Integrative Zoology

WILEY

INTEGRATIVE ZOOLOGY

ORIGINAL ARTICLE OPEN ACCESS

Allometric Constraint Predominates Over the Acoustic Adaptation Hypothesis in a Radiation of Neotropical Treefrogs

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Received: 30 March 2024 | **Revised:** 2 August 2024 | **Accepted:** 10 October 2024

Funding: This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, ref. 140815/2018-0); Society of Systematic Biology (SSB-GSRA 2018); Programa para el Desarrollo de la Ciencias Básicas (PEDECIBA); Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, 23/2551-0001938-8); Maxwell/hanrahan Foundation; Fonds voor Wetenschappelijk Onderzoek (FWO12A7614N and FWO12A7617N); Marie Skłodowska-Curie Actions (MSCA, 101022238/HOSTILE); Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, #2018/18473-6 and #2022/02789-0); Junta de Andalucía (EMERGIA20_38444).

Keywords: acoustic communication | anura | bioacoustics | macroevolution | phenotypic evolution

ABSTRACT

Male frogs emit stereotypical advertisement calls to attract mates and deter conspecific rivals. The evolution of these calls is thought to be linked to anatomical constraints and the acoustic characteristics of their surroundings. The acoustic adaptation hypothesis (AAH) posits that species evolve calls that maximize propagation distance and reduce signal degradation in the environment where they are emitted. We applied phylogenetic comparative analyses to study the association of body size, vegetation density, type of

Integrative Zoology, 2025; 20:763–773
https://doi.org/10.1111/1749-4877.12920

aquatic ecosystem, and calling site on the evolution of acoustic traits in Cophomantini, a large radiation of Neotropical treefrogs (Hylidae). We obtained and analyzed body size, acoustic, and habitat data from a total of 112 species (58% of Cophomantini), using the most inclusive available phylogeny. We found a significant negative correlation between peak frequency, body size, and calling site, but contrary to the predictions of the AAH, we did not find support for associations among call traits and environmental characteristics. Although spectral allometry is explained by an anatomical constraint, it could also be maintained by female choice. We recommend that future studies strive to incorporate factors such as female mate preferences, eavesdropping by predators or parasites, and genetic drift.

1 | Introduction

Most breeding anuran males emit advertisement calls to attract mates and repel conspecific males from their territory (Wells 2007). Such calls are genetically determined, highly stereotyped, and play an important role in species recognition and mate choice (Ryan 1980; Howard and Young 1998; Wells 2007). The advertisement call is a complex trait usually described by its spectral and temporal properties (Duellman and Trueb 1994). The spectral properties determine the sound frequency of a call, while temporal properties include, among other parameters, the duration of the call, notes, and silent intervals, and their repetition rates (Köhler et al. 2017). Evolutionary processes acting upon these acoustic traits, such as natural and sexual selection or genetic drift, could drive call divergence, potentially leading to reproductive isolation and speciation (Vences and Wake 2007; Wilkins, Seddon, and Safran 2013).

Current explanations for variation in temporal and spectral properties of advertisement calls involve intrinsic and extrinsic factors. Anatomical constraints of the organism emitting the call (Ryan et al. 1988) could drive the evolution of peak frequency (PF; i.e., the frequency emitted with the highest energy) because it is negatively correlated with the body size of the emitter (Zweifel 1968; Ryan and Kime 2003; Wells 2007; Gingras et al. 2013; Muñoz et al. 2020; Tonini et al. 2020). This correlation is attributed to the association between body size and the mass of the vocal cords, the resonant element in anurans (Martin 1971). However, although PF is constrained by this allometric relationship, this trait and others could also be influenced by extrinsic factors, like the environment (Goutte et al. 2016, 2018; Muñoz et al. 2020; Nakamura, Escalona, and Pinheiro 2024).

The acoustic characteristics of habitats represent selective forces that favor the propagation of calls with specific features that reduce their degradation (Morton 1975). According to the acoustic adaptation hypothesis (AAH hereafter; Morton 1975), species that inhabit grassland areas (i.e., areas with low woody vegetation density) are expected to produce acoustic signals with the following characteristics: shorter duration, higher frequency modulation (FM), higher peak frequencies, and broader frequency bandwidth. These characteristics are thought to reduce the attenuation and degradation of calls compared to species inhabiting forested areas (i.e., areas with high vegetation density; Ryan and Kime 2003; Boncoraglio and Saino 2007; Ey and Fischer 2009; Erdtmann and Lima 2013; Da Rosa et al. 2023). Although the original AAH was formulated to explain variation in acoustic signals related to vegetation types, it is now clear that other environmental variables should be considered. For example, background noise can mask acoustic signals in certain environments, such as lotic systems with a strong water current. In such habitats, high-frequency advertisement calls can be positively selected to avoid masking interference from background noise (Feng et al. 2006; Preininger, Bockle, and Hodl 2007; Goutte et al. 2016, 2018; Brunner and Guayasamin 2020). Calls with shorter sequences of notes, separated by long periods of silence, and with pure-tone notes emitted within a narrow frequency bandwidth have been hypothesized as advantageous for communication in systems with a noisy water current (Dubois and Martens 1984).

