo-Fisher
Hyalinobatrachium orocostale	7_V3_04	Santiago Castroviejo-Fisher	Venezuela: Guárico: Cerro Platillón, Hacienda Picachitos (1200 m)	9.85639	-67.50253	Santiago Castroviejo-Fisher
Hyalinobatrachium pallidum	Hyalinobatrachium pallidum Las Antenas 03-2008	Fernando Rojas-Runjaic	Venezuela: Zulia: Cerro Las Antenas, cuenca del río Lajas, 1430 m	10.34345	-72.56141	Rojas-Runjaic *et al.* (2012)

Dataset S1. Continued.

Species	Code (track_name)	Autorship	Locality	Lat	Long	Coordinates source
Hyalinobatrachium pallidum	Hyalinobatrachium pallidum Qda 2da Antena 12-07-2008	Fernando Rojas-Runjaic	Venezuela: Zulia: Cerro Las Antenas, cuenca del río Lajas, 1430 m	10.34345	-72.56141	Rojas-Runjaic *et al.* (2012)
Hyalinobatrachium pellucidum	MZ000032 (Hyalinobatrachium bergeri near San Jose)	Santiago Castroviejo-Fisher	Peru: San Martín: Cainarachi valley near Tarapoto, 523 m	-6.42131	-76.29125	Twomey *et al*. (2014)
Hyalinobatrachium pellucidum	pellucidum_San Jose_23C	Evan Twomey	Peru: San Martín: Cainarachi valley near Tarapoto, 523 m	-6.42131	-76.29125	Twomey *et al*. (2014)
Hyalinobatrachium tatayoi	3_V3_05	Santiago Castroviejo-Fisher	Venezuela: Zulia: Quebrada en la toma de agua de El Tokuko, sierra de Perijá	9.84174	-72.82046	Santiago Castroviejo-Fisher
Hyalinobatrachium tatayoi	4_V3_05	Santiago Castroviejo-Fisher	Venezuela: Zulia: Quebrada en la toma de agua de El Tokuko, sierra de Perijá	9.84174	-72.82046	Santiago Castroviejo-Fisher
Hyalinobatrachium tatayoi	5_V3_05	Santiago Castroviejo-Fisher	Venezuela: Zulia: Quebrada en la toma de agua de El Tokuko, sierra de Perijá	9.84174	-72.82046	Santiago Castroviejo-Fisher
Hyalinobatrachium taylori	Fonozoo 10_67523	Christian Marty & Philippe Gaucher	French Guiana: ?	?	?	Coordinates from records in GBIF
Hyalinobatrachium taylori	Fonozoo 34_73179	Christian Marty & Philippe Gaucher	French Guiana: ?	?	?	Coordinates from records in GBIF
Hyalinobatrachium tricolor	Hyalinobatrachium cf. nouragensis Wapou 2005 stéréo	Michel Blanc	French Guiana: Kaw: Crique Wapou, 2 m	4.43333	-52.15000	Castroviejo-Fisher *et al*. (2011)
Hyalinobatrachium tricolor	Hyalinobatrachium cf. nouragensis Wapou 2005 stéréo échantillon	Michel Blanc	French Guiana: Kaw: Crique Wapou, 2 m	4.43333	-52.15000	Castroviejo-Fisher *et al*. (2011)

Dataset S1. Continued.

Species	**Code (track_name)**	**Autorship**	**Locality**	**Lat**	**Long**	**Coordinates source**
Hyalinobatrachium tricolor	Hyalinobatrachium cf. nouragensis Wapou 2005 stéréo échantillon	Michel Blanc	French Guiana: Kaw: Crique Wapou, 2 m	4.43333	-52.15000	Castroviejo-Fisher *et al*. (2011)
Hyalinobatrachium valerioi	Fonozoo 193-8125	W. E. Duellman	Ecuador: Pichincha: 4 km N Dos Rios, 1140 m	-0.30526	-78.88081	Coordinates from AmphibiaWebEcuador (QCAZA49925) (http://zoologia.puce.edu.ec/vertebrados/anfibios/Mapa.aspx?Id=1215)
Ikakogi tayrona	Ikakogi tayrona 1015 canto	Fernando Vargas-Salinas	Colombia: Magdalena: Sierra Nevada de Santa Marta, Vereda San Lorenzo, 2200 msnm	11.11520	-74.05090	Vargas-Salinas *et al.* (2015)
Nymphargus bejaranoi	Fonozoo 200_76053	J. Köhler & S. Lötters	Bolivia: Santa Cruz: Remate, Caballero, Parque Nacional Amboró	-17.58330	-63.65000	Coordinates from http://mapasamerica.dices.net/bolivia/mapa.php?nombre=Rio-Cheyo&id=15073
Nymphargus griffithsi	18822_griffithsi2-2	Carl Hutter	Ecuador: Pichincha: Reserva Las Gralarias, Heloderma creek	-0.02076	-78.70617	Hutter & Guayasamin (2012)
Nymphargus griffithsi	18854_griffithsi2	Carl Hutter	Ecuador: Pichincha: Reserva Las Gralarias, Hercules Giant Tree frog creek	-0.02551	-78.70405	Hutter & Guayasamin (2012)
Nymphargus griffithsi	18885_griffithsi2_2	Carl Hutter	Ecuador: Pichincha: Reserva Las Gralarias, Five frog creek	-0.03118	-78.70597	Hutter & Guayasamin (2012)
Nymphargus griffithsi	?	Carl Hutter	Ecuador: Pichincha: Reserva Las Gralarias, Kathy’s Creek	-0.02335	-78.72953	Hutter & Guayasamin (2012)
Nymphargus griffithsi	34970_griffithsi2	Carl Hutter	Ecuador: Pichincha: Reserva Las Gralarias, Five frog creek	-0.03118	-78.70597	Hutter & Guayasamin (2012)
Nymphargus griffithsi	Fonozoo 185-7914	W. E. Duellman	Ecuador: Pichincha: 3.5 km NE Mindo, 1540 m	-0.03987	-78.75286	Georreferenced from Google Earth

Dataset S1. Continued.

Species	**Code (track_name)**	**Autorship**	**Locality**	**Lat**	**Long**	**Coordinates source**
Nymphargus griffithsi	Fonozoo 192-8123	W. E. Duellman	Ecuador: Pichincha: 9 km SE Tandayapa, 2150 masl	-0.04663	-78.63378	Georreferenced from Google Earth
Nymphargus griffithsi	Fonozoo 192-8124	W. E. Duellman	Ecuador: Pichincha: 4 km N Dos Rios, 1140 m	-0.30526	-78.88081	Coordinates from AmphibiaWebEcuador (QCAZA49925) (http://zoologia.puce.edu.ec/vertebrados/anfibios/Mapa.aspx?Id=1215)
Nymphargus lasgrarias	?	Carl Hutter	Ecuador: Pichincha: Reserva Las Gralarias, Kathy's Creek	-0.02335	-78.72953	Hutter & Guayasamin (2012)
Nymphargus lasgrarias	?	Carl Hutter	Ecuador: Pichincha: Reserva Las Gralarias, Heloderma creek	-0.02076	-78.70617	Hutter & Guayasamin (2012)
Nymphargus lasgrarias	?	Carl Hutter	Ecuador: Pichincha: Reserva Las Gralarias, Five frog creek	-0.03118	-78.70597	Hutter & Guayasamin (2012)
Nymphargus phenax	Fonozoo 185-7907	W. E. Duellman	Peru: Ayacucho: Tutumbaro, 1840 m	-12.72364	-73.94801	Georreferenced from Google Earth
Nymphargus pluvialis	Cocind35-99(1)	Alessandro Catenazzi	Peru: Cusco: near Buenos Aires, along the road Cusco-Pilcopata, 2360 m	-13.15109	-71.59088	Catenazzi *et al*. (2009)
Nymphargus pluvialis	Cocind35-99(2)	Alessandro Catenazzi	Peru: Cusco: near Buenos Aires, along the road Cusco-Pilcopata, 2360 m	-13.15109	-71.59088	Catenazzi *et al*. (2009)
Nymphargus siren	QCAZ46663	Elicio Tapia y Samael Padilla	Ecuador: Provincia Sucumbíos: vía Santa Bárbara - La Bonita	0.45113	-77.54565	Coordinates from AmphibiaWebEcuador (QCAZA46683) (http://zoologia.puce.edu.ec/Vertebrados/anfibios/Mapa.aspx?Id=1209)
Nymphargus truebae	Coctru149-99(8)	Alessandro Catenazzi	Peru: Cusco: near El Mirador along the Cusco-Pilcopata road, 1800 m	-13.07289	-71.55727	Catenazzi *et al*. (2009)

Dataset S1. Continued.

