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Body size, habitat, and sexual selection affect call evolution in Cophomantini treefrogs (Anura: Hylidae: Hylinae)

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ABSTRACT

Advertisement calls are emitted by anuran males to attract females or repel competitors. Call frequencies are coupled with the vocal apparatus and, thus, likely with body size due to allometric constraints. Physical properties of the habitat might affect the evolution of advertisement calls through natural selection, with high- and low-frequency calls expected in lotic and lentic environments, respectively. Conversely, call frequencies may be influenced by sexual selection because low-frequency sounds are predicted to be perceived as more intimidating in intrasexual competition and more attractive in female choice. In addition, although the evolution of dominant frequencies has been investigated in anurans, little is known about fundamental frequencies. Here we investigated whether body size, habitat, and sexual selection are associated with the evolution of dominant and fundamental frequencies of the vocalizations in the Neotropical hylid tribe Cophomantini, using phylogenetic comparative methods. We found that body size and habitat predict dominant frequency across the phylogeny, whereas only body size is correlated with fundamental frequency. Reproduction in lotic environments is plesiomorphic for the tribe, with changes to lentic environments concentrated in some *Boana* clades. Intensity of sexual selection is negatively correlated with dominant frequency in *Bokermannohyla*, in which low-frequency calls are expected under strong sexual selection. Our results illustrate how the interplay between body size, habitat, and sexual selection may affect the evolution of spectral traits.

Keywords: Acoustic Adaptation Hypothesis; communication; Phylogenetic Generalized Least Squares (PGLS); phylogenetic comparative methods; sexual size dimorphism

INTRODUCTION

Advertisement calls are important during anuran communication, mainly for female attraction and male competition (Wells 1977, 2007, Gerhardt 1994, Owings and Morton 1998, Barnard 2004). As traits mediated by physical, biological, and/or sexual components, advertisement calls are under size-frequency allometry (Morton 1977, Bowling *et al.* 2017, Tonini *et al.* 2020). The larynx size is expected to be positively correlated with body size and the acoustic signals are physically shaped by laryngeal components (McClelland *et al.* 1996, but see Purgue 1997, Narins *et al.* 2001). Consequently, a negative correlation between body size and spectral traits [e.g. dominant frequency (DF) and fundamental frequency (FF)] has been reported in most phylogenetic comparative studies, suggesting that smaller anuran species vocalize at higher frequencies than larger species (e.g. Gingras *et al.* 2013, Goutte *et al.* 2016, Escalona *et al.* 2019, Bezerra, Carvalho-e-Silva and Gonzaga 2021). As such, low-frequency sounds could be index signals (i.e. honest signals due to biological constraints) for larger male body sizes (Maynard-Smith and Harper 1995, 2003, Escalona *et al.* 2019).

Female-biased 'sexual size dimorphism' (SSD) is predominant in 90% of anuran species due to natural selection (i.e. fecundity selection, in which larger females have larger clutches of eggs; Shine 1989, Han and Fu 2013, Nali *et al.* 2014,

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Pincheira-Donoso *et al.* 2021). However, in a few species, competition among males and female choice are two sexual pressures expected to invert the female-biased SSD towards a male-biased SSD (Wells 1978, 2007, Shine 1979, Ryan 1988a, Katsikaros and Shine 1997). In these cases, lower DF and FF emitted by larger males are likely more intimidating in male-male acoustic competition (i.e. intrasexual selection; Davies and Halliday 1978, Wagner 1989) and more attractive in female choice (i.e. intersexual selection) when sexual selection is intense (Ryan 1985, Bee *et al.* 2000). Although the role of sexual selection on call frequencies is usually tested within a single species in anurans (e.g. Gerhardt *et al.* 1996), it has not been tested across anuran species as it has been evaluated in other vertebrates (e.g. Mikula *et al.* 2021, Marcolin *et al.* 2022, but see Bernardy *et al.* 2024). As such, it is imperative to assess whether an intense sexual selection favors low-frequency calls in anurans.

In addition to body size and sexual selection, environmental features also might affect spectral traits. When acoustic signals travel from sender to receiver, the physical properties of the habitat might constrain communication (Forrest 1994). For instance, the noise of flowing water in rivers is often characterized by high intensity and low-frequency bands (Goutte *et al.* 2013). Because two sounds that share a similar frequency range may overlap, stream noise might mask low-frequency vocalizations of frogs (Hödl 1977, Arak and Eiriksson 1992, Feng *et al.* 2006, Erdtmann and Lima 2013, Muñoz and Penna 2016). In this regard, the Acoustic Adaptation Hypothesis (AAH) predicts that natural selection maximizes the transmission and content integrity of an acoustic signal (Morton 1975). Thus, the background noise of lotic waters is expected to favor higher DF and FF in anuran calls through natural selection (Ryan 1988a, Wilkins *et al.* 2013, Röhr *et al.* 2016).

Spectral evolution of calls has been investigated using DF in anurans, but FF is largely unexplored. The FF is the base frequency of a natural sound, being the lowest frequency produced by the vocal cord, also called the first harmonic (Bradbury and Vehrencamp 2011). In this sense, it is constrained by physical properties of an organism (i.e. vocal cord length and mass), reflecting some aspects of its morphology, being a potential trait to be explored in an evolutionary framework. However, the identification of the FF in a spectrogram might be a hard task, because there are cases where other harmonics might be more energetic than the FF, generating ambiguities in its identification (Köhler *et al.* 2017). Given the existence of such ambiguities, using FF in evolutionary and taxonomic studies can be complicated. In contrast, the DF is the peak frequency of the call containing the highest sound energy, which might coincide with the FF or not (Cocroft and Ryan 1995, Köhler *et al.* 2017). Given its usually unambiguous identification, DF is widely employed in several studies (e.g. Escalona *et al.* 2019, 2021, Tonini *et al.* 2020). However, DF might be found in different harmonics, and some species are capable of changing it (e.g. Bee *et al.* 2000, Hepp *et al.* 2012). In some taxa, the harmonic band in which the DF is located might vary intraspecifically (e.g. Escalona *et al.* 2021). These facts impose challenges on the establishment of homology hypotheses using DF as a character. As such, given the pros and cons of DF and FF, both traits should be studied to better understand anuran call evolution.

The tribe Cophomantini (Anura: Hylidae: Hylinae) of Neotropical treefrogs is an interesting focal group to study the evolution of advertisement calls. Cophomantini was recognized by Faivovich *et al.* (2005) and currently includes ~190 species grouped into six genera (Pinheiro *et al.* 2019, 2021, Lyra *et al.* 2020, Marinho *et al.* 2022, Brunes *et al.* 2023, Sánchez-Nivicela *et al.* 2023): *Aplastodiscus*, *Boana*, *Bokermannohyla*, *Hyloscirtus*, *Myersiohyla*, and *Nesorohyla*. This tribe exhibits a high variation of advertisement calls, body size (snout-vent length: SVL), sexual size dimorphism, and habitat. For example, *Boana wavrini* (SVL: 89–113 mm, DF: 673–730 Hz; Hoogmoed 1990) is a large tree-frog with low-frequency calls associated with lentic waters, whereas *Boana jimenezi* is an example of small species with high-frequency calls associated with lotic environments (SVL: 27.6–35 mm, DF: 3010–3488 Hz; Señaris and Ayarzagüena 2006).

Recent studies on anuran call evolution (e.g. Röhr *et al.* 2016, Tonini *et al.* 2020) only included a few Cophomantini species, with a low-density sampling of its internal clades. On the other hand, Cophomantini is a clade whose internal relationships are relatively well supported and stable across distinct studies (Faivovich *et al.* 2005, 2013, Wiens *et al.* 2005, 2006, 2010, Pyron and Wiens 2011, Pyron 2014, Duellman *et al.* 2016, Jetz and Pyron 2018, Pinheiro *et al.* 2019, Lyra *et al.* 2020, Dubois *et al.* 2021). Among these, Lyra *et al.* (2020) provided the most comprehensive phylogenetic hypothesis for the tribe, including representatives of all internal groups of species currently recognized in an infrageneric level. Hence, comparative methods and a well-supported phylogeny of Cophomantini might improve our understanding of how advertisement calls evolved in this tribe.

Here we investigated how body size, habitat, and intensity of sexual selection are correlated with spectral parameters (DF and FF) of advertisement calls in Cophomantini, using phylogenetic comparative methods. Specifically, our predictions are: (i) species in which males call along lotic habitats will tend to call at higher frequencies than species in which males call from lentic habitats; (ii) species under intense sexual selection will tend to exhibit low-frequency calls; (iii) body size and call frequency will be negatively correlated across lineages. We tested these hypotheses at tribe- and genus-level to evaluate general and clade-specific patterns. Since DF and FF are not always coincident in Cophomantini, we also assessed the correlation between each of these two response variables and other variables separately (body size, habitat, and sexual-size dimorphism), and reconstructed ancestral character states of the dominant harmonic to discuss implications of using either DF or FF as response variable to test ecological hypotheses.