Calling sites can also have implications for the emitter communication success (Schwartz et al. 2016). The calling site chosen by the emitter may exert selective pressure on calls because sounds degrade more rapidly when broadcasted at ground level than when emitted higher up (Marten and Marler 1977a, 1977b; Piercy, Embleton, and Sutherland 1977; Forrest 1994; Kime, Turner, and Ryan 2000). For example, low-frequency (< 2 kHz) sounds are less attenuated than high-frequency ones when emitted at heights above than 1 m (Marten and Marler 1977a, 1977b; Kime, Turner, and Ryan 2000), while they are more attenuated when emitted between the ground and heights up to 1 m (i.e., ground effect “window,” Marten and Marler 1977a, 1977b). On the other hand, species that vocalize perched on vegetation could benefit from the amplification effect of leaves (Wells and Schwartz 1982; Muñoz and Halfwerk 2022) when the frequency of the calls matches the frequency of leaf resonance (4–6 kHz; Muñoz and Halfwerk 2022). Thus, we expect that species that vocalize on the ground should emit calls at different frequencies than species that vocalize while perched on vegetation.

The Neotropical tribe Cophomantini constitutes a monophyletic group of 193 species of treefrogs (Hylidae) comprised of six genera (Faivovich et al. 2005; Pinheiro et al. 2019; Lyra et al. 2020; Frost 2023; Figure 1). They are primarily nocturnal and arboreal, and they inhabit a variety of environments, ranging from open habitats and dense forests to locations near lotic or lentic environments, such as lakes, ponds, and temporary rain pools (Caramaschi and Cruz 1999; Duellman 2001; Duellman, Marion, and Hedges 2016; Centeno, Vivancos, and Andrade 2021). Species vary in size (snout–vent length [SVL] from 3 to 12 cm; Nakamura, Escalona, and Pinheiro 2024), and calling site, but males of most species vocalize while perched on vegetation (Duellman 2001). The phylogenetic relationships among Cophomantini species are relatively well-explored and stable (Faivovich et al. 2005; Wiens et al. 2005, 2006, 2010; Faivovich, McDiarmid, and Myers 2013; Duellman, Marion, and Hedges 2016; Jetz and Pyron 2018; Pinheiro et al. 2019; Lyra et al. 2020; Dubois, Ohler, and Pyron 2021), with the most taxonomic inclusive phylogeny to date