Species	Code (track_name)	Autorship	Locality	Lat	Long	Coordinates source
Nymphargus truebae	Coctru149-99(9)	Alessandro Catenazzi	Peru: Cusco: near El Mirador along the Cusco-Pilcopata road, 1800 m	-13.07289	-71.55727	Catenazzi *et al*. (2009)
Nymphargus truebae	Coctru76-99(5)	Alessandro Catenazzi	Peru: Cusco: near El Mirador along the Cusco-Pilcopata road, 1800 m	-13.07289	-71.55727	Catenazzi *et al*. (2009)
Rulyrana flavopunctata	QCAZ MR-LS110307	Morley Read	Ecuador: Provincia Napo: Parque Nacional Wawa Sumaco. Morley Read (Coordenadas tentativas; esperar respuesta de Morley)	-0.47035	-77.60092	Georreferenced from Google Earth
Rulyrana spiculata	Cocspi150-99(1)	Alessandro Catenazzi	Peru: Cusco: near San Pedro along the road Cusco-Pilcopata, 1525 m	-13.06077	-71.55118	Catenazzi *et al*. (2009)
Sachatamia albomaculata	Sachatamia_albomaculata_AMNH REEL 226 (track 2)	Charles W. Myers	Panama: Bocas del Toro: Río Changuinola, nr Quebrada El Guabo, [16 km airline W Almirante]: 90 m elev	9.29574	-82.55532	Georreferenced from Google Earth
Teratohyla midas	Fonozoo 198_75654	Morley Read	Ecuador: Provincia Orellana: Boanamo, Saladero Venado	-1.25166	-76.38969	http://www.ais.aviacioncivil.gob.ec/designador/SEBW
Teratohyla midas	LS110878-AmpWeEcu	Morley Read	Ecuador: Provincia Orellana: Boanamo, Saladero Venado	-1.25166	-76.38969	http://www.ais.aviacioncivil.gob.ec/designador/SEBW
Teratohyla midas	Another call of Cochranella yellow spots	Yvain	?	?	?	
Teratohyla midas	MZ000013	Santiago Castroviejo-Fisher	Colombia: Amazonas: Biological station Zafire, 14.4 km SW from Tanimboca	-4.00304	-69.89500	Santiago Castroviejo-Fisher

Dataset S1. Continued.

Species	Code (track_name)	Autorship	Locality	Lat	Long	Coordinates source
Teratohyla midas	T_midas_EJRA_GGU_0688	Giussepe Gagliardi-Urrutia	Peru: Iquitos: El Aguajal, Estación biológica José Álvarez Alonso	-3.96825	-73.41731	http://iiap.org.pe/ebjaa/spanish.html
Teratohyla midas	T_midas_EJRA_GGU_0689	Giussepe Gagliardi-Urrutia	Peru: Iquitos: El Aguajal, Estación biológica José Álvarez Alonso	-3.96825	-73.41731	http://iiap.org.pe/ebjaa/spanish.html
Teratohyla midas	Teratohyla midas - Upper Shunte 70 F	Evan Twomey	Peru: Amazonas: Pongo de Rentema, 557 m	-5.29547	-78.40616	Twomey *et al*. (2014)
Teratohyla pulverata	Fonozoo 21_66961	Roberto Ibañez D., A, Stanley Rand, Michael J. Ryan y Cesar A. Jaramillo A.	Panama: ?	?	?	Coordinates from records in GBIF
Vitreorana castroviejoi	10_V4a_04	Santiago Castroviejo-Fisher	Venezuela: Sucre: Cerro El Humo	10.69964	-62.61669	Santiago Castroviejo-Fisher
Vitreorana castroviejoi	11_V4a_04	Santiago Castroviejo-Fisher	Venezuela: Sucre: Cerro El Humo	10.69964	-62.61669	Santiago Castroviejo-Fisher
Vitreorana eurygnatha	FNJV 11743	Adão Cardoso	Brasil: Sao Paulo: São José do Barreiro, Parque Nacional da Serra da Bocaina	-23.03030	-44.70320	Georreferenced from Google Earth
Vitreorana eurygnatha	FNJV 11744	Adão Cardoso	Brazil: Minas Gerais: Poços de Caldas, Morro do Ferro	-21.91667	-46.51667	https://www.mindat.org/maps.php?id=30588
Vitreorana eurygnatha	FNJV 11747	Adão Cardoso	Brazil: Minas Gerais: Poços de Caldas, Morro do Ferro	-21.91667	-46.51667	https://www.mindat.org/maps.php?id=30588
Vitreorana eurygnatha	FNJV 11748	Adão Cardoso	Brazil: Minas Gerais: Poços de Caldas, Morro do Ferro	-21.91667	-46.51667	https://www.mindat.org/maps.php?id=30588

Dataset S1. Continued.

Species	Code (track_name)	Autorship	Locality	Lat	Long	Coordinates source
Vitreorana gorzulae	6_V2_05	Santiago Castroviejo-Fisher	Venezuela: Bolívar: Quebrada Km.127 Crta. El Dorado - Sª Elena	5.96700	-61.39412	Santiago Castroviejo-Fisher
Vitreorana helenae	1_V2_05	Santiago Castroviejo-Fisher	Venezuela: Bolívar: Quebrada Jaspe, San Ignacio de Yuruaní, Parque Nacional Canaima	4.91738	-61.09344	Georreferenced from Google Earth
Vitreorana helenae	3_V2_05	Santiago Castroviejo-Fisher	Venezuela: Bolívar: Quebrada 150 m debajo del salto Karawai	5.69079	-61.86106	Santiago Castroviejo-Fisher
Vitreorana ritae	Co_oyampiensis_SUR	Philippe Kok	?	?	?	
Vitreorana ritae	FNJV_0011063_Vitreorana_ritae_Presidente Figueiredo_AM_Marcio R. Martins	Marcio Martins	Brazil: Amazonas: Presidente Figueiredo, Usina Hidrelétrica de Balbina - Vila Residencial	-1.93549	-59.43026	Coordinates from Google Earth
Vitreorana ritae	Fonozoo 11_67524	Christian Marty & Philippe Gaucher	French Guiana: ?	?	?	
Vitreorana uranoscopa	FNJV 11750	Adão Cardoso	Brazil: São Paulo: Santo André, Paranapiacaba	-23.77429	-46.29569	Coordinates from Google Earth
Vitreorana uranoscopa	FNJV 12951	Cynthia Prado	Brazil: São Paulo: Ilha do Cardoso	-25.16051	-47.97151	Coordinates from Google Earth
Vitreorana uranoscopa	FNJV 12952	Cynthia Prado	Brazil: São Paulo: Ilha do Cardoso	-25.16051	-47.97151	Coordinates from Google Earth
Vitreorana uranoscopa	FNJV 12955	Cynthia Prado	Brazil: São Paulo: Ilha do Cardoso	-25.16051	-47.97151	Coordinates from Google Earth
Vitreorana uranoscopa	Fonozoo 129-6168	Axel Kwet	Brazil: Santa Catarina: Florianópolis (Corrego Grande)	-27.58333	-48.51667	Axel Kwet

Dataset S1. Continued.