MATERIAL AND METHODS

Data collection

We searched for advertisement call spectral parameters [fundamental frequency (FF) and dominant frequency (DF)], snout-vent length (SVL), and habitat data of Cophomantini species in a variety of sources: natural history books and fauna inventories (e.g. Duellman 1970, 1997, Lutz 1973, Heyer *et al.* 1990), online life-history database (AmphibiaWeb 2022), and taxonomic literature (see Supporting Information S1). Given the pros and cons for both FF and DF (as explained in the Introduction), we opted to employ both traits as response variables in our study. We also evaluated the character 'dominant harmonic', which considers the harmonic band on which the DF is located (i.e. the most energetic harmonic). For an illustration of FF and DF in a spectrogram, see Supporting Information S2: Fig. S2.1.

Morphological traits included male and female SVL (continuous variables used as a proxy for body size). Following Mikula *et al.* (2021), we used the sexual size dimorphism index (SDI) as a proxy for the intensity of sexual selection: SDI = log (male SVL/female SVL). Positive and negative values of SDI indicate male- and female-biased SSD species, respectively. Thus, the higher the SDI, the more intense the sexual selection (see the rationale in Gage *et al.* 2002, Morrow and Fricke 2004, Charlton and Reby 2016, Mikula *et al.* 2021). As an alternative way to estimate SSD, we also computed residuals from the correlation between male and female SVL (positive and negative values indicate male- and female-biased SSD, respectively). Computed residuals were also implemented in the phylogenetic generalized least squares (PGLS) analyses described below. When only the range of phenotypic values was available (i.e. SVL, DF, and FF values), midpoint values were calculated as a proxy for mean values. Because minimum and maximum values are unavailable for several species and no differences between the use of mean and maximum values were detected according to Pearson's correlations (Supporting Information S2: Fig. S2.2), we used mean values to maximize the sample size. All compiled data can be found in Supporting Information S1.

We also scored habitat as lentic (slow-flowing water; e.g. lakes, temporary ponds, and swamps) or lotic (rapid-flowing water; e.g. torrential streams, rivers, and waterfalls). Because some species are reported in both lentic and lotic habitats (Supporting Information S1), those taxa were scored as ambiguous (i.e. polymorphic). However, it is not possible to ascertain if these reports correspond to a true polymorphism or if they are a result of biased interpretation by the respective original authors. Because it is not possible to disentangle these ambiguities, we only considered species with unambiguous data for habitat in PGLS analyses, following previous studies testing the AAH in anurans (e.g. Röhr *et al.* 2016, Zhao *et al.* 2021).

Phylogeny

Phenotypical data from different species cannot be treated as statistically independent because species are part of a hierarchically structured phylogeny (Felsenstein 1985). Here we employed the maximum likelihood (ML) phylogenetic hypothesis of Cophomantini from Lyra *et al.* (2020), as this is the taxonomically most inclusive hypothesis available for the group. The original tree included 128 terminals of Cophomantini, being 119 recognized species, with representatives of all current genera and their respective internal species groups. After its publication, modifications in terminal names of *Boana* followed recent taxonomic changes: (i) *Boana latistriata* is now considered a junior synonym of *Boana polytaenia* (Faivovich *et al.* 2021) and was pruned from the tree; (ii) *Boana* clade H and *Boana* clade J were named as *Boana courtoisae* and *Boana steinbachi*, respectively, as updated by Fouquet *et al.* (2021); (iii) the sample referred to as *Boana cinerascens* by Lyra *et al.* (2020) was named as *Boana gracilis*, as updated by Sturaro *et al.* (2020); (iv) terminals of *Boana* candidate G1 and *Boana hutchinsi* were named as *Boana appendiculata* and *Boana ventrimaculata*, respectively, as updated by Caminer and Ron (2020). The tree was ultrametricized and time-scaled using the *chronos* function from 'ape' (Paradis *et al.* 2004) before downstream analyses. Before each analysis, we checked whether the terminals from the tree matched with the rows from the dataset. Terminals with missing data were pruned from the tree.

Phylogenetic signal

We calculated the phylogenetic signal to test if our data shows a significant tendency of related species to resemble one another (Blomberg *et al.* 2003). For habitat (discrete character), we estimated the D value (Fritz and Purvis 2010) using the *phylo.d* function of the package 'caper' (Orme *et al.* 2018) in R v.4.0.1 (R Core Team 2020). If $D > 1$, the binary trait is overdispersed across the phylogeny; if $D = 0$, the trait is distributed as expected under the Brownian motion; if $D < 0$, the trait is more conserved than the Brownian expectation. Because the D value is only used for binary characters, we removed taxa with ambiguous values on habitat from the dataset.

For body size, DF, FF, and SDI (continuous characters), we estimated Pagel's (1997, 1999) lambda ($\lambda \approx 0$ indicates no phylogenetic signal; $\lambda \approx 1$ indicates a strong phylogenetic signal) and Blomberg's K ($K \approx 1$ indicates that trait similarity is proportional to divergence under the Brownian motion model; $K > 1$ indicates that the traits are more similar; and $K < 1$ indicates that traits are more different than expected under Brownian motion; Blomberg *et al.* 2003) using the *phylosig* function in the R package 'phytools' (Revell 2012). Although Pagel's λ and Blomberg's K assume the Brownian motion model of trait evolution (i.e. random walk divergence in species resemblance), these metrics are not correlated with each other (Diniz-Filho *et al.* 2012), and thus we calculated both. To evaluate the significance of phylogenetic signals, we used the *phylosig* function (Revell 2012) to calculate the *P*-value of the likelihood ratio test for Pagel's lambda and *P*-value from the randomization test for Blomberg's K.

Ancestral character state reconstruction

We performed ancestral character states reconstruction for DF, FF, body size (male and female SVL), SDI, and habitat. For this, we employed ML ancestral states, Fitch's optimization, and squared-change parsimony as follows. Maximum Likelihood ancestral states for continuous characters (DF, FF, SDI, male and female SVL) were estimated with 95% confidence intervals using the *fastAnc* function in the 'phytools' R-package (Revell 2012); for discrete characters (dominant harmonic and habitat), we used the *make.simmap* function (nsim = 1000) by fitting three evolutionary models: (i) the equal-rates model (ER), in which a single parameter drives all transition rates at the same rate (Pagel 1994, Lewis 2001); (ii) the symmetric model (SYM), in which only forward and reverse transitions share the same rate; and (iii) the all-rates-different model (ARD), in which each rate is a unique parameter (Paradis *et al.* 2004). Using the *fitDiscrete* function of the 'geiger' R-package (Harmon *et al.* 2008), we selected the model that best describes the evolution of dominant harmonic and habitat use with Akaike weights (AICw). Additionally, we also employed Fitch's optimization (Fitch 1971) for categorical variables (dominant harmonic and habitat) and squared-change parsimony (Maddison 1991) for continuous variables (DF, FF, SDI, male and female SVL) using Mesquite v.3.03 (Maddison and Maddison 2015).

Phylogenetic Generalised Least Squares

We ran PGLS analyses to account for the statistical non-independence of phylogeny-structured data (*corPagel* function from the 'ape' package; Paradis *et al.* 2004), using the *gls* function from the 'nlme' package (Pinheiro *et al.* 2023). To account for the best fitting evolutionary model as error structure, we used the *fitContinuous* function in 'geiger' (Harmon *et al.* 2008) to compare three models: (i) Brownian motion (BM), which assumes that the correlation among trait values is proportional to the extent of shared ancestry for pairs of species (Felsenstein 1973); (ii) the Ornstein–Uhlenbeck model (OU), which is used to model trait evolution with the tendency towards a central value, such as under stabilizing selection (Butler and King 2004); (iii) the Early Burst model (EB), which assumes that traits begin diversifying rapidly, followed by a reduction in their diversification rates (Yoder *et al.* 2010), as suggested to be a product of species entering new adaptive zones (Simpson 1945). We calculated Akaike Information Criterion corrected for small sample size (AICc) and Akaike weights (AICw) for all models to assess model support. The lowest AICc and highest AICw indicated the best fitting model, which was incorporated as error structure in PGLS analyses.

All PGLSs accounted for both topology and branch lengths from the phylogeny. Like terminals without data were pruned from the tree, taxa that have data available but are not in the tree were removed from the dataset for PGLS analyses. To check for multicollinearity between predictors, variance-inflation factors (VIFs) were calculated using the vif function in the 'car' package (Fox and Weisberg 2019). Because all VIFs were less than five (VIFmax = 1.51) as recommended by Ringle *et al.* (2015), we kept all predictor variables. Following Mundry (2014), all PGLS analyses were verified to assess their adherence to the underlying assumptions (e.g. calculating Shapiro–Wilk and Breusch–Pagan tests for normality and homogeneity of the residuals, respectively, plus visual inspections; Supporting Information S2 and S3: Figs S2.3, S3.1). PGLS models were run in two strategies: (i) considering all species of Cophomantini; and (ii) considering each genus separately to evaluate general and clade-specific patterns, respectively. *Myersiohyla* and *Nesorohyla* were not analysed separately in the second strategy due to sample size limitations (i.e. a minimum of three species with SDI and DF is required to perform PGLS and linear regressions).