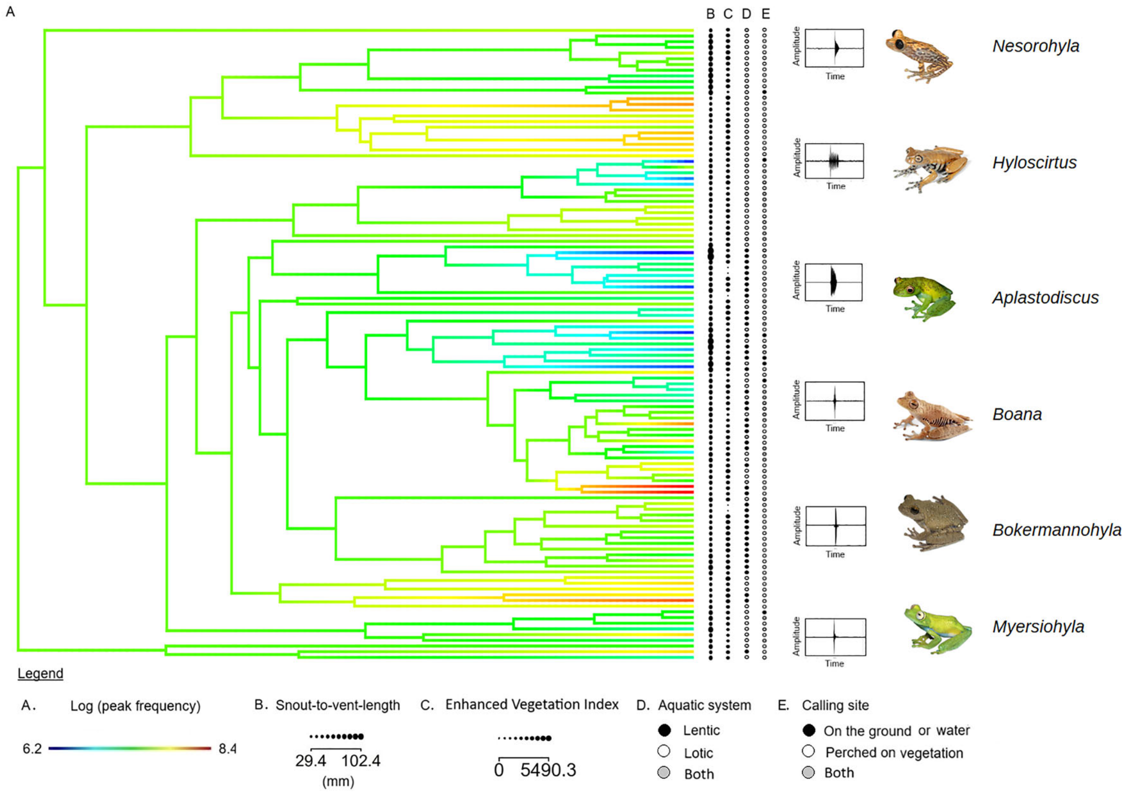

FIGURE 1 | Ultrametric phylogenetic tree (Lyra et al. 2020) representing the variation of phenotypic data and the reconstruction of peak frequency among 112 analyzed Cophomantini species. Black circles on nodes delimit genera, and internal branch colors represent ML reconstructions of log of peak frequency. Oscillograms (x-axis = 1 s) and dorsolateral photos from one representative species per genus. Oscillograms were created with Seewave package (Sueur, Aubin, and Simonis 2008). Photos: Davi Lee Bang, Santiago R. Ron (Anfibios del Ecuador), Paulo Pinheiro, and Philippe J. R. Kok.

including approximately 60% of its currently recognized species diversity (Lyra et al. 2020). Additionally, advertisement calls of many species of the clade have already been described (~ 65%). Thus, this group represents an excellent study model to address different questions regarding the evolution of advertisement calls.

In this study, we applied phylogenetic comparative analyses to test in Cophomantini the intrinsic allometric relationship between body size and call frequency and three predictions of an extended AAH: (i) species that vocalize in open environments emit shorter calls, with more amplitude and frequency modulation, higher peak frequencies, and broader frequency bandwidth than those that vocalize in forest environments; (ii) species that vocalize in lotic environments emit shorter calls, with higher peak frequencies and narrower frequency bandwidths; and (iii) species that vocalize from the ground have calls with different frequencies than those that vocalize perched on vegetation. We discuss the implications of our results for the AAH and how alternative evolutionary drivers could explain the patterns of acoustic evolution in frogs.

2 | Materials and Methods

2.1 | Sampling and Body Size

We examined the recordings of advertisement calls and preserved adult male specimens from personal and museum collections (Supporting Information S1 and S2, respectively). Taxon sampling was focused on the species used by Lyra et al. (2020), who generated the most complete phylogeny for Cophomantini in terms of taxa and characters to date. We analyzed 468 advertisement calls from 100 species (up to 5 calls per species randomly chosen when available) and compiled acoustic data from the literature for 12 species for which we did not get access to sound recordings (Supporting Information S1). We measured the SVL of 648 museum adult male specimens of 91 species (1–5 specimens per species) and complemented this dataset with body size data of 22 species obtained from the literature (Supporting Information S2). We measured SVL using Mitutoyo digital calipers with precision to the nearest 0.1 mm. Although in some cases the recorded and measured specimens were from the same population, our objective was to estimate the mean SVL for species and not the SVL of the recorded males. In total, our sampling covered 58% of Cophomantini species and included representatives of all genera.

2.2 | Acoustic Data

We measured a total of eight acoustic traits. Six of these follow the definitions and terminology of Köhler et al. (2017): call duration (CD), number of notes (NN), PF, frequency bandwidth (BW), call structure (CS), and note type (NT). We measured BW as the difference between the upper and lower frequency boundaries of the notes 6 dB below the PF. This threshold excluded background noise while preserving most of the spectral information related to the notes. We categorized CS as pulsed (when at least one note presented sequential 100% amplitude modulation [AM] between pulses), pulsatile (when at least one note presented sequential < 100% AM between pulses), or tonal (when no sequential AM was present within any note that formed the advertisement call), and NT as simple or complex (one note vs. more than one note per call). In addition to the six variables proposed by Köhler et al. (2017), we measured AM as the difference in relative amplitude between the end and the beginning of the call and FM as the difference in PF between the end and the beginning of the call. For all meristic and continuous variables, we used the average value for each call property measured for all individuals of each species. In the few species in which categorical traits (CS and NT) were variable, we considered the state that occurred most frequently (in all cases > 75% of observations).

We measured temporal traits from oscillograms and spectral variables from power spectra. We estimated power spectra using a fast Fourier transform (FFT) analysis with a Blackman window of 5 ms, 80% of overlap in the time grid, and a DFT size of 512 samples in the frequency grid. We measured relative amplitude from power spectra using the peak power measurement. We carried out all bioacoustical analyses with Raven Pro v. 1.5 (K. Lisa Yang Center for Conservation Bioacoustics 2014).