Species	**Code (track_name)**	**Autorship**	**Locality**	**Lat**	**Long**	**Coordinates source**
Vitreorana uranoscopa	Fonozoo 129-6173	Axel Kwet	Brazil: Santa Catarina: Florianópolis (Corrego Grande)	-27.58333	-48.51667	Axel Kwet
Vitreorana uranoscopa	Fonozoo 130-6192	Axel Kwet	Brazil: Santa Catarina: Florianópolis (Corrego Grande)	-27.58333	-48.51667	Axel Kwet
Vitreorana uranoscopa	Fonozoo 139-6425	Axel Kwet	Brazil: Santa Catarina: Sao Pedro de Alcantara	-27.56667	-48.78333	Axel Kwet
Vitreorana uranoscopa	Fonozoo 139-6438	Axel Kwet	Brazil: Santa Catarina: Vargem Grande near Águas Mornas	-27.66667	-48.83333	Axel Kwet
Vitreorana uranoscopa	Fonozoo 658_99316	Célio F. B. Haddad, Joâo G. R. Giovanelli, Luis O. Menta Giasson & Luis Felipe Toledo	Brazil: Saulo Paulo: Ribeirâo Branco	-24.21585	-48.76569	ww.fonozoo.com
Vitreorana uranoscopa	Fonozoo 13_99858	Axel Kwet	Brazil: Parana: forest pond in Mata Atlantica, at ca. 600 m, near BR 376, Municip. Guaratuba	-25.55000	-49.00000	Axel Kwet

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Dataset S2. Advertisement call data for Centrolenidae species for which literature data was used.

Species	Structure	Source	#Notes	CallDur(s)	PeakFreq(Hz)	Source	Locality
Celsiella revocata	Tonal	Señaris & Ayarzagüena (2005)	1	0.0252	4084.1000	Señaris & Ayarzagüena (2005)	Venezuela: Aragua: Nacientes del río Tuy, Colonia Tovar
Centrolene geckoideum	Pulsed	Grant *et al*. (1998)	9	0.2951	3874.4087	Grant *et al*. (1998)	Colombia: Valle del Cauca: Dagua, El Queremal
Centrolene hesperium	Pulsed	Cadle & McDiarmid (1990)	2	0.1200	3630.0000	Cadle & McDiarmid (1990)	Peru: Cajamarca: near the basecamp on trail betweem Monte Seco and Chorro Blanco, about 2.5 km NE Monte Seco, Río Zaña, 1800 m
Centrolene venezuelense	Pulsed	Señaris & Ayarzagüena (2005)	4	1.1149	4316.2000	Señaris & Ayarzagüena (2005)	Venezuela: Mérida: La Mucuy
Hyalinobatrachium guairarepanense	Tonal	Señaris & Ayarzagüena (2005)	1	0.1950	3923.0000	Señaris & Ayarzagüena (2005)	Venezuela: Distrito Capital: vertiente sur del Parque Nacional El Ávila
Hyalinobatrachium talamancae	Pulsed	Kubicki *et al*. (2015)	1	0.3000	4900.0000	Kubicki (2007)	Costa Rica: El Limón: Región El Guayacán, 500 masl
Sachatamia ilex	?	Kubicki (2007)	1	0.0500	6000.0000	Kubicki (2007)	Costa Rica: ?
Vitreorana antisthenesi	Pulsed	Wen *et al*. (2012)	1	0.0400	5431.5000	Wen *et al*. (2012)	Venezuela: Cojedes: cerca de San Carlos, arroyo que intersecta la carretera Manrique-La Sierra (9°51'44" N, 68°32'58" W; 564 m a.s.l.)
Hyalinobatrachium mesai	Pulsed	Wen *et al*. (2012)	1	0.0750	4414.5000	Castroviejo-Fisher *et al.* (2011)	Venezuela: Bolívar: southern slope of Sarisariñama tepui, 420 m
Hyalinobatrachium iaspidiense	Pulsed	Wen *et al*. (2012)	1	0.0900	4386.6000	Castroviejo-Fisher *et al.* (2011)	Venezuela: Bolívar: Gran Sabana, Quebrada Jaspe, 800-1000 m
Hyalinobatrachium iaspidiense	Pulsed	Wen *et al*. (2012)	1	0.0700	4628.5000	Castroviejo-Fisher *et al.* (2011)	Guyana: Mabura Hill Forest Reserve, Maiko creek, 60 m
Hyalinobatrachium iaspidiense	Pulsed	Wen *et al*. (2012)	1	0.0600	4810.6000	Castroviejo-Fisher *et al.* (2011)	French Guiana: ?

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Dataset S3. Morphometric and natural history data for Centrolenidae species considered in the phylogenetic comparative analysis.

Species	Sex predominant parental caring	SVL (mm)	Reference	HW (mm)	Reference	TL (mm)	Reference	Calling site	Reference	Note
Celsiella revocata	?	23.6	Señaris & Ayarzagüena (2005)	8.59	Señaris & Ayarzagüena (2005)	12.65	Señaris & Ayarzagüena (2005)	Abaxial	Señaris & Ayarzagüena (2005)	
Celsiella vozmedianoi	Male	28.10	Señaris & Ayarzagüena (2005)	10.31	Señaris & Ayarzagüena (2005)	17.70	Ayarzagüena & Señaris (1996)	Adaxial	Señaris & Ayarzagüena (2005)	HL estimated from holotype
Centrolene altitudinale	?	23.20	Señaris & Ayarzagüena (2005)	8.49	Señaris & Ayarzagüena (2005)	13.46	Señaris & Ayarzagüena (2005)	Adaxial	Señaris & Ayarzagüena (2005)	
Centrolene ballux	Female	20.60	Duellman & Burrowes (1989)	7.60	Duellman & Burrowes (1989)	12.40	Duellman & Burrowes (1989)	Abaxial	Duellman & Burrowes (1989)	"All individuals were found at night on the upper surfaces of leaves of bushes"
Centrolene buckleyi	Female	29.00	Guayasamin *et al*. (2017)*; Lynch & Duellman (1973)	10.63	Lynch & Duellman (1973)	14.85	Lynch & Duellman (1973)	Adaxial	Guayasamin *et al*. (2017)	*Guayasamín, J. M., Frenkel, C., Varela-Jaramillo, A. y Santiago R. Ron 2017. Centrolene buckleyi En: Ron, S. R., Yanez-Muñoz, M. H., Merino-Viteri, A. Ortiz, D. A. (Eds). Anfibios del Ecuador. Version 2018.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. https://bioweb.bio/faunaweb/amphibiaweb/FichaEspecie/Centrolene%20buckleyi, acceso Miércoles, 10 de Enero de 2018.
Centrolene condor	?	27.60	Cisneros-Heredia & Morales-Mite (2008)	9.50	Cisneros-Heredia & Morales-Mite (2008)	14.90	Cisneros-Heredia & Morales-Mite (2008)	Adaxial	Guayasamín, *et al.* (2010)	Guayasamín, J. M., Varela-Jaramillo, A. y Frenkel, C 2010. Centrolene condor. En: Ron, S. R., Guayasamin, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. y Nicolalde, D. A. 2016. AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. <http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=6871>, acceso febrero 17, 2017.