The full models considered log DF (Hz) and log FF (Hz) as response variables and log male body size (mm), habitat (lentic and lotic), log SDI, and their interactions as predictor variables. Simpler models were built by removing the least significant predictors until reaching the null model (log DF ~ 1 and log FF ~ 1). The following model was selected according to the Akaike Information Criterion (AIC = -63.37):

$$\begin{aligned} \textit{Call frequencies} = \beta_0 + \beta_1 \times \textit{maleSVL} + \beta_2 \times \textit{habitat} \\ + \beta_3 \times \textit{SDI} + \beta_4 \times \textit{maleSVL} \times \textit{habitat} \end{aligned}$$

In the above model, call frequencies (DF and FF) are the response variables, while male SVL, habitat, SDI, and the interaction between male SVL and habitat are predictors (β_0 is the intercept; β_{1-4} are the angular coefficients for each predictor). Given that DF and FF are not significantly correlated with the same predictors, we explored the differences in distributions of both spectral traits. As such, we analysed the correlation between DF and FF, labelling each species when a coincidence between DF and the first harmonic band (= FF) is present, absent, or ambiguous (i.e. when the DF and FF are reported as either matched or mismatched in literature).

We also performed two additional analyses. First, for 33 species (Supporting Information S1), we were only able to rely on a single measurement of FF from spectrogram figures because the original authors did not report the values of FF from multiple specimens; thus, we conducted PGLS analyses with and without these species. Second, although only 19% (six out of 31 species) of *Bokermannohyla* are present in the tree (Lyra *et al.* 2020), SSD and DF data are available for 80% (25/31) of this genus. Thus, we performed linear models without considering phylogenetic non-independence for single-genus analyses to maximise sample size (especially in the case of *Bokermannohyla*)—these were performed as exploratory analyses only and we are aware that only our PGLS results should be considered due to the non-independence of phylogeny-structured data.

RESULTS

From the 128 terminals originally included in the phylogeny of Cophomantini, ten were pruned out from the tree, resulting in a final dataset including 118 species. From the 116 species evaluated for habitat, 56 (48%) call at lotic habitats, 45 (39%) call at lentic habitats, and 15 (13%) can be found calling in both lentic and lotic habitats (Table 1).

The mean DF among Cophomantini frogs was 1865 Hz, ranging from 272 Hz in *Boana rosenbergi* (Duellman 1970) to 6447 Hz in *B. polytaenia* (as *Hypsiboas* aff. *polytaenius*; Pinheiro *et al.* 2012). Mean FF was 1624 Hz, ranging from 114 Hz in *B. boans* (Duellman 1970) to 6447 Hz in *B. polytaenia* (Pinheiro *et al.* 2012). Mean SVL was 46.65 mm for adult males, ranging from 27.05 mm in *B. buriti* (Caramaschi and Cruz 1999) to 119.9 mm in *B. boans* (Duellman 1970), and 52.69 mm for adult females, ranging from 27.5 mm in *B. alemani* (Rivero 1964) to 116.9 mm in *B. boans* (Duellman 1970). Sexual size dimorphism is female-biased (log SDI < 0, *N* = 93) for most species, with some few male-biased exceptions (log SDI > 0, *N* = 11). Log SDI ranged from -0.2055 in *B. claresignata* (Lutz and

Table 1. Dominant frequency (DF: Hz), fundamental frequency (FF: Hz), and male body size (SVL: mm) categorized into habitat types. Sample size (*N*), mean, and standard deviation are provided for each variable

Habitat	*N*	Mean DF	SD DF	Mean FF	SD FF	Mean male SVL	SD male SVL
Lentic	45	1815.08	1085.96	1505.51	1131.26	45.70	16.26
Lotic	56	2050.79	747.31	1633.05	664.98	46.62	12.96
Lentic and lotic	15	1646.71	836.25	1232.75	555.05	47.69	22.76

Orton 1946) to 0.1122 in *B. wavrini* (Hoogmoed 1990). The mean DF found for lotic habitats was 2050 Hz, and for lentic habitats was 1815 Hz (Table 1). *Bokermannohyla* is the genus with average larger males ($F = 4.0846$; $P < 0.01$; Supporting Information S2: Fig. S2.4) and with average higher SDI ($F = 7.7682$; $P < 0.001$; Supporting Information S2: Fig. S2.6). Additional exploratory data analyses are illustrated in Supporting Information S2.

Phylogenetic signal

Estimates of phylogenetic signal indicated a Pagel's λ significantly different from zero ($P < 0.05$) for DF ($\lambda = 0.73$), FF ($\lambda = 0.93$), male SVL ($\lambda = 0.96$), and female SVL ($\lambda = 0.81$). Furthermore, estimates of Blomberg's K indicated a significant phylogenetic signal for DF ($K = 0.37$), FF (0.49), male SVL ($K = 0.52$), and female SVL ($K = 0.40$; Table 2). Estimates of D value indicated that the habitat ($D = -036$, pRandom = 0, pBrownian = 0.566) is highly conserved across phylogeny.

Ancestral character state reconstruction

The reconstructed ancestor of Cophomantini exhibited a high DF (DF$_{\text{Max. Likelihood}}$ = 2170.09 Hz; Figs 1, 2; DF$_{\text{Parsimony}}$ = 2266.1–2863.4 Hz; Supporting Information S4: Fig. S4.1A–B), with a lower mean FF than DF (FF$_{\text{Max. Likelihood}}$ = 788.01 Hz; FF$_{\text{Parsimony}}$ = 1355.6–1904.9 Hz; Supporting Information S4: Fig. S4.1C–D), and a female-biased sexual size dimorphism (male SVL$_{\text{Max. Likelihood}}$ = 43.71 mm; female SVL$_{\text{Max. Likelihood}}$ = 51.51 mm; SDI$_{\text{Max. Likelihood}}$ = -0.015; male SVL$_{\text{Parsimony}}$ = 38.45–47.5 mm; female SVL$_{\text{Parsimony}}$ = 47.22–55.93 mm; SDI$_{\text{Parsimony}}$ = -0.0168–-0.0148; Supporting Information S4: Figs S4.2, S4.3). All ancestral character state reconstructions are illustrated in Supporting Information S4.

The all-rates-different model (ARD; AICc = 195.5707) fits better than both the equal-rates model (ER; AICc = 200.6887) and the symmetric model (SYM; AICc = 199.6318) for habitat. The reconstructed ancestor of Cophomantini was found to vocalize at lotic habitats according to Fitch's optimization (parsimony approach; Supporting Information S4: Fig. S4.4A), whereas the ARD model indicated ambiguous probabilities between lotic and lentic habitats (Fig. 3A; Supporting Information S4: Fig. S4.4B). SYM (AICc = 219.3043) fits better than ER (AICc = 226.2394) and ARD (AICc = 226.4467) for the dominant harmonic. The reconstructed ancestor of Cophomantini was found to vocalize with the highest energy concentration at the first harmonic according to Fitch's optimization (Supporting Information S4: Fig. S4.5A) and the SYM model (Fig. 4A; Supporting Information S4: Fig. S4.5B).

Phylogenetic Generalised Least Squares

Evolutionary trajectory of all variables was best explained by the Ornstein–Uhlenbeck model (Table 3), which was incorporated as an error structure. PGLS models for DF ($R^2 = 0.2271$; $F = 7.17$; $P < 0.001$) and FF ($R^2 = 0.3051$; $F = 10.11$; $P < 0.001$) as response variables are significant (Table 4). We found a statistically significant negative correlation between DF and male SVL ($R^2 = 0.1221$; $\beta = -1.11$; $t = -5.23$; $P = < 0.001$; Fig. 3B). Dominant frequency is statistically associated with habitat, in which lotic breeders tend to call at higher-frequency bands (mean DF = 2050.79 Hz), whereas lentic breeders call at lower-frequency bands (mean DF = 1815.08 Hz; $t = -2.77$; $P < 0.01$; Fig. 3C). Furthermore, the interaction between male SVL and habitat is a significant predictor of DF ($\beta = 0.86$; $t = 2.79$; $P < 0.01$), in which the negative slope between DF and male SVL is lower in lentic than lotic species (Fig. 3C). This interaction also revealed that call frequency differences between lotic and lentic species are lower at smaller sizes, whereas lotic males tend to call at higher frequencies than lentic males at larger body sizes.