2.3 | Habitat Data

We approached the average habitat vegetation density for each species using the Enhanced Vegetation Index (EVI; Liu and Huete 1995; Gao et al. 2000) from georeferenced localities obtained from the Global Biodiversity Information Facility (GBIF; Supporting Information S3). The EVI is a spectral-derived index from surface reflectance images that has been empirically validated, provides differences in vegetation density between environments (Huete et al. 2002), and has been used to assess similar questions in bioacoustics (Medina-García, Araya-Salas, and Wright 2015; Mendoza-Henao et al. 2023). We acknowledge that this index is a proxy to habitat structure, which should ideally be described in a more detailed way (e.g., Goutte, Dubois, and Legendre 2013; Escalona, Castroviejo-Fisher, and Simões 2023). However, this is not feasible for a comparative dataset at a continental scale. We obtained the raster files with Landsat monthly EVI values layers between 2000 and 2017 (a total of 211 files; Masek et al. 2006) using the "getHdf" function of the MODIS package (Mattiuzzi et al. 2014). We stacked all the layers and extracted the raster value per locality. We used the mean value per site of all layers calculated using the "stack" function of the RASTER package (Hijmans et al. 2015). We compared statistically the EVI values of a subset of open vegetation localities (i.e., georeferenced sites at which species of Cophomantini have been found) with those of forest localities to validate the applicability of EVI to our dataset (Supporting Information S4). All analyses were conducted using the R software, version 4.3.1 (R Core Team 2023).

We compiled data of the predominant calling site (i.e., perched on vegetation, from ground/water, or both) and the type of aquatic ecosystem (i.e., lotic, lentic, or both) in which each species vocalizes from the literature and complemented it with field observations directly provided by researchers and with our own field observations (Supporting Information S3). Although calling height is a continuous variable, it is not available, as such, for most species. Thus, we decided to include it as a categorical variable and capture some of the variation instead of excluding it altogether.

2.4 | Phylogeny

We used the phylogeny of Cophomantini produced by Lyra et al. (2020) in our comparative analysis framework. This phylogeny includes 115 of the 193 currently named species (60% of Cophomantini species richness) and includes representatives of all currently recognized genera. The phylogeny was inferred from DNA sequences of up to seven mitochondrial and six nuclear genes (totaling 7486 bp), using maximum likelihood analysis under a GTRGAMMA model of the concatenated dataset (Lyra et al. 2020). We updated species identifications according to recent systematic studies (Caminer and Ron 2020; Sturaro et al. 2020; Faivovich et al. 2021; Fouquet et al. 2021). We trimmed the original tree by removing the species for which we had no phenotypic data (Figure 1), using the "drop.tip" function as implemented in the package APE (Paradis, Claude, and Strimmer 2004) in R. We made the tree ultrametric and dichotomous by using the "chronos" and "multi2di" functions of the package APE. For illustrative purposes, we reconstructed the evolution of PF along the branches using the "contMap" function of PHYTOOLS (Revell 2012, 2013) and mapped the variation of SVL, EVI, calling site, and type of aquatic ecosystem on sampled species (Figure 1).

2.5 | Phylogenetic Comparative Analysis

We coded categorical variables (i.e., CS, type of calling site, and type of aquatic ecosystem) as discrete numeric variables. We log or square root-transformed continuous traits to fulfill the requirements of the statistical methods (i.e., normality of the residuals; Freckleton 2009). We carried out a phylogenetic principal component analysis (phylogenetic PCA; Revell 2009) to rule out associations among the acoustic traits. For this, we standardized the variables using the "scale" function of R and then used the "phyl.pca" function of the package PHYTOOLS (Revell 2012), setting a correlation matrix.

We used phylogenetic generalized least-squares (PGLS) models (Martins and Hansen 1997) to assess the correlation between continuous acoustic and environmental variables. PGLS models incorporate phylogenetic relationships among species into the error structure of the linear model, considering a model of phenotypic evolution that best fits the observed data (Grafen 1989; Martins and Hansen 1997). We included SVL as a covariable for the models assessing the correlation with PF and BW as response variables due to the allometric correlation (Tonini et al. 2020; Section 3). We also assessed a model incorporating the interaction of body size with the calling site. We performed the models using the "pgls" function implemented in the package CAPER (Orme et al. 2013), under an extension of the Brownian motion model of trait evolution, estimating the maximum likelihood value of the lambda (λ) parameter, which provides an estimate of the observed covariance among residuals (Freckleton, Harvey, and Pagel 2002; Revell 2010). We quantified the number of

TABLE 1 | Loadings of the phylogenetic principal component analysis of acoustic traits of Cophomantini frogs ($N = 98$). Variance is explained by each principal component in parentheses. Lambda = 0.25.