Dataset S3. Continued.

Species	**Sex predominant parental caring**	**SVL (mm)**	**Reference**	**HW (mm)**	**Reference**	**TL (mm)**	**Reference**	**Calling site**	**Reference**	**Note**
Centrolene daidaleum	Male	21.70	Ruiz-Carranza & Lynch (1991)	7.62	Ruiz-Carranza & Lynch (1991)	11.87	Ruiz-Carranza & Lynch (1991)	Abaxial	Cardozo-Urdaneta & Señaris (2010)	
Centrolene geckoideum	Male	75.17	Rueda-Almonacid (1994)	27.93	Rueda-Almonacid (1994)	39.51	Rueda-Almonacid (1994)	Rocks	Rueda-Almonacid (1994)	
Centrolene heloderma	?	31.50	Duellman (1981)	10.74	Duellman (1981)	17.45	Duellman (1981)	Abaxial	Duellman (1981)	
Centrolene hesperium	Female	25.5	Cadle & McDiarmid (1990)	9.2	Cadle & McDiarmid (1990)	14.5	Cadle & McDiarmid (1990)	Adaxial	Cadle & McDiarmid (1990)	Holotype measurements
Centrolene lynchi	Female	24.70	Duellman (1980)	8.62	Duellman (1980)	14.77	Duellman (1980)	Abaxial	Duellman (1980)	
Centrolene peristictum	Female	19.70	Lynch & Duellman (1973)	6.42	Lynch & Duellman (1973)	10.80	Lynch & Duellman (1973)	Adaxial	Maldonado (2012)	
Centrolene sabini	?	30.53	Catenazzi *et al*. (2012)	9.73	Catenazzi *et al*. (2012)	16.65	Catenazzi *et al*. (2012)	outro	Catenazzi *et al*. (2009)	
Centrolene savagei	Male	21.09	Ruiz-Carranza & Lynch (1991)	7.12	Ruiz-Carranza & Lynch (1991)	11.68	Ruiz-Carranza & Lynch (1991)	Abaxial	Vargas-Salinas *et al*. (2007)	
Centrolene venezuelense	?	30.4	Señaris & Ayarzagüena (2005)	10.52	Señaris & Ayarzagüena (2005)	16.39	Señaris & Ayarzagüena (2005)	Adaxial	Señaris & Ayarzagüena (2005)	
Chimerella corleone	?	19.18	Twomey *et al*. (2014)	7.20	Twomey *et al*. (2014)	9.95	Twomey *et al*. (2014)	Abaxial	Twomey *et al*. (2014)	

Dataset S3. Continued.

Species	**Sex predominant parental caring**	**SVL (mm)**	**Reference**	**HW (mm)**	**Reference**	**TL (mm)**	**Reference**	**Calling site**	**Reference**	**Note**
Chimerella mariaelenae	Female	19.5	Guayasamin *et al*. (2013)*	7.40	Cisneros-Heredia & McDiarmid (2006)	10.80	Cisneros-Heredia & McDiarmid (2006)	Adaxial	Guayasamin *et al*. (2013)*	*Guayasamín, J. M., Frenkel, C., Varela-Jaramillo, A. y Cisneros-Heredia, D. F. 2013. Chimerella mariaelenae. En: Ron, S. R., Guayasamin, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. y Nicolalde, D. A. 2016. AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. <http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1572>, acceso abril 18, 2017.
Cochranella erminea	?	23.40	Torres-Castello *et al*. (2007)	7.60	Torres-Castello *et al*. (2007)	13.60	Torres-Castello *et al*. (2007)	Abaxial	Twomey *et al*. (2014)	
Cochranella euknemos	Female	23.90	Savage & Starret (1967)	7.67	Savage & Starret (1967)	16.10	*Estimated from Fig. 1 in Savage & Starret (1967)	Adaxial	Kubicki (2007)	
Cochranella granulosa	Female	25.75	Savage (2002)	9.36	Santiago Castroviejo-Fisher	15.21	Santiago Castroviejo-Fisher	Adaxial	Kubicki (2007)	
Cochranella guayasamini	?	23.60	Twomey *et al*. (2014)	8.10	Twomey *et al*. (2014)	12.40	Twomey *et al*. (2014)	Adaxial	Twomey *et al*. (2014)	
Cochranella mache	?	23.90	Guayasamin *et al*. (2010)*	8.05	Guayasamin & Bonaccorso (2004)	14.45	Guayasamin & Bonaccorso (2004)	Adaxial	Guayasamin *et al*. (2010)*	*Guayasamín, J. M., Varela-Jaramillo, A., Frenkel, C. y Pazmiño-Armijos, G. 2010. Cochranella mache. En: Ron, S. R., Guayasamin, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. y Nicolalde, D. A. 2016. AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. <http://zoologia.puce.edu.ec/vertebrados/anfibios/

Dataset S3. Continued.

Species	Sex predominant parental caring	SVL (mm)	Reference	HW (mm)	Reference	TL (mm)	Reference	Calling site	Reference	Note
Cochranella nola	?	20.93	Harvey (1996)	7.77	Harvey (1996)	12.37	Harvey (1996)	Adaxial	Lötters & Köhler (2000)	
Cochranella resplendens	Female	27.30	Lynch & Duellman (1973)	9.50	Lynch & Duellman (1973)	14.61	Lynch & Duellman (1973)	Adaxial	Teran-Valdez *et al*. (2009)	Eggs on upper surface of leaves
Espadarana andina	?	26.40	Señaris & Ayarzagüena (2005)	8.90	Señaris & Ayarzagüena (2005)	15.29	Señaris & Ayarzagüena (2005)	Adaxial	Señaris & Ayarzagüena (2005)	Eggs on upper surface of leaves
Espadarana audax	Female	23.30	Lynch & Duellman (1973)	8.81	Lynch & Duellman (1973)	12.58	Lynch & Duellman (1973)	Adaxial	Duellman (1975) *in* Guayasamin *et al.* (2018)	Guayasamín, J. M., Frenkel, C., Varela-Jaramillo, A. y Cisneros-Heredia, D. F. 2018. Espadarana audax En: Ron, S. R., Yanez-Muñoz, M. H., Merino-Viteri, A. Ortiz, D. A. (Eds). Anfibios del Ecuador. Version 2018.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. https://bioweb.bio/faunaweb/amphibiaweb/FichaEspecie/Espadarana%20audax, acceso Sábado, 9 de Junio de 2018.
Espadarana prosoblepon	Female	24.10	Lynch & Duellman (1973)	8.22	Lynch & Duellman (1973)	13.16	Lynch & Duellman (1973)	Adaxial	Savage (2002)	
Hyalinobatrachium aff. *bergeri*	?	22.23	Santiago Castroviejo-Fisher	7.90	Santiago Castroviejo-Fisher	11.70	Santiago Castroviejo-Fisher	Abaxial	Santiago Castroviejo-Fisher	
Hyalinobatrachium anachoretus	?	21.00	Twomey *et al*. (2014)	8.10	Twomey *et al*. (2014)	10.85	Twomey *et al*. (2014)	Abaxial	Twomey *et al*. (2014)	
Hyalinobatrachium bergeri	?	21.50	Santiago Castroviejo-Fisher	7.70	Santiago Castroviejo-Fisher	11.80	Santiago Castroviejo-Fisher	Abaxial	Cannatella (1980)	
Hyalinobatrachium cappellei	?	21.80	Castroviejo-Fisher *et al*. (2011)	8.54	Castroviejo-Fisher *et al*. (2011)	11.97	Castroviejo-Fisher *et al.* (2011)	Abaxial	Castroviejo-Fisher *et al*. (2011)	
Hyalinobatrachium carlesvilai	?	22.85	Castroviejo-Fisher *et al*. (2009)	8.38	Castroviejo-Fisher *et al*. (2009)	11.84	Castroviejo-Fisher *et al*. (2009)	Abaxial	Castroviejo-Fisher *et al*. (2009)	

Dataset S3. Continued.