Dominant frequency and sexual size dimorphism index (SDI) are not associated with each other when we consider all species in PGLS ($P > 0.05$; $N = 94$; Fig. 5A), but a negative correlation was found for *Bokermannohyla* when we considered each genus separately ($P < 0.01$; $N = 6$; Fig. 5B). *Aplastodiscus* ($P > 0.05$; $N = 8$), *Boana* ($P > 0.05$; $N = 62$), and *Hyloscirtus* ($P > 0.05$; $N = 18$) did not corroborate that result. PGLS using the residuals between male and female SVL as an alternative proxy for sexual dimorphism were congruent with those using SDI (Supporting Information S2: Table S2.1). The results of the linear regressions (Supporting Information S2: Table S2.1) corroborate the PGLS results found for *Bokermannohyla*, indicating a negative correlation between SDI and DF.

Although a high correlation between DF and FF was found ($R^2 = 0.5485$, $P < 0.001$; Fig. 4B–C), the significant predictors of DF and FF models are different (Table 4). PGLS revealed that FF, differently from what is described above for DF, is negatively correlated only with male SVL ($\beta = -1.34$; $t = -5.69$, $P = < 0.001$) but it is not correlated with other traits (habitat: $t = -1.22$; $P > 0.05$; SDI: $t = -0.01$; $P > 0.05$; see scatter plots in Supporting Information S3: Figs S3.2 and S3.3). PGLS analyses excluding data of 33 species relying on single measurements of fundamental frequency were congruent with those including all data, that is, only male body size is correlated with FF (Supporting Information S3: Table S3.1).

Table 2. Phylogenetic signal of quantitative traits (Pagel's λ and Blomberg's K) for dominant frequency [log DF (Hz)], fundamental frequency [log FF (Hz)], body size [log Male SVL (mm) and log Female SVL (mm)], and sexual dimorphism (SDI). Note the *P*-value < 0.05 for all parameters presented

Variable	*N*	Pagel's λ	*P*-value	Blomberg's K	*P*-value
log DF	108	0.73	1.48112×10^{-6}	0.37	0.001
log FF	108	0.93	3.20711×10^{-9}	0.49	0.001
log Male SVL	117	0.96	2.23881×10^{-14}	0.52	0.001
log Female SVL	104	0.81	6.44652×10^{-9}	0.40	0.003
log SDI	103	0.93	1.8452×10^{-10}	0.28	0.003

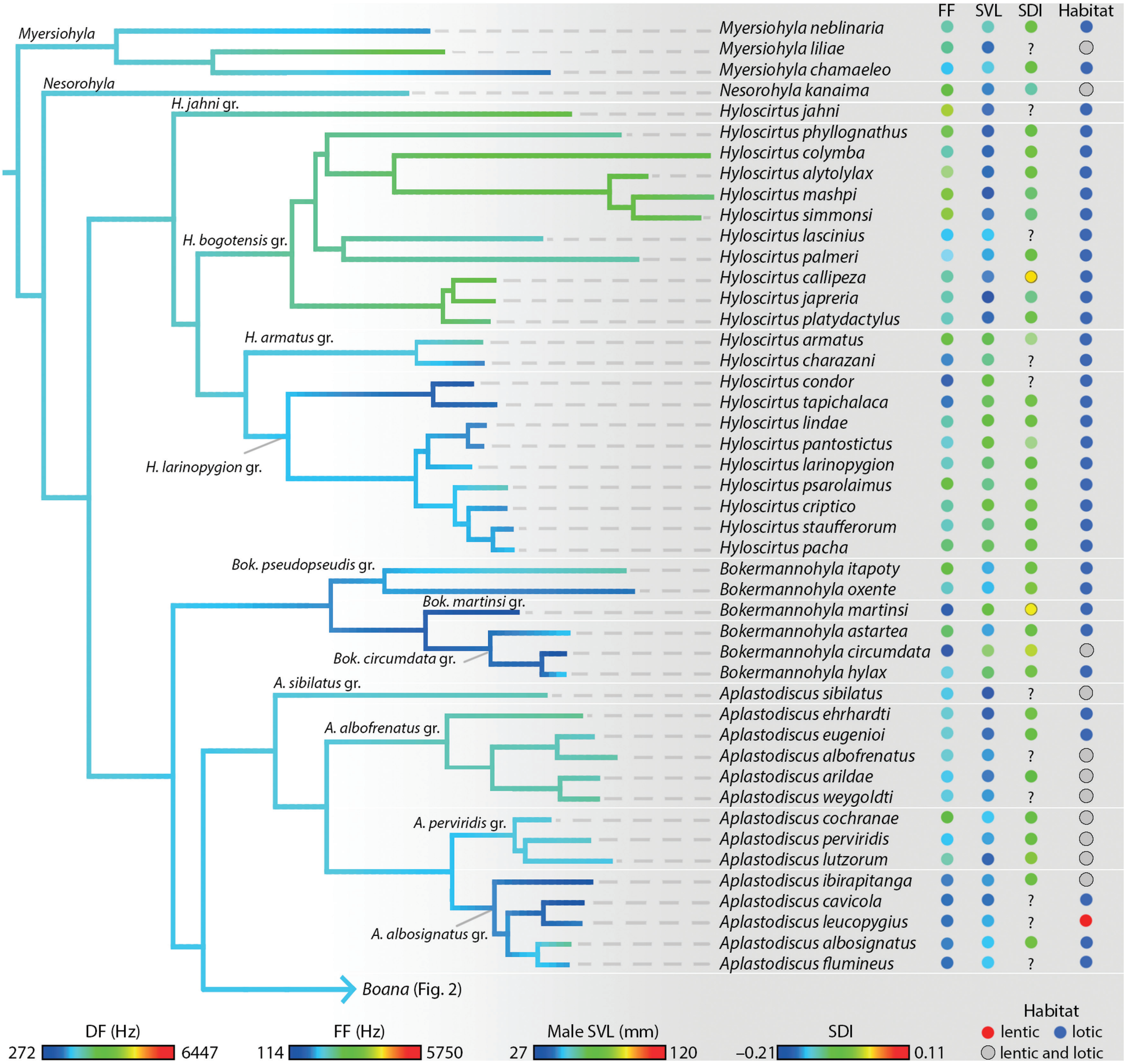

Figure 1. Maximum likelihood (ML) ancestral state reconstruction of dominant frequency in Cophomantini [pruned ML tree from Lyra *et al.* (2020), with 109 species]. The distribution of fundamental frequency, male body size, sexual size dimorphism, and habitat is shown on the right. This tree was ultrametricized and time-scaled before PGLS analyses.

DISCUSSION

The hypothesis that closely related species tend to emit similar vocalizations in Anura has been well supported by several studies (e.g. Cocroft and Ryan 1995, Robillard *et al.* 2006, Goicoechea *et al.* 2010, Gingras *et al.* 2013, Escalona *et al.* 2019, Tonini *et al.* 2020). This is the first time that it was quantitatively evaluated for Cophomantini. Our results indicated that the phylogenetic signal is significant for DF, FF, habitat use, and male and female SVL (Table 2). However, phylogenetic signal does not let us infer about the evolutionary processes underlying these traits. Historically, high phylogenetic signals used to be interpreted as low rates of evolutionary change, whereas low phylogenetic signals were interpreted as rapid evolution rates (e.g. Gittleman *et al.* 1996, Blomberg *et al.* 2003). Revell *et al.* (2008) demonstrated that these evolutionary patterns are not always true and, thus, other analyses are required to better understand the evolution of a specific trait (e.g. fitting different evolutionary models).

Best fitting models (Table 3) indicated that DF, FF, habitat, and body size of Cophomantini follow the Ornstein–Uhlenbeck model (OU), which suggests that these traits evolved under stabilizing selection, but lineages might shift optimum trait values when occupying new selective regimes (Butler and King 2004, O'Meara 2012). Our analyses revealed that both body size (male and female SVL) and vocalization spectral traits (DF and FF) present high phylogenetic signals, which is consistent with the idea that random walk with central tendency in OU