	PC1 (1.43)	PC2 (1.18)	PC3 (1.10)
Note type	−0.56	0.50	−0.15
Number of notes	−0.72	0.38	−0.25
Call structure	−0.61	−0.39	0.24
Call duration	−0.69	0.05	−0.12
Peak frequency	0.22	0.68	−0.13
Bandwidth frequency	−0.55	−0.36	0.33
Frequency modulation	−0.03	0.37	0.70
Amplitude modulation	0.04	0.32	0.66

independent evolution events of the less frequent calling site state (i.e., calling from the ground/water) using stochastic character mapping (Huelsenbeck, Nielsen, and Bollback 2003; Supporting Information S4).

3 | Results

The final dataset contained 112 species, of which 77 (69%) vocalized perched on vegetation, seven (6%) from the ground or water (Figure 1), and 28 (25%) indistinctly from both sites. Although most sampled Cophomantini frogs vocalize perched on vegetation, calling from the ground/water evolved through 10 independent evolutionary events (Supporting Information S4), justifying our question and subsequent analysis and results. Forty-four species vocalized in lentic ecosystems (39%), 56 vocalized associated with lotic ones (50%), and 12 (11%) were reported indistinctly in both types of aquatic ecosystems. Thirty-two species had tonal advertisement calls, whereas 72 species had pulsed calls. The most frequently occurring NN was 1 (range: 1–28). Mean ± standard deviation (SD), estimated among all species pooled, was 0.42 ± 0.61 s (range: 0.02–3.76 s) for CD, 1898.5 ± 795.4 Hz (range: 468.8–4500.0 Hz) for PF, 446.4 ± 263.1 Hz (range: 109.3–1330.1 Hz) for BW, 7.8 dB ± 19.3 (range: 0.0–178.2 dB) for AM, 183.9 ± 424.2 Hz (range: 0.0–1612.5 Hz) for FM, and 45.9 ± 14.1 mm (range: 29.4–102.4 mm) for SVL. Acoustic, morphometric, and habitat data are available in Dataset 1.

In the phylogenetic principal components analysis (Table 1; Supporting Information S4), we found an association among NT, NN, CD and CS in the first component; CS and BW in the first, second, and third components; and NT and NN in the first, second, and third components. Thus, we did not include NT, NN, and CS in the PGLS models.

As expected, we found a negative correlation between PF and male SVL ($R^2 = 0.30$, $\beta = -45.28 \pm 6.55$ SE, $\lambda = 0.37$, $p \leq 0.001$; Table 2), with body size explaining 30% of the variation of PF. Conversely, we found no statistically significant association between CD, PF, BW, FM, and AM with vegetation density (Table 3). In addition, we did not find an association between PF and lotic environments (Table 4). We found a significant association, although with low explanatory capacity (≤ 7%), between CD, BW, and aquatic ecosystem. We found that the model considering the interaction between body size and calling site explains better PF than the other models (see Table 5). This model shows a negative correlation between PF and calling site interacting with male SVL ($R^2 = 0.38$, $\lambda = 0.39$, $p \leq 0.001$; Table 6), explaining 38% of the variation of PF.

4 | Discussion

Our study is one of the most inclusive, in terms of species sampling, of a Neotropical clade with considerable variation in call, body size, and habitat (Figure 1), with new acoustic and morphometric data. As an ecologically diverse group of treefrogs, we expected that Cophomantini advertisement calls evolved in correlation with body size and, at least partially, in response to selective pressures imposed by the transmission characteristics of the environments in which they are emitted, following predictions derived from the AAH. Below, we discuss our results and provide guidelines for future research on the evolution of acoustic signals and the AAH.

4.1 | Body Size as an Intrinsic Factor of Evolution of Advertisement Calls

The allometric relationship between body size and PF is conserved in frogs (Tonini et al. 2020). The causal link between these variables is the positive correlation between body size and the resonant elements in anurans (Martin 1971). We found a negative relationship between body size and PF (Table 2), which implies a constraint on the evolution of this spectral trait. This result is consistent with those of previous studies in vertebrates (Ryan and Brenowitz 1985; Barclay and Brigham 1991; Fitch 1997; Podos 2001), including anurans (Gingras et al. 2013; Goutte et al. 2016; Escalona et al. 2019; Tonini et al. 2020; De Mello Bezerra, de Carvalho-e-Silva, and Gonzaga 2021; Nakamura, Escalona, and Pinheiro 2024). Our results indicate that PF evolved in correlation with body size in Cophomantini frogs, and it suggests that advertisement calls are index signals (Maynard Smith and Harper 1995, 2003), carrying information about the body size of the emitter, regardless of the vegetation structure. Importantly, PF variation explained by body size in Cophomantini is lower than that found for other anuran clades (30 vs. 38%–66%; Erdtmann and Amézquita 2009; Gingras et al. 2013; Röhr et al. 2016; Tonini

TABLE 2 | Results of a phylogenetic generalized least squares model between peak frequency and snout–vent length (SVL) for Cophomantini frogs.