Species	Sex predominant parental caring	SVL (mm)	Reference	HW (mm)	Reference	TL (mm)	Reference	Calling site	Reference	Note
Hyalinobatrachium chirripoi	Male	24.50	Taylor (1958)	8.70	Taylor (1958)	13.75	Taylor (1958)	Adaxial	Guayasamín *et al.* (2010)	Guayasamín, J. M., Varela-Jarami A., Frenkel, C. y Pazmiño-Armijos, 2010. Hyalinobatrachium chirripoi. Ron, S. R., Guayasamin, J. M., Yanez-Muñoz, M. H., Merino-Viteri Ortiz, D. A. y Nicolalde, D. A. 2016 AmphibiaWeb Ecuador. Version 2016.0. Museo de Zoología, Pontif Universidad Católica del Ecuador. <http://zoologia.puce.edu.
Hyalinobatrachium colymbiphyllum	Male	25.60	Taylor (1949)	9.23	Taylor (1949)	13.93	Taylor (1949)	Abaxial	Savage (2002)	
Hyalinobatrachium duranti		21.90	Señaris & Ayarzagüena (2005)	8.19	Señaris & Ayarzagüena (2005)	11.69	Señaris & Ayarzagüena (2005)	Abaxial	Señaris & Ayarzagüena (2005)	
Hyalinobatrachium fleischmanni	Male	20.40	Lynch & Duellman (1973)	8.00	Lynch & Duellman (1973)	11.24	Lynch & Duellman (1973)	Abaxial	Varela-Jaramillo *et al*. (2010)	Varela-Jaramillo, A., Frenkel, C. y Pazmiño-Armijos, G. 2010. Hyalinobatrachium fleischmanni. E Ron, S. R., Guayasamin, J. M., Yanez-Muñoz, M. H., Merino-Viter A., Ortiz, D. A. y Nicolalde, D. A. 2016. AmphibiaWebEcuador. Vers 2016.0. Museo de Zoología, Pontif Universidad Católica del Ecuador. <http://zoologia.puce.edu.ec/vertel os/anfibios/FichaEspecie.aspx?Id= 1>, acceso enero 31, 2017.
Hyalinobatrachium fragile	?	20.30	Señaris & Ayarzagüena (2005)	7.29	Señaris & Ayarzagüena (2005)	10.66	Señaris & Ayarzagüena (2005)	Abaxial	Señaris & Ayarzagüena (2005)	
Hyalinobatrachium guairarepanense	?	22.6	Señaris & Ayarzagüena (2005)	8.68	Señaris & Ayarzagüena (2005)	12.48	Señaris & Ayarzagüena (2005)	Adaxial	Señaris & Ayarzagüena (2005)	
Hyalinobatrachium iaspidiense	?	20.1	Castroviejo-Fisher *et al*. (2011)	7.6	Castroviejo-Fisher *et al*. (2011)	11.6	Castroviejo-Fisher *et al*. (2011)	Abaxial	Castroviejo-Fisher *et al*. (2011)	

Dataset S3. Continued.

Species	**Sex predominant parental caring**	**SVL (mm)**	**Reference**	**HW (mm)**	**Reference**	**TL (mm)**	**Reference**	**Calling site**	**Reference**	**Note**
Hyalinobatrachium kawense	?	20.00	Castroviejo-Fisher *et al*. (2011)	6.90	Castroviejo-Fisher *et al*. (2011)	10.00	Castroviejo-Fisher *et al*. (2011)	Adaxial	Castroviejo-Fisher *et al*. (2011)	
Hyalinobatrachium mesai	?	20.0	Castroviejo-Fisher *et al*. (2011)	8.0	Castroviejo-Fisher *et al*. (2011)	11.2	Castroviejo-Fisher *et al*. (2011)	Adaxial	Castroviejo-Fisher *et al*. (2011)	Only one male observed
Hyalinobatrachium mondolfii	Male	21.30	Señaris & Ayarzagüena (2005)	8.25	Señaris & Ayarzagüena (2005)	11.72	Señaris & Ayarzagüena (2005)	Abaxial	Señaris & Ayarzagüena (2005)	
Hyalinobatrachium orientale	Male	21.50	Señaris & Ayarzagüena (2005)	8.06	Señaris & Ayarzagüena (2005)	11.89	Señaris & Ayarzagüena (2005)	Abaxial	Señaris & Ayarzagüena (2005)	
Hyalinobatrachium orocostale	?	20.10	Castroviejo-Fisher *et al*. (2008)	7.44	Castroviejo-Fisher *et al*. (2008)	10.63	Castroviejo-Fisher *et al*. (2008)	Abaxial	Castroviejo-Fisher *et al*. (2008)	
Hyalinobatrachium pallidum	Male	21.90	Señaris & Ayarzagüena (2005)	8.12	Señaris & Ayarzagüena (2005)	11.80	Señaris & Ayarzagüena (2005)	Abaxial	Cardozo-Urdaneta & Señaris (2012)	
Hyalinobatrachium pellucidum	Male	20.82	Guayasamin *et al.* (2010)*	7.64	Santiago Castroviejo-Fisher	11.67	Santiago Castroviejo-Fisher	Abaxial	Guayasamin *et al.* (2010)*	*Guayasamín, J. M., Ron, S. R., Varela-Jaramillo, A. y Frenkel, C. 2010. Hyalinobatrachium pellucidum. En: Ron, S. R., Guayasamin, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. y Nicolalde, D. A. 2016. AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. <http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1213>, acceso enero 31, 2017.
Hyalinobatrachium talamancae	Male	22.0	Taylor (1958)	8.6	Taylor (1958)	12.2	Taylor (1958)	Abaxial	Taylor (1958)	
Hyalinobatrachium tatayoi	?	22.36	Castroviejo-Fisher *et al*. (2007)	8.36	Castroviejo-Fisher *et al*. (2007)	12.23	Castroviejo-Fisher *et al*. (2007)	Abaxial	Castroviejo-Fisher *et al*. (2007)	

Dataset S3. Continued.