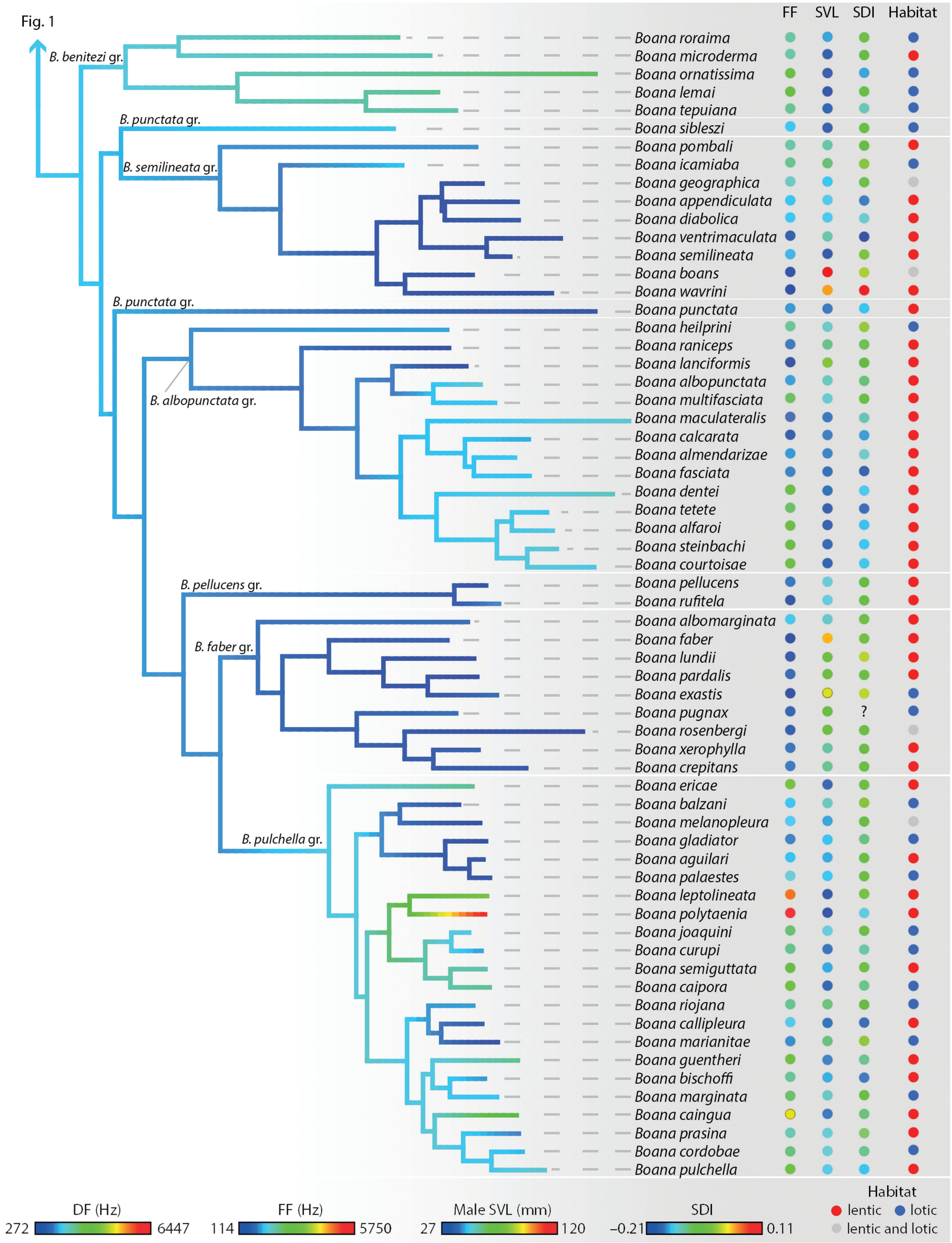

Figure 2. Maximum Likelihood (ML) ancestral state reconstruction of dominant frequency in Cophomantini (continued from Fig. 1).

model leads to higher λ and K values as the stabilizing pressure becomes stronger (e.g. Zanne *et al.* 2005, Ossi and Kamilar 2006, Swenson *et al.* 2007). Although morphology usually exhibits a higher phylogenetic signal than behavior (Blomberg *et al.* 2003), FF presented a phylogenetic signal as high as body size—probably due to larynx constraints (e.g. McClelland *et al.*

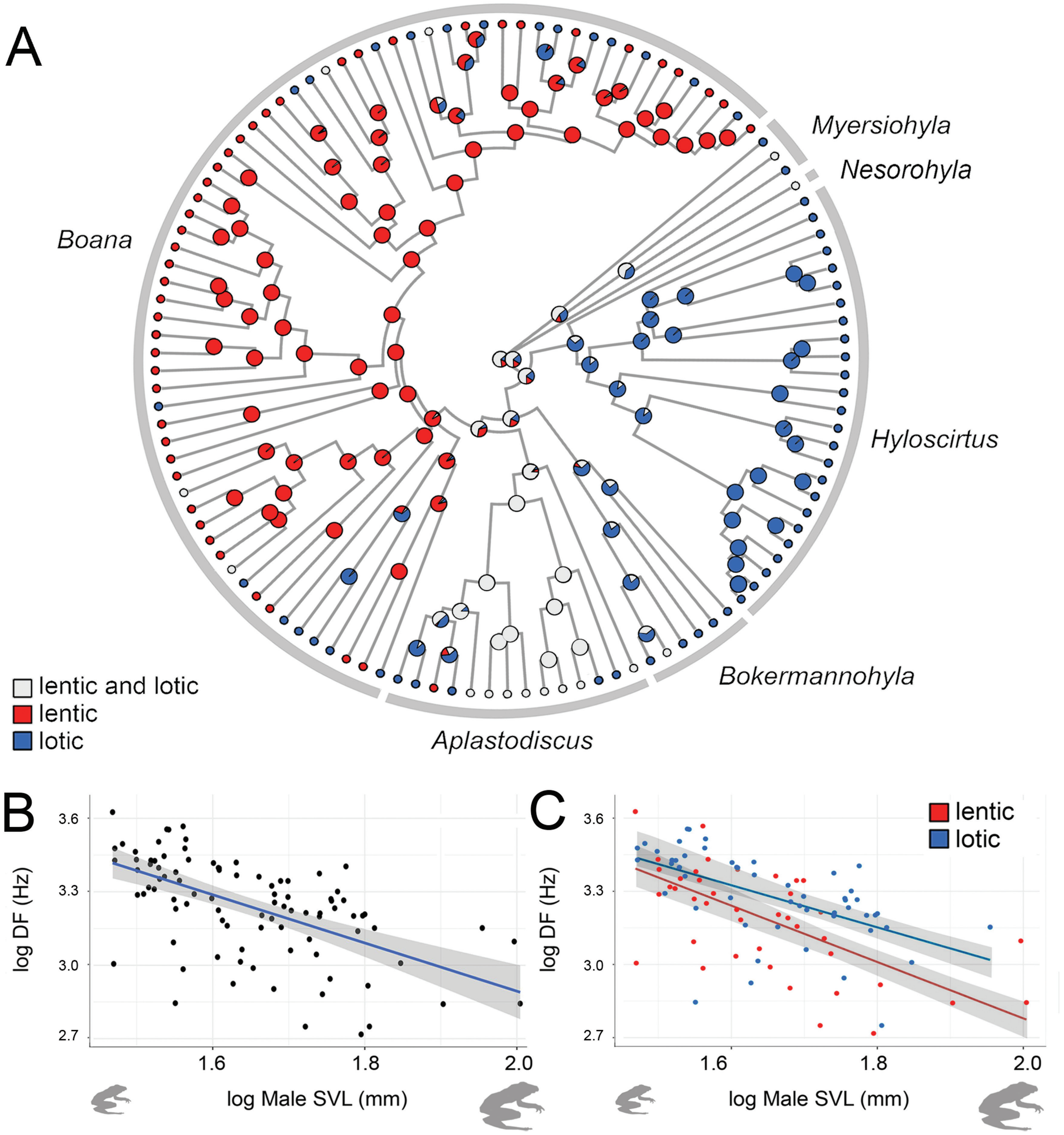

Figure 3. Relationship between dominant frequency, male body size, and habitat in Cophomantini. (A) Ancestral character states reconstruction of habitat using the ARD model (AICc = 195.5707). (B) Scatter plots showing PGLS between male body size (male SVL: x-axis) and dominant frequency (DF: y-axis; $P < 0.05$). (C) Same data as (B), but considering the statistical interaction with habitat ($P < 0.05$).

1996)—and thus is possibly less labile than other behavioral traits with choice-based plasticity. In contrast, DF exhibited a slightly weaker phylogenetic signal than body size, which is consistent with the results found by Escalona *et al.* (2019) for centrolenids.

In addition to phylogenetic signals and fitting evolutionary models, we tested how some morphological and environmental traits are associated with DF and FF. Below, we discuss some interpretations of why body size and habitat use present a phylogenetic signal, and how these traits, in addition to sexual size dimorphism index, might explain the evolution of spectral traits across Cophomantini.