	N	λ	R^2	$\beta \pm$ SE	t value	p value
Sqrt (Peak frequency)	112	0.37	0.30			
Intercept				118.37 ± 10.91	10.85	< 0.001*
Predictor: log (SVL)				−45.28 ± 6.55	−6.91	< 0.001*

Asterisks (*) indicate significant values ($p < 0.05$).

TABLE 3 | Results of phylogenetic generalized least squares model between call traits and enhanced vegetation index (EVI) for Cophomantini frogs.

	N	λ	R^2	$\beta \pm$ SE	t value	p value
Sqrt (Call duration)	107	0	0.005			
Intercept				2.39 ± 1.53	1.57	0.12
Predictor: log (EVI)				−0.51 ± 0.42	−1.22	0.23
Sqrt (Peak frequency)	107	0.38	0.29			
Intercept				158.25 ± 38.90	4.07	< 0.001*
Predictor: log (SVL)				−45.70 ± 6.78	−6.74	< 0.001*
Predictor: log (EVI)				−10.83 ± 9.85	−1.10	0.27
Sqrt (Bandwidth freq.)	96	0.33	−0.007			
Intercept				1.92 ± 1.29	1.50	0.14
Predictor: log (SVL)				0.23 ± 0.21	1.13	0.26
Predictor: log (EVI)				0.07 ± 0.33	0.22	0.83
Amplitude modulation	96	0	0.006			
Intercept				135.37 ± 99.93	1.35	0.18
Predictor: log (EVI)				−34.61 ± 27.54	−1.26	0.21
Frequency modulation	96	0.07	−0.010			
Intercept				−139.71 ± 1893.07	−0.07	0.94
Predictor: log (EVI)				122.88 ± 521.98	0.24	0.81

Asterisks (*) indicate significant values ($p < 0.05$).

TABLE 4 | Results of phylogenetic generalized least squares model between call traits and aquatic ecosystems (lotic vs. lentic ecosystems) for Cophomantini frogs.

	N	λ	R^2	$\beta \pm$ SE	t value	p value
Log (Call duration)	112	0.88	0.06			
Intercept				−1.10 ± 2.42	−4.53	< 0.001*
Predictor: aquatic ecosyst. 1				0.18 ± 0.11	1.65	0.10
Predictor: aquatic ecosyst. 2				0.39 ± 0.13	2.91	< 0.005*
Sqrt (Peak frequency)	112	0.42	0.28			
Intercept				117.86 ± 11.18	10.53	< 0.001*
Predictor: aquatic ecosyst. 1				−0.84 ± 1.83	−0.46	0.65
Predictor: aquatic ecosyst. 2				−1.69 ± 2.32	−0.73	0.47
Predictor: log (SVL)				−44.48 ± 6.71	−6.63	< 0.001*
Sqrt (Bandwidth freq.)	98	0.13	0.07			
Intercept				10.37 ± 7.64	1.36	< 0.001*
Predictor: aquatic ecosyst. 1				−3.28 ± 1.26	−2.61	0.01*
Predictor: aquatic ecosyst. 2				−0.48 ± 1.70	−0.28	0.78
Predictor: log (SVL)				7.05 ± 4.59	1.54	0.13

Asterisks (*) indicate significant values ($p < 0.05$).

TABLE 5 | Phylogenetic generalized least-squares models fitted to explain the evolution of peak frequency for Cophomantini frogs.

Model	R^2	K	AIC
Sqrt (Peak frequency) ~ log (SVL)	0.30	3	753.77
Sqrt (Peak frequency) ~ log (EVI) + log (SVL)	0.29	4	722.74
Sqrt (Peak frequency) ~ Aquatic system + log (SVL)	0.28	4	757.22
Sqrt (Peak frequency) ~ Calling site	0.12	3	766.25
Sqrt (Peak frequency) ~ Calling site + log (SVL)	0.34	4	748.26
Sqrt (Peak frequency) ~ Calling site × log (SVL)	0.38	4	743.45

Note: $N = 112$.
Abbreviations: R^2: adjusted R^2; K: number of estimated parameters; AIC: Akaike's Information Criterion.

TABLE 6 | Result of phylogenetic generalized least squares model between peak frequency and calling site (water or ground, perched on vegetation or both), considering the interaction with body size, for Cophomantini frogs.