Species	**Sex predominant parental caring**	**SVL (mm)**	**Reference**	**HW (mm)**	**Reference**	**TL (mm)**	**Reference**	**Calling site**	**Reference**	**Note**
Hyalinobatrachium taylori	?	19.12	Señaris & Ayarzagüena (2005)	7.18	Señaris & Ayarzagüena (2005)	10.36	Señaris & Ayarzagüena (2005)	Adaxial	Señaris & Ayarzagüena (2005)	
Hyalinobatrachium tricolor	?	20.50	Castroviejo-Fisher *et al*. (2011)	8.30	Castroviejo-Fisher *et al*. (2011)	10.85	Castroviejo-Fisher *et al*. (2011)	Abaxial	Castroviejo-Fisher *et al*. (2011)	
Hyalinobatrachium valerioi	Male	21.54	Santiago Castroviejo-Fisher	7.90	Santiago Castroviejo-Fisher	11.63	Santiago Castroviejo-Fisher	Abaxial	Savage (2002); Vockenhuber *et al*. (2008)	
Ikakogi tayrona	Female	29.5	Ruiz-Carranza & Lynch (1991)	10.89	Ruiz-Carranza & Lynch (1991)	16.46	Ruiz-Carranza & Lynch (1991)	Abaxial	Vargas-Salinas *et al*. (2015)	
Nymphargus bejaranoi	?	24.20	José M. Padial	8.85	José M. Padial	13.60	José M. Padial	Adaxial	Köhler (2000)	
Nymphargus grandisonae	Female	27.20	Duellman (1980)	9.30	Duellman (1980)	15.67	Duellman (1980)	Adaxial	Duellman (1980)	
Nymphargus griffithsi	Female	24.10	Lynch & Duellman (1973)	7.78	Lynch & Duellman (1973)	13.30	Lynch & Duellman (1973)	Adaxial	Lynch & Duellman (1973)	
Nymphargus lasgralarias	Female	25.34	Hutter & Guayasamin (2012)	8.69	Hutter & Guayasamin (2012)	14.83	Hutter & Guayasamin (2012)	Adaxial	Hutter & Guayasamin (2012)	
Nymphargus phenax	?	21.30	Cannatella & Duellman (1982)	?	-	12.13	Cannatella & Duellman (1982)	Adaxial	Cannatella & Duellman (1982)	
Nymphargus pluvialis	?	27.08	Juan C. Chaparro	9.30	Juan C. Chaparro	16.53	Juan C. Chaparro	Adaxial	Cannatella & Duellman (1982)	

Dataset S3. Continued.

Species	**Sex predominant parental caring**	**SVL (mm)**	**Reference**	**HW (mm)**	**Reference**	**TL (mm)**	**Reference**	**Calling site**	**Reference**	**Note**
Nymphargus siren	?	20.80	Lynch & Duellman (1973)	7.22	Lynch & Duellman (1973)	11.92	Lynch & Duellman (1973)	Adaxial	Duellman *in* Guayasamin *et al.* (2010)	Guayasamín, J. M., Varela-Jaramillo, A. y Frenkel, C. 2010. Nymphargus siren. En: Ron, S. R., Guayasamin, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. y Nicolalde, D. A. 2016. AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. <http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1209>, acceso febrero 01, 2017.
Nymphargus truebae	?	23.30	Duellman (1976)	8.42	Duellman (1976)	13.44	Duellman (1976)	Adaxial	Duellman (1976)	
Rulyrana flavopunctata	?	21.60	Lynch & Duellman (1973)	7.82	Lynch & Duellman (1973)	12.53	Lynch & Duellman (1973)	Adaxial	Lynch & Duellman (1973)	
Rulyrana saxiscandens	?	22.00	Duellman & Schulte (1993)	7.70	Duellman & Schulte (1993)	12.90	Duellman & Schulte (1993)	Adaxial	Duellman & Schulte (1993); E. Twomey (*pers. com.*)	
Rulyrana spiculata	?	22.20	Duellman (1976)	8.04	Duellman (1976)	12.65	Duellman (1976)	Adaxial	Duellman (1976)	
Sachatamia albomaculata	Female	22.58	Santiago Castroviejo-Fisher	7.55	Santiago Castroviejo-Fisher	13.83	Santiago Castroviejo-Fisher	Adaxial	Kubicki (2007)	
Sachatamia ilex	?	29.34	Santiago Castroviejo-Fisher	10.11	Santiago Castroviejo-Fisher	17.54	Santiago Castroviejo-Fisher	Adaxial	Kubicki (2007); Guayasamin *et al.* (2017)	Guayasamin, J. M., Varela-Jaramillo, A. y Frenkel, C. 2017. Sachatamia ilex En: Ron, S. R., Yanez-Muñoz, M. H., Merino-Viteri, A. Ortiz, D. A. (Eds). Anfibios del Ecuador. Version 2018.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. https://bioweb.bio/faunaweb/amphibiaweb/FichaEspecie/Sachatamia%20ilex

Dataset S3. Continued.

Species	Sex predominant parental caring	SVL (mm)	Reference	HW (mm)	Reference	TL (mm)	Reference	Calling site	Reference	Note
Teratohyla pulverata	Female	24.23	José M. Padial	8.32	José M. Padial	14.57	José M. Padial	Adaxial	Bustamante *et al*. (2007)	
Teratohyla midas	Female	18.40	Lynch & Duellman (1973)	6.62	Lynch & Duellman (1973)	10.62	Lynch & Duellman (1973)	Adaxial	Guayasamin *et al.* (2013)	Guayasamin, J. M., Frenkel, C., Ron, S. R. y Ortiz, D. A. 2013. Teratohyla midas. En: Ron, S. R., Guayasamin, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. y Nicolalde, D. A. 2016. AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. <http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1206>, acceso febrero 02, 2017.
Teratohyla spinosa	Female	19.50	José M. Padial	6.91	José M. Padial	11.60	José M. Padial	Adaxial	Guayasamin *et al.* (2010)	Guayasamín, J. M., Varela-Jaramillo, A., Frenkel, C. y Pazmiño-Armijos, G. 2010. Teratohyla spinosa. En: Ron, S. R., Guayasamin, J. M., Yanez-Muñoz, M. H., Merino-Viteri, A., Ortiz, D. A. y Nicolalde, D. A. 2016. AmphibiaWebEcuador. Version 2016.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. <http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1210>, acceso febrero 02, 2017.
Vitreorana antisthenesi	?	25.41	Señaris & Ayarzagüena (2005)	9.10	Señaris & Ayarzagüena (2005)	14.15	Señaris & Ayarzagüena (2005)	Adaxial	Señaris & Ayarzagüena (2005)	
Vitreorana castroviejoi	?	22.50	Señaris & Ayarzagüena (2005)	7.97	Señaris & Ayarzagüena (2005)	13.10	Ayarzaguena & Señaris 1996	Adaxial	Ayarzaguena & Señaris 1996	HL estimated from the holotype
Vitreorana eurygnatha	?	20.40	Heyer *et al*. (1990)	7.34	Heyer *et al*. (1990)	11.22	Heyer *et al*. (1990)	Adaxial	Heyer *et al*. (1990)	
Vitreorana gorzulae	?	20.60	Señaris & Ayarzagüena (2005)	8.38	Señaris & Ayarzagüena (2005)	12.38	Señaris & Ayarzagüena (2005)	Adaxial	Señaris & Ayarzagüena (2005)	
Vitreorana helenae	?	19.10	Ayarzaguena 1992	7.70	Ayarzaguena 1992	11.10	Ayarzaguena 1992	Adaxial	Señaris & Ayarzagüena (2005)	

Dataset S3. Continued.