Dominant frequency and body size

In spite of nutritional and environmental factors during development affecting the adult body size (Monaghan

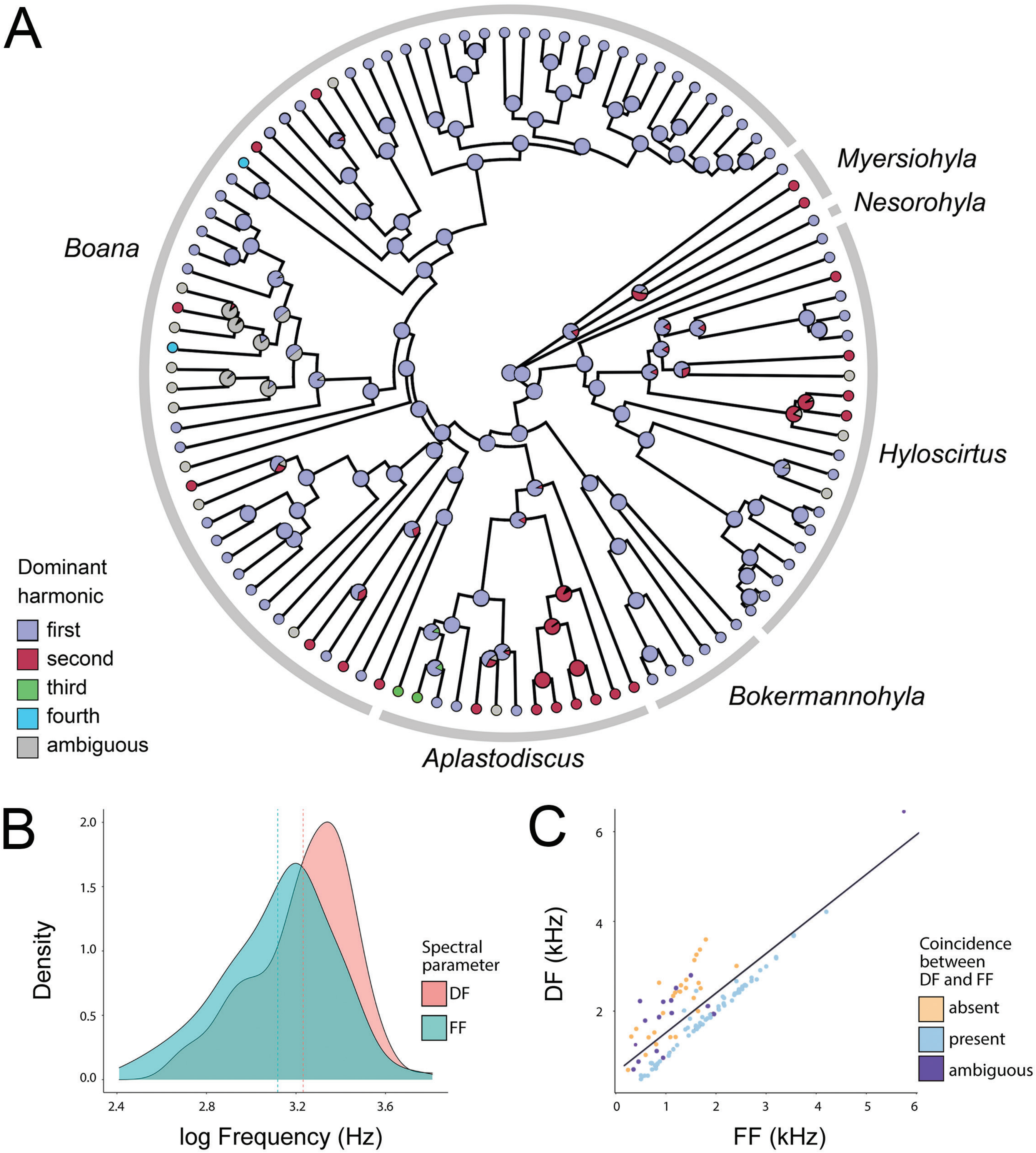

Figure 4. Relationship between dominant and fundamental frequencies. (A) Ancestral character state reconstruction of dominant harmonic using the SYM model (AICc = 219.3043). (B) Density plot showing that mean DF is slightly higher than mean FF. (C) Scatter plot showing the significant correlation between DF and FF ($R^2 = 0.5485$, $P < 0.001$), with colors pointing whether DF and FF are in the same harmonic (present coincidence), different harmonics (absent coincidence), or ambiguous (i.e. not stable, when DF and FF are reported in different harmonics in literature).

2008), a high phylogenetic signal of body size is extensively reported in frogs (e.g. Vidal-García *et al.* 2014, Escalona *et al.* 2019, Bezerra *et al.* 2021). This is consistent with its additive genetic basis (Berven 1982). As expected, DF variation is explained by male SVL (Fig. 3B), probably because larynx structures are correlated with body size (McClelland *et al.* 1996); since vocal folds and the vocal tract are longer in larger frogs, their oscillation

Table 3. Comparison of the three evolutionary models employed (BM: Brownian Motion; EB: Early Burst; OU: Ornstein–Uhlenbeck) for dominant frequency (DF), fundamental frequency (FF), sexual size dimorphism index (SDI), and male and female snout-vent length (SVL) across the phylogeny of Cophomantini, using the Akaike weights (AICw) for small sample size as scores of goodness-of-fit. σ^2 is the evolutionary rate parameter that determines how fast traits will randomly walk-through time in BM/OU or the initial rate in EB; α is the evolutionary constraint in OU that moves trait values back to the optimum; θ is the optimal value in OU; r is the rate change in EB. The best fitting model for each variable is highlighted in bold

Models	Parameters	DF	FF	Male SVL	Female SVL	SDI
BM	AICw	0.009	0.126	0.149	0.041	0.125
	log-likelihood	12.238	12.605	103.550	89.116	84.472
	σ^2	0.451	0.453	0.098	0.102	0.108
EB	AICw	0.003	0.044	0.052	0.014	0.134
	log-likelihood	103.55	12.605	103.550	89.116	84.472
	σ^2	0.448	0.452	0.097	0.103	0.109
	r	9×10^{-7}	1×10^{-6}	1×10^{-6}	8×10^{-7}	1×10^{-6}
OU	AICw	**0.987**	**0.829**	**0.797**	**0.944**	**0.819**
	log-likelihood	17.943	15.541	106.276	93.307	87.337
	σ^2	0.548	0.578	0.126	0.127	0.139
	a	2.911	2.919	2.718	2.721	2.1719
	Θ	3.293	3.177	1.609	1.682	1.623

Table 4. Summary of phylogenetic generalised least square (PGLS) results for the best fitting model, including dominant (DF) and fundamental frequency (FF) as response variables, and male snout-vent length (SVL), habitat, sexual size dimorphism index (SDI), and the interaction between male SVL and habitat as predictors. Significant predictors are indicated in bold. All estimates are from log-transformed data. Intercept indicates the expected value of log DF or log FF when continuous predictors are equal to zero and the categorical variable is lentic

Response	Predictor	Coefficient	SE	t-value	*P*-value
DF[a]	**Intercept**	5.0787	0.3681	13.7963	2.2×10^{-16}
	Male SVL	-1.1008	0.2105	-5.2290	1.3×10^{-6}
	Habitat	1.4079	0.5083	-2.7699	0.0069
	SDI	-0.007	0.3281	-0.0223	0.9823
	Male SVL:Habitat	0.8561	0.3058	2.7988	0.0064
FF[b]	**Intercept**	5.3577	0.4118	13.0101	2.2×10^{-16}
	Male SVL	-1.3423	0.2357	-5.6950	2.0×10^{-7}
	Habitat	-0.6851	0.5684	-1.2051	0.2318
	SDI	-0.0056	0.3704	-0.0152	0.9879
	Male SVL:Habitat	0.4287	0.3421	1.2535	0.2137

[a] General statistics for the DF model: $R^2 = 0.2271$; F = 7.17; $P = 5.497 \times 10^{-5}$.
[b] General statistics for the FF model: $R^2 = 0.3051$; F = 10.1; $P = 1.174 \times 10^{-6}$.

and vibrations result in lower frequency sounds (Nevo and Schneider 1976, Ryan 1988a, b).

As a consequence of the phylogenetic correlation between DF and male SVL, a reasonable question is whether DF is an honest signal of body size in Cophomantini. Under the Handicap Principle, honest signals of fitness are costly otherwise low-quality individuals could exaggerate them to gain the benefits of attracting mates (Zahavi 1975). However, Penn and Számadó (2019) criticized the Handicap Principle because selection minimizes signalling costs, and thus honest signals are maintained by trade-offs. These authors distinguished honest signals that emerged due to phenotypic plasticity from actual constraints. In the latter case, index signals such as vocalizations are honest signals due to physical, physiological, or other constraints (Maynard-Smith and Harper 1995, 2003, Bradbury and Vehrencamp 2011). Hence, DF might be considered an index signal of body size in Cophomantini, as suggested for centrolenid frogs (Escalona *et al.* 2019), although changes in DF could occur in situations of intense sexual selection (e.g. Nali and Prado 2014).