	N	λ	R^2	$\beta \pm$ SE	*t* value	*p* value
Sqrt (Peak frequency)	112	0.39	0.38			
Intercept				0.71 ± 50.94	0.01	0.99
Predictor: Calling site 1				127.78 ± 51.66	2.47	0.01*
Predictor: Calling site 2				83.47 ± 52.50	1.59	0.11
Predictor: log (SVL)				8.64 ± 12.73	0.68	0.50
Predictor: Calling site 1 × log (SVL)				−30.75 ± 12.95	−2.37	0.02*
Predictor: Calling site 2 × log (SVL)				−20.15 ± 13.17	−1.53	0.12

Asterisks (*) indicate significant values ($p < 0.05$).

et al. 2020), although higher than in glassfrogs (16%; Escalona et al. 2019) and that found by Nakamura, Escalona, and Pinheiro (2024) relying only on bibliographic data for Cophomantini (12%). This suggests that other factor(s) besides body size explain the evolution of PF. For example, our analyses show that the interaction of body size with the calling site explains better the variation of PF (Table 5, up to 38%), indicating that the calling site was also relevant in the evolution of PF in Cophomantini frogs as discussed in the next section.

4.2 | The AAH in Cophomantini

The AAH was initially proposed to explain variation in bird acoustic signals in relation to broad vegetation types: grasslands versus forests (Morton 1975). The different structural properties of these environments should act as strong selective forces on acoustic signals, leading species inhabiting each vegetation type to produce vocalizations with spectral and temporal properties that minimize their degradation. In other words, sounds should be adapted to the predominant type of vegetation to transmit information effectively. This hypothesis offers several broad predictions, considering variation in vegetation structure and its impact on the degradation of acoustic signals: increased obstruction of the transmission path by vegetation should be associated with a decrease in PF and FM of calls, transmission in narrower BW, and an increase in CD (Morton 1975; Ryan and Kime 2003; Boncoraglio and Saino 2007; Ey and Fischer 2009; Erdtmann and Lima 2013). Our results do not support any of these predictions in Cophomantini frogs. We did not find differences in terms of CD, PF, FM, BW, NT, or AM among species that inhabit areas with denser vegetation compared to those inhabiting areas with less vegetation density (Table 3).

We acknowledge that our geographic scale may be too coarse to capture patterns that might be occurring at a finer scale (Goutte, Dubois, and Legendre 2013; Goutte et al. 2016; Escalona, Castroviejo-Fisher, and Simões 2023). Additionally, if we consider the largely heterogeneous origin of our dataset, we need to recognize that there are potential biases not considered in our analyses, such as different recording equipment (e.g., microphones, recorders) and conditions (e.g., distance from organisms producing the sound, temperature at the time of recording, social context). However, given the size of our dataset, we argue that any biologically meaningful pattern should have been picked up by our analyses.

A broader evaluation of habitat characteristics, beyond vegetation structure, is needed to explain the evolution of acoustic signals (Goutte, Dubois, and Legendre 2013; Goutte et al. 2018). Torrent habitats could positively select for advertisement calls with higher peak frequencies, which would decrease masking interference with background noise (Feng et al. 2006; Goutte et al. 2016, 2018). We did not find any correlation between spectral call traits and vocalizing from lotic environments (Table 3). Thus, our results are opposite of those of Röhr et al. (2016), Goutte et al. (2016, 2018), and Zhao et al. (2021) that found correlated evolution of spectral traits and vocalization in lotic habitats for anurans in general, ranids, and bufonids. Recently, Nakamura, Escalona, and Pinheiro (2024) found a correlated evolution of PF and vocalization in lotic habitats for Cophomantini frogs. However, the later study used bibliographic data that could lead to a false positive, since different methods and authors could generate different frequency values (Köhler et al. 2017). Thus, our results indicate that call evolution in Cophomantini is different from other anuran clades, and although the reason needs to be investigated in depth, it could be explained by different selective regimes.

The relative position of the calling site could also affect sound integrity because sounds degrade more when broadcasted at the ground level when compared to the same acoustic signal emitted from a higher point above the ground (Forrest 1994; Marten and Marler 1977a, 1977b; Kime, Turner, and Ryan 2000). Our results show that in Cophomantini frogs, the evolution of PF is correlated with the calling site, with an interaction of the later variable and body size (Table 6). The partial slope of our model, considering the interaction of body size and PF (Table 6), indicates that species that vocalize from vegetation are bigger and emit calls at lower PF. Although this result should be treated cautiously due to the scarce variation of calling sites in Cophomantini frogs and our simplification of this variable (i.e., discrete), it mirrors the results of recent independent studies (Tonini et al. 2020; Muñoz et al. 2020). Further research considering the height of the calling site of species can provide more insights about the role of calling position in the evolution of advertisement calls.