Species	Sex predominant parental caring	SVL (mm)	Reference	HW (mm)	Reference	TL (mm)	Reference	Calling site	Reference	Note
Vitreorana oyampiensis	Female	18.40	Señaris & Ayarzagüena (2005)	7.62	Señaris & Ayarzagüena (2005)	10.54	Señaris & Ayarzagüena (2005)	Adaxial	Señaris & Ayarzagüena (2005)	
Vitreorana uranoscopa	?	21.20	Heyer *et al*. (1990)	7.63	Heyer *et al*. (1990)	11.87	Heyer *et al*. (1990)	Adaxial	Machado *et al*. (2014); Zaracho (2014); Haga *et al*. (2014)	

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4. ANEXOS – Pareceres da banca de avaliação da dissertação

Pontifícia Universidade Católica do Rio Grande do Sul
FACULDADE DE BIOCIÊNCIAS

DISSERTATION EVALUATION FORM

TITLE OF THESIS/DISSERTATION: The evolution of acoustic mating signals in Glassfrogs (Centrolenidae Taylor, 1951)

CANDIDATE: Moisés D. Escalona Sulbarán

EVALUATOR: Dr. Walter Hödl

1. FORMAL PRESENTATION OF THE TEXT:

1.1. Quality of figures and tables:
(x) Fully Satisfactory ( ) Satisfactory ( ) Insufficient

1.2. Correctness of spelling and grammar:
(x) Fully Satisfactory ( ) Satisfactory ( ) Insufficient

1.3. Language quality regarding precision and objectivity:
(x) Fully Satisfactory ( ) Satisfactory ( ) Insufficient

2. ORIGINALITY AND RELEVANCE OF THE RESEARCH:

2.1. Does the study qualify as an original research project?
(x) Yes ( ) No

2.2. Is the study scientifically relevant?
(x) Yes ( ) No

3. IS THE LITERATURE REVIEW SUFFICIENT AND ADEQUATE TO MEET THE PROPOSED OBJECTIVES?
(x) Yes ( ) Partially ( ) No

4. ARE THE METHODS ADEQUATE TO MEET THE OBJECTIVES OF THE STUDY?
(x) Yes ( ) Partially ( ) No

5. ARE THE RESULTS RELEVANT AND RELIABLE?
(x) Yes ( ) Partially ( ) No

6. DISCUSSION AND CONCLUSIONS:

6.1. Are the results discussed employing relevant points and consistent logic?
(x) Yes ( ) Partially ( ) No

6.2. As the conclusions supported by the data?
(x) Yes ( ) Partially ( ) No

7. QUALITY OF THE DEFENSE (For Ph.D. Dissertations only).
( ) Fully Satisfactory ( ) Satisfactory ( ) Insufficient

8. PLEASE COMPOSE A WRITTEN REVIEW OF THE DISSERTATION/THESIS, AND ENCLOSE IT WITH THIS FORM.

The evolution of acoustic mating signals in glassfrogs (Centrolenidae Tayler, 1951)

In his master`s dissertation, performed in a two year project, the author analyzed the association of shared ancestry, body size, calling site, and environmental noise and parental care with temporal and spectral call parameters in the anuran family Centrolenidae, by creating phenotypic databases and applying modern phylogenetic comparative methods.

The thesis represents an innovative approach to analyze the causes for the variation of acoustic signals among the glassfrogs, a family comprising over 150 species. Acoustic data were compiled for 72 species including representatives of all genera. Data were mainly taken from audio recordings of museum collections. Morphometric and calling site data as well as phylogenetic data were taken from the literature. Thus the real value of the thesis comes from the compilation of a large data set and its application in assessing the associations between acoustic traits and phenotypic as well as environmental variables. Highly remarkable –although still very speculative- is the effort to analyze the temporal dynamic of phenotypic evolution.

I truly congratulate the author to this fantastic overview of the literature on acoustic, morphological, ecological and phylogenetic aspects with regard to anuran behavior. The thesis is a perfect introduction to the evolutionary aspects of (acoustic) anuran behavior . I just wish that with these sound theoretical analyses, readers (or the author himself?) will use it as a starting point for studying the life history and behavior of a single species, as many of the discussed aspects derive only from (very) anecdotal data.

Since I am not very familiar with phylogenetic comparative methods I just will comment on one aspect, which is related to the lack of ecological and/or life history data in the glass frog family.

Contrary to several recent papers on a similar topic - none of the call traits tested in this thesis correlated with potential environmental noise. Even though the author considered a proxy for the occurrence of potentially noisy streamside environments by the Terrain ruggedness index, streamside environments of glassfrogs do not necessarily provide noisy habitats. I agree with the author and I am actually not surprised. In the contrary: glassfrogs are generally the only reproductively active anurans at – often slow-flowing and almost silent - streams whereas f.e. many pond breeding frogs have to deal with the sound produced by co-occuring species, covering a large range of frequencies. Torrent frogs (and I wouldn`t include glassfrogs to this ecologically defined group) such as Hylodes spp. or Staurois spp. breeding at fast-flowing, turbulent streams or even water falls, however, do show clear adaptations to the very noisy environment by producing high pitched sounds (even species with relatively large body sizes!) and occasionally even in combination with visual signals.

With regard to the title I am not convinced that the term acoustic mating signals is fully appropriate. Mating signals (or mating calls) are per definition signals which lead to or finally end with mating. I am not convinced that all analyzed calls have actually led to mating. Most likely the calls function to attract females and/or to keep off males. For these calls the term advertisement call has been accepted ever since Well`s famous Anim.Beh. 1977 paper.

In summary I congratulate the author (and his advisor!) to have considered such an attractive and innovate topic. The paper is well written and the methods –to the best of my knowledge- well applied . The discussion and the presentation of the analytical results is clear. Personally I like the thought- and carefulness which is reflected throughout the paper with regard to a speculative topic such as the evolution of acoustic traits in a little known frog family.

Walter Hödl Vienna, 23.3.2018

9. As a global assessment, please fill in the box below with the statement "PASS" if you consider the Dissertation or Thesis to have fulfilled the requirements for a Ph.D. or M.Sc. degree, respectively. If you do not consider the Dissertation/Thesis to have achieved such level, fill in the word "FAIL".

PASS

PLACE AND DATE: Vienna, 23.3.2018

Walter Hödl

Signature:____________________ _____________

Pontifícia Universidade Católica do Rio Grande do Sul
FACULDADE DE BIOCIÊNCIAS

DISSERTATION EVALUATION FORM

TITLE OF THESIS/DISSERTATION: The evolution of acoustic mating signals in Glassfrogs (Centrolenidae Taylor, 1951)

CANDIDATE: Moisés D. Escalona Sulbarán

EVALUATOR: Dr. Sandra M. Goutte

1. FORMAL PRESENTATION OF THE TEXT:

1.1. Quality of figures and tables:
( ) Fully Satisfactory (x) Satisfactory ( ) Insufficient
1.2. Correctness of spelling and grammar:
(x) Fully Satisfactory ( ) Satisfactory ( ) Insufficient
1.3. Language quality regarding precision and objectivity:
(x) Fully Satisfactory ( ) Satisfactory ( ) Insufficient

2. ORIGINALITY AND RELEVANCE OF THE RESEARCH:

2.1. Does the study qualify as an original research project?
(x) Yes ( ) No
2.2. Is the study scientifically relevant?
(x) Yes ( ) No

3. IS THE LITERATURE REVIEW SUFFICIENT AND ADEQUATE TO MEET THE PROPOSED OBJECTIVES?

( ) Yes (x) Partially ( ) No

4. ARE THE METHODS ADEQUATE TO MEET THE OBJECTIVES OF THE STUDY?

(x) Yes ( ) Partially ( ) No

5. ARE THE RESULTS RELEVANT AND RELIABLE?

( ) Yes (x) Partially ( ) No

6. DISCUSSION AND CONCLUSIONS:

6.1. Are the results discussed employing relevant points and consistent logic?
( ) Yes (x) Partially ( ) No
6.2. As the conclusions supported by the data?
(x) Yes ( ) Partially ( ) No

7. QUALITY OF THE DEFENSE (For Ph.D. Dissertations only)**.**

( ) Fully Satisfactory ( ) Satisfactory ( ) Insufficient

8. PLEASE COMPOSE A WRITTEN REVIEW OF THE DISSERTATION/THESIS, AND ENCLOSE IT WITH THIS FORM.

9. As a global assessment, please fill in the box below with the statement "PASS" if you consider the Dissertation or Thesis to have fulfilled the requirements for a Ph.D. or M.Sc. degree, respectively. If you do not consider the Dissertation/Thesis to have achieved such level, fill in the word "FAIL".