Enquist (1985) also differentiated signal variation due to individual intention or performance (index signals) and cited toads as an example of signalling constrained by anatomical traits (Davies and Halliday 1978). Even when we consider the volitional variation in tension of vocal cords by contracting or relaxing muscles associated to the arytenoid cartilages (Martin 1971, Schmid 1978, Ryan 1988a), call frequencies are anatomically restricted within a largely stereotyped species-specific range (see Supporting Information S1). Therefore, spectral traits in vocalizations can be considered index signals shaped by larynx

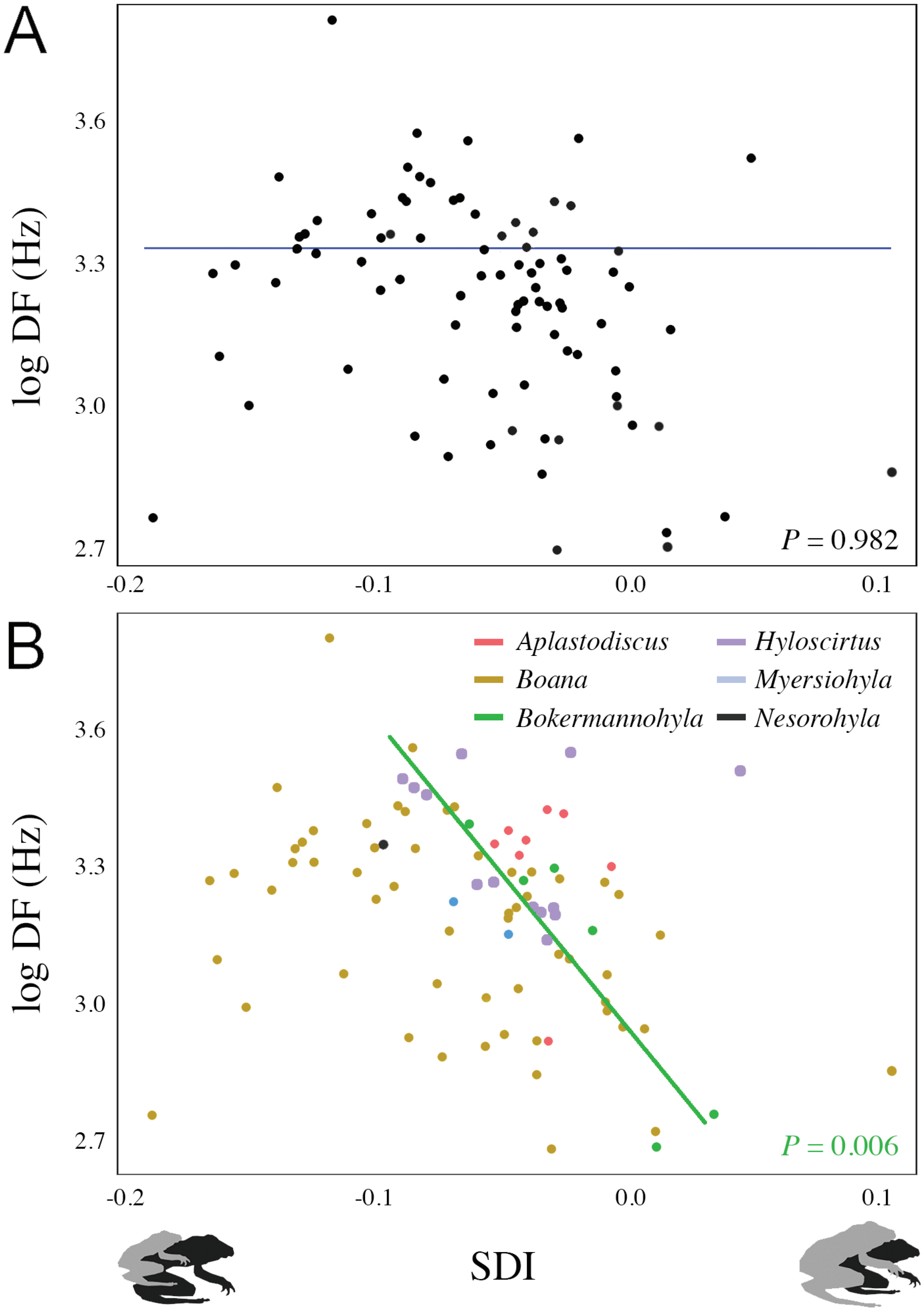

Figure 5. Relationship between dominant frequency (DF) and intensity of sexual selection [using sexual size dimorphism index (SDI) as proxy]. (A) PGLS plot considering all species ($P > 0.05$). (B) PGLS plot considering each genus separately. Only trend line of *Bokermannohyla* is plotted, as only this genus exhibits a significant correlation ($P < 0.01$). Estimates β, t- and P-values for each genus are summarized in Supporting Information, Table S2.1.

structures (i.e. the less massive the vocal cords, the higher the frequency) but with a slight plasticity shaped by tension (i.e. the more tense the vocal cords due to muscle contraction, the higher the frequency; Ryan 1988a).

If a low DF is typically an index signal of a larger body size, but tension might result in frequency alteration within a certain range, why do frogs not cheat more often? Although frequency alteration may emerge intentionally (Bee *et al.* 2000), it should be rare because the benefits of a weak male pretending to be strong might be smaller than the potential cost of fighting against stronger individuals (Enquist 1985, Penn and Számadó 2019). Thus, the DF might be an index signal of body size in most species due to size-frequency allometry (Tonini *et al.* 2020). Further studies about DF alteration, cheating, and muscles associated with tension of vocal cords are required to clarify the effects of intentional modulation of signals in the evolution of advertisement calls in Cophomantini. For instance, Araujo-Vieira *et al.* (2023) scored 11 characters related to larynx, cricoid, and arytenoid anatomy of Scinaxini, suggesting that their data will be useful to study the correlation between laryngeal morphology and call evolution. For Cophomantini, this approach is not possible yet because larynx diversity is poorly understood, except for a few studies (e.g. Arenas-Rodríguez and Hoyos 2022).

Dominant frequency and habitat

Although some species call in both lentic and lotic habitats—mostly in *Aplastodiscus* (e.g. Bokermann 1967, Heyer *et al.* 1990, Garcia *et al.* 2001, Abrunhosa *et al.* 2005, Pinheiro *et al.* 2021)—the significant phylogenetic signal of habitat in vocalization contexts suggests that males of closely related species tend to use similar microenvironments to attract females. Moreover, Fitch's optimization indicates an ancestral lotic habitat (Supporting Information S4: Fig. S4.4A), which is retained in *Myersiohyla* [Rivero 1961, Ayarzagüena and Señaris 1994, Faivovich *et al.* 2013; but see Kok (2006) for *Myersiohyla liliae*], *Nesorohyla* (Pinheiro *et al.* 2019), *Hyloscirtus* (e.g. Coloma *et al.* 2012, Guayasamin *et al.* 2015, Rojas-Runjaic *et al.* 2018), and *Bokermannohyla* (e.g. Lugli and Haddad 2006, Pinheiro *et al.* 2014). However, some changes to lentic environments are concentrated among species of *Boana* (Supporting Information S4: Fig. S4.4A–B). Consequently, despite its significant phylogenetic signal that reflects niche conservatism in closely related species, there is a variation of habitat (i.e. lotic vs. lentic) among some Cophomantini clades. This variation implies different environmental selective pressures, such as differences in environmental noise levels. For example, the higher noise levels of a lotic habitat would have a different effect on vocalizations compared to the lower noise levels of a lentic habitat.

Low frequency background noise is one environmental pressure predicted to affect DF (e.g. Goutte *et al.* 2016, Röhr *et al.* 2016, Zhao *et al.* 2021). Our PGLS results are consistent with the Acoustic Adaptation Hypothesis (AAH; Morton 1975), because lotic breeders call at higher frequencies, whereas lentic breeders call at lower frequencies (Fig. 3C), which supposes a response to avoid masking interference by torrent noise. Furthermore, we found a statistical interaction between body size and habitat on call frequencies, which revealed that differences in call frequencies between habitats are higher in larger frogs, whereas the habitat effect on call frequencies is attenuated in small frogs. This is explained when we consider that background noise frequencies are usually below 1.5 kHz (Goutte *et al.* 2013), and thus low-frequency calls from large species are more masked by noise than high frequencies from small species. As such, the response to natural selection should be more intense in larger species due to the overlap between their calls and the environmental noise. Conversely, little overlap between background noise and high-frequency calls from small species is expected, and thus a low response to selection should occur. Goutte *et al.* (2018) emphasized the importance of simultaneously assessing the role of body size and habitat on call frequencies while testing the AAH, but some previous studies did not find a significant interaction between these two variables (e.g. Röhr *et al.* 2016, Zhao *et al.* 2021).

Although the AAH was initially investigated in the context of call adaptations to vegetation structure in birds, subsequent research has extended its application to test how anurans adapted their calls in response to anthropophony (i.e. human noise; e.g. Cunnington and Fahrig 2010), geophony (i.e. environmental noise such as that emitted by rapid-flowing water; e.g. Castellano *et al.* 2003, Preininger *et al.* 2007, Vargas-Salinas and Amézquita 2014, Caldart *et al.* 2016, Goutte *et al.* 2016, 2018, Rhör *et al.* 2016, Zhao *et al.* 2021), and vegetation (i.e. forests vs. open habitats; e.g. Goutte *et al.* 2016, 2018, Bezerra *et al.* 2021, da Rosa *et al.* 2023, Gillard and Rowley 2023, Mendoza-Henao *et al.* 2023, Bernardy *et al.* 2024). However, a few authors found no difference in DF between lentic and lotic environments (e.g. Vargas-Salina and Amézquita 2014). Here, the AAH is a valid explanation to geophony constraints, in which lotic breeders call at high-frequency bands in Cophomantini, with few exceptions in this group. For example, *Bokermannohyla martinsi* (DF: 291–986 Hz; Pinheiro *et al.* 2014) and *Aplastodiscus cavicola* (DF: 680–790 Hz; Abrunhosa *et al.* 2005) vocalize associated with mountain streams, but they emit low-frequency calls. On the other hand, *Boana caingua* (DF: 3234–3609 Hz; Batista *et al.* 2015), *B. leptolineata* (DF: 3500–5200 Hz; Caorsi *et al.* 2017), and *B. polytaenia* (DF: 4147–7070 Hz; Pinheiro *et al.* 2012) are some examples of lentic water breeders that call at high-frequency bands. However, the call frequency in these cases can be explained by size-frequency allometry.