Overall, our results are not compatible with the original AAH. The general lack of corroboration for the AAH in our study mirrors the results of previous research with anuran advertisement calls (Ryan, Cocroft, and Wilczynski 1990; Bosch and De la Riva 2004; Penna et al. 2006; Erdtmann and Lima 2013; Goutte et al. 2018; Velásquez et al. 2018; De Mello Bezerra, de Carvalho-e-Silva, and Gonzaga 2021; Hardt and Benedict 2021; Da Rosa et al. 2023; Gillard and Rowley 2023; Mendoza-Henao et al. 2023; Nali, Zamudio, and Prado 2023). A recent meta-analysis of terrestrial vertebrates also failed to find support for a universal role of vegetation structure in the evolution of acoustic communication (Freitas et al. 2024). Does this mean that we should abandon this hypothesis, or that it is false? As we argue below, we defend that rather than rejecting the AAH altogether, it would be more productive to work with a general and expanded version of this hypothesis while recognizing its limitations.

If an expanded version of the AAH is broadly defined as "acoustic characteristics of habitats determine selective forces that favor the propagation of calls with specific features that reduce their degradation," we could potentially invoke many ad hoc scenarios to protect the hypothesis making it difficult to test. Thus, we concur with Goutte et al. (2018) that rather than focusing on falsifying the AAH, the most relevant task is to expand research on acoustic signal evolution by incorporating potentially relevant biological and environmental processes and factors such as evolutionary and developmental constraints (Gould 1980; Smith et al. 1985; Stearns 1986), eavesdropping by predators or parasites (Ryan, Tuttle, and Rand 1982; Bernal, Rand, and Ryan 2006), acoustic partitioning (Weir, Wheatcroft, and Price 2012; Röhr et al. 2016; Sugai et al. 2021; Allen-Ankins and Schwarzkopf 2022), drift (Wilkins, Seddon, and Safran 2013; Da Rosa et al. 2023), and sexual selection (Ryan 1980). In other words, the signals and the environment are decomposable in multiple variables allowing a myriad of potential cause-and-effect relations that should be studied within the expanded version of the AAH at all spatial, temporal, and taxonomic scales.

Finally, adaptation to environmental factors is hardly the only evolutionary cause of variation of phenotypic characters. In our study, we found evidence of correlated evolution between PF, calling site, and male body size in Cophomantini frogs, but no support for most of the predictions of AAH. Although this correlated evolution is explained by an anatomical constraint of body size on the larynx, as well as by sound attenuation by the ground, it could be maintained by female choice (but see Bernardy et al. 2024). Future research could strive to incorporate factors such as female mate preferences, eavesdropping by predators or parasites, and genetic drift to explain the evolution of acoustic traits.

Acknowledgments

We acknowledge the following people for helping us with this study: Alberto Sánchez-Vialas, André Antunes, Antoine Fouquet, Ariovaldo Giaretta, Axel Kwet, Célio F. B. Haddad, David Kizirian, Diego Baldo, Felipe Toledo, Giussepe Gagliardi-Urrutia, José M. Padial, Jörn Köhler, Juliano Romanzini, Karl-Heinz Jungfer, Marco Rada, Miguel Trefaut Rodrigues, Rafael Márquez, Raíssa Furtado, Renan Oliveira, and Renato Recoder. We also thank the Fonoteca Zoológica (www.FonoZoo.com), Museo Nacional de Ciencias Naturales de Madrid (CSIC), the Fonoteca Neotropical Jacques Villard (UNICAMP), the American Museum of Natural History, the Anfibios del Ecuador (Museo de Zoología de la Pontificia Universidad Católica del Ecuador; https://bioweb.bio/faunaweb/amphibiaweb), the Macaulay Library (Cornell University), and the SAPOTECA (biblioteca de sons e vídeos de anuros amazônicos, Centro para Estudos Integrados da Amazônia—CENBAM) for kindly providing sound recordings. We thank Davi Lee Bang and Santiago Ron (Anfibios del Ecuador) for the photos. We thank Eduardo Eizirik, Laura Alencar, and Rafael Márquez for many helpful comments that improved our manuscript. Moisés Escalona received a doctoral scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, ref. 140815/2018-0), a grant from the Society of Systematic Biology (SSB-GSRA 2018), and a postdoctoral fellowship from the Programa para el Desarrollo de la Ciencias Básicas (PEDECIBA) and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, 23/2551-0001938-8). Pedro Peloso was supported by fellowships from CNPq (PCI/MPEG) and by an award from the Maxwell/hanrahan Foundation. Philippe J. R. Kok was supported by research grants from the Fonds voor Wetenschappelijk Onderzoek (FWO12A7614N and FWO12A7617N) and by a Marie Skłodowska-Curie Actions (MSCA) grant (101022238/HOSTILE). Paulo D. P. Pinheiro was supported by the Smithsonian Institution with a Peter Buck postdoctoral fellowship. Daniel Yudi Miyahara Nakamura thanks Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grants #2018/18473-6 and #2022/02789-0). Santiago Castroviejo-Fisher is financed by a research grant from Junta de Andalucía, Spain (EMERGIA20_38444).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.