PASS

PLACE AND DATE: Abu Dhabi, 27 March 2018

Signature:________________________________

In his Masters dissertation, Mr Moisés D. Escalona Sulbarán studies call evolution in relation to morphology, habitat, reproductive behavior and shared ancestry in 72 species of glassfrogs. Overall, the dissertation is very well written and agreeable to read, especially for a Masters dissertation. I believe that this manuscript, given some moderate modifications, will be easily published as an article in a scientific journal. The study is overall well designed and complete, although I think that it could have benefitted from being more focused on less questions (see detailed comments below). Mr Escalona Sulbarán clearly put a lot of work in this dissertation, conducting several analyses and sampling an important number of species. This serious work should definitively confer Mr Escalona Sulbarán a M. Sc. degree.

I list below my main comments and attach the annotated manuscript with detailed comments.

Many interesting questions structure this study, and Mr. Escalona Sulbarán came up with six hypotheses to be tested; that might be too many for a single dissertation though. This results in sections that would deserve to be expanded (see comments on the manuscript) and some unsatisfactory tests (hypothesis 2 and 3).

The choice of certain variables would deserve further explanations: Mr Escalona Sulbarán explains that snout-vent length is linked to call dominant frequency, so it does make sense to use it in the analysis. However, the two other morphological measurements, head width and tibia length don't seem directly correlated with acoustic signal production. At several points in the manuscript, one can wonder why these measurements were used. If I understand correctly, those measurements were used especially because they are not linked to call production, in order to compare the evolution rates of morphology and calls. If so, this should be more clearly explained in the methods. It was also not clear to me weather the two measures were corrected for size in the analysis. If that was not the case, then they would mostly reflect size differences between species rather than shape.

I seriously question the use of Terrain Ruggedness Index as a proxy for ambient noise level. Ambient noise level is highly variable both in time and space, with variations between points centimeters away or measures taken at the same point hours from one another. For example, a heavy rain will instantaneously increase noise level. If a stream frog calls next to a boulder, its perceived noise level will highly depend on whether the boulder is in between the stream and the frog or is the frog is between the stream and the boulder. The Terrain Ruggedness Index therefore does not seem an appropriate proxy for ambient noise level. If this work is to be submitted for publication, I would suggest to remove this section altogether; the data is simply insufficient to test hypotheses on call adaptation to noise level (and there is enough data and analyses to make for an interesting paper without this section). This work has some nice results that could be better highlighted by removing unnecessary analyses.

For the call frequency bandwidth, I wonder why Mr. Escalona Sulbarán chose a -20 dB threshold, which is very low.

My evaluation of Mr. Escalona Sulbarán's work was hindered by the fact that I did not have access to any of the data (raw or transformed) in any form. Three datasets are cited in the text but not given. In addition, the Results section is missing a paragraph describing the data: what is a call diversity? What about the morphological measurements? In a Masters dissertation, one would expect to have data and description of the various datasets in an organized manner. Also, I would have liked to see more photos of the frogs, calling in the different positions for example!

I ignore if the length of the dissertation is a requirement, but the manuscript would benefit from expansions, in the methods, results and discussion sections. For example, the phylogenetic comparative analyses are described very briefly and vaguely. Overall, this is a very good dissertation and will be a solid publication after a few changes.

Pontifícia Universidade Católica do Rio Grande do Sul
FACULDADE DE BIOCIÊNCIAS

DISSERTATION EVALUATION FORM

TITLE OF THESIS/DISSERTATION: The evolution of acoustic mating signals in Glassfrogs (Centrolenidae Taylor, 1951)

CANDIDATE: Moisés D. Escalona Sulbarán

EVALUATOR: Dr. Liam J. Revell

1. FORMAL PRESENTATION OF THE TEXT:

1.1. Quality of figures and tables:
(X) Fully Satisfactory ( ) Satisfactory ( ) Insufficient

1.2. Correctness of spelling and grammar:
(X) Fully Satisfactory ( ) Satisfactory ( ) Insufficient

1.3. Language quality regarding precision and objectivity:
(X) Fully Satisfactory ( ) Satisfactory ( ) Insufficient

2. ORIGINALITY AND RELEVANCE OF THE RESEARCH:

2.1. Does the study qualify as an original research project?
(X) Yes ( ) No

2.2. Is the study scientifically relevant?
(X) Yes ( ) No

3. IS THE LITERATURE REVIEW SUFFICIENT AND ADEQUATE TO MEET THE PROPOSED OBJECTIVES?
( ) Yes (X) Partially ( ) No

4. ARE THE METHODS ADEQUATE TO MEET THE OBJECTIVES OF THE STUDY?
(X) Yes ( ) Partially ( ) No

5. ARE THE RESULTS RELEVANT AND RELIABLE?
(X) Yes ( ) Partially ( ) No

6. DISCUSSION AND CONCLUSIONS:

6.1. Are the results discussed employing relevant points and consistent logic?
(X) Yes ( ) Partially ( ) No

6.2. As the conclusions supported by the data?
( ) Yes (X) Partially ( ) No

7. QUALITY OF THE DEFENSE (For Ph.D. Dissertations only)**.**
( ) Fully Satisfactory ( ) Satisfactory ( ) Insufficient

8. PLEASE COMPOSE A WRITTEN REVIEW OF THE DISSERTATION/THESIS, AND ENCLOSE IT WITH THIS FORM.

9. As a global assessment, please fill in the box below with the statement "PASS" if you consider the Dissertation or Thesis to have fulfilled the requirements for a Ph.D. or M.Sc. degree, respectively. If you do not consider the Dissertation/Thesis to have achieved such level, fill in the word "FAIL".

PASS

PLACE AND DATE:

28 March 2018

Revell

Signature:______________________________

Written review of thesis: Moisés D. Escalona Sulbarán

I found this thesis on the evolution of acoustic signaling in glass frogs (Centrolenidae) to be generally very well-written and interesting. I made a number of comments on the text; however, these were provided to improve the writing not as a reflection of serious deficiency. To the contrary, compared to other Master's theses I have reviewed, this one was remarkably well-written and clear.

The study used a unique dataset of acoustic traits for the frog call of centrolenids combined with a variety of other morphological, behavioral, ecological, and proxy ecological traits to attempt to understand the basis for call variation among species. In general, the author found high phylogenetic signal and strong evidence for the effect of body size on call attributes, but less evidence for a direct effect of environmental conditions, such as terrain ruggedness, or call position, such as above or below the leaf.

I unequivocally recommend an evaluation of 'pass;' however, I will nonetheless emphasize some comments which I also noted in my review.

1) For a thesis in which the phylogeny is so integral, there is very little information presented about how the tree was estimated. This is an oversight that should be addressed.
2) Many important references underlying different methods employed in the study are not included. For instance, Paradis (2014) is cited as a general reference for phylogenetic comparative methods, and the phylogenetic regression is attributed to Martins & Hansen (1997).
3) The author should be careful not to over-interpret the results of this study. For instance, in one place the author obtains a 'marginally' ($0.05<P<0.10$) result, notes it as such, and then proceeds to discuss it for a paragraph as if real. There are other less blatant instances.
4) Finally, I'm of the mind that it is almost always better to anticipate criticism than to respond to it. To that end, I would encourage the author to think carefully about the limitations of both data and method. For instance, one possible explanation for the absence of a correlation between TRI and call attributes is that no such correlation exists, or furthermore that relevant variation does not exist because all glass frogs are torrent-adapted (as suggested by the author). Another is that TRI simply does not capture any relevant ecological variation in the environmental attribute (noisiness) of principal interest.

To re-emphasize, however, I think that this document is certainly worthy of being considered a more than worthy Masters' thesis contribution. I look forward to seeing it published.

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