Dominant frequency and sexual selection

The estimated SDI for the common ancestor of Cophomantini indicates a female-biased SSD in both ML and squared-change parsimony (Supporting Information S4: Figs S4.2C, S4.3C). This pattern is typically attributed to increased fecundity in anurans (Wells 2007, Nali *et al.* 2014). However, there are some exceptions in Cophomantini, mainly in *Bokermannohyla*. In this genus, 60% of the species exhibit a male-biased SSD (15 species out of 25) if we consider only the species with SVL available for both male and female. If all 31 species are considered, this value drops to 48.4% (15 species out of 31; Supporting Information S1 and S2: Fig. S2.6). While previous studies tested the relationship between SDI and body size, temporal breeding patterns, clutch size, territoriality, egg-laying site, climatic variables, and female frequency preferences (Han and Fu 2013, Nali *et al.* 2014, Monroe *et al.* 2015, Portik *et al.* 2020, Silva *et al.* 2020, Pincheira-Donoso *et al.* 2021, Dugo-Cota *et al.* 2022, Bernardy *et al.* 2024), to our knowledge, this is the first study to test the relationship between DF and SSD in amphibians.

PGLS revealed that SDI (our proxy for intensity of sexual selection) is statistically associated with DF only in *Bokermannohyla* (Fig. 5B), but this correlation is not supported when we evaluate it at the tribe level (Fig. 5A). The same pattern is congruent between PGLS and linear models maximizing sample size per genus (remembering that linear models were done only as an exploratory approach; Supporting Information S2: Table S2.1). As such, the more intense the sexual selection (higher value of SDI), the lower the call frequencies in *Bokermannohyla*. Shine (1979) suggested that larger species usually exhibit male-biased SSD due to higher selective pressures for male territoriality when compared with smaller species (in which female-biased SSD is commonly present). Although some clades of *Boana* exhibit exceptionally large species (e.g. *B. boans* and *B. wavrini* in the *B. semilineata* group; Duellman 1970, Hoogmoed 1990), *Bokermannohyla* contains the average largest species in terms of male SVL (Supporting Information S2: Fig. S2.4). Furthermore, it is the only genus with average log SDI > 0 (Supporting Information S2: Fig. S2.6), concentrating several male-biased SSD species (e.g. Magalhães *et al.* 2018, Centeno *et al.* 2021). Hence, spectral parameters of advertisement calls might play an important role in clades with male-biased SSD under strong intrasexual selection (i.e. larger males are predicted to win male-male combats or intimidate competitor with their low-frequency calls; Wells 1978, Katsikaros and Shine 1997) and intersexual selection (larger males with low DF usually are chosen by females; Ryan 1980, 1988a, Wells 2007). This is consistent with the aggressive behavior in some species of this genus (Magalhães *et al.* 2018, Pinheiro *et al.* 2022). In the particular case of *Bokermannohyla ibitiguara*, experiments using playback demonstrated that males change their DF to lower frequencies in situations simulating male-male competition (Nali and Prado 2014).

Another possible reason for the increase in the proportion of male-biased SSD species (and thus the increase in SDI) is parental care, which promotes larger males and reduces female-biased SSD, as observed in *Eleutherodactylus* frogs (Dugo-Cota *et al.* 2022) and other tetrapods such as mammals (Lindenfors *et al.* 2007), birds (Székely *et al.* 2007), and lizards (Cox *et al.* 2007). Parental care was recently reported in this genus for *Bokermannohyla caramaschii*, in which adults exhibit egg and tadpole guarding in water-filled rocky cavities (Alves *et al.* 2021). However, the current data available for parental care in *Bokermannohyla* precludes a phylogenetic comparative test for this hypothesis.

Although the average large body size and the male-biased SSD may predict low DFs in *Bokermannohyla*, most species of this genus reproduce in torrential streams (e.g. Faivovich *et al.* 2009, Brandão *et al.* 2012, Magalhães *et al.* 2016, 2018). Hence, a trade-off between lower-frequency (favored by sexual selection) and higher-frequency (favored by natural selection in lotic environments) is expected to affect the evolution of their advertisement calls (Röhr *et al.* 2016).

Dominant and fundamental frequencies

Dominant and fundamental frequencies are highly correlated (Fig. 4C), but the mean DF is slightly higher than the mean FF (Fig. 4B). R^2 indicates that 54% of the variation in FF explains the variation in DF, while the remaining 46% is explained by other factors such as changes in DF from the first harmonic band (i.e. FF) to the upper harmonic bands (Fig. 4A; Supplementary Information S4: Fig. S4.5). Some groups such as the *Boana pulchella* group and *Nesorohyla* mostly exhibit the DF in the first harmonic, whereas other clades present more variation on the placement of the dominant frequency in the first, second, third, or fourth harmonics (Supporting Information S1). The exact mechanisms underlying these changes in energy concentration throughout the harmonic series of Cophomantini are unknown. Wilczynski *et al.* (1993) hypothesized that one way to enhance DF is reducing the size of resonators (e.g. cavities inside the head), but Rand and Dudley (1993) did not find resonators in the anuran head (neither in the vocal sac nor in the supralaryngeal structures). Additionally, Foratto *et al.* (2021) suggested that natural selection favors DF in upper harmonics to avoid masking interference with environmental noise in *Dendropsophus minutus*, but this hypothesis remains untested in Cophomantini.

In a theoretical framework, establishing the homology of FF is relatively simpler than DF because FF is the first harmonic band directly resulted from the air passage from lungs through vocal cords vibrating them, while DF might be in upper harmonics either due to a mix of resonators in cavities of frogs or by control of the calling itself (Martin 1971, Wilczynski *et al.* 1984, Ryan 1988b, Rand and Dudley 1993, Wilkzynski *et al.* 1993, Bee *et al.* 2000). In an operational framework, however, Köhler *et al.* (2017) noted that FF is methodologically difficult to identify in some conditions: (i) harmonics with low energy (including the FF in some cases) may not be visible in oversaturated recordings when the microphone is too close to the sound source; (ii) upper harmonics with high energy sound are usually detectable on spectrograms of long-distance recordings, whereas lower harmonics (including the FF in some cases) might not be visible; (iii) false harmonics and spectrogram settings may confuse the correct determination of FF. It is also poorly known whether pulse structure affects FF estimation, although it is known that a high pulse rate can lead to false harmonics on the spectrogram (see a brief discussion in Köhler *et al.* 2017). The abovementioned biases in FF data might produce statistical noise and thus are possible explanations for why PGLS did not reveal significant correlations between FF and habitat or intensity of sexual selection (Supporting Information S3: Fig. S3.2). As such, although the role of body size, habitat, and sexual selection on DF has been addressed in frogs, the evolution of FF must be better assessed in the future.

CONCLUSION

We investigated how body size, habitat, and sexual size dimorphism affect fundamental (FF) and dominant frequencies (DF) of advertisement calls in Cophomantini treefrogs. Using phylogenetic comparative methods, we found that male body size drives the evolution of DF and FF. Moreover, DF is associated with habitat, pointing out that natural selection might be favoring a higher DF in lotic environments to avoid masking interference from stream noise. We found correlated evolution between SDI and DF in *Bokermannohyla*, which possibly implies a trade-off between low-frequencies (favored by sexual selection) and high-frequencies (favored by natural selection in lotic environments). To our knowledge, this is the first study to investigate the effect of SSD on spectral traits of anuran calls. We are also unaware of other studies discussing the use of DF and FF in a comparative approach. We hope our efforts highlight the importance of considering not only body size, habitat, and DF but also sexual selection and FF in comparative acoustic studies of anurans.

SUPPORTING INFORMATION

Supplementary data is available at *Biological Journal of the Linnean Society* online.

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CREDIT STATEMENT

Daniel Yudi Miyahara Nakamura (Conceptualization, Investigation, Formal analysis, Data curation, Writing—original draft, Writing—review and editing, Visualization), Moisés Escalona (Investigation, Writing—review and editing), and Paulo Durães Pereira Pinheiro (Conceptualization, Investigation, Data curation, Writing—review and editing, Visualization, Supervision)

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All acoustic, morphometric, and habitat data are available in Supporting Information 1.

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