RRH: Evolution of vireonid song

LRH: Mejías et al.

Relationships of song structure to phylogenetic history, habitat, and morphology in the vireos,

greenlets, and allies (Passeriformes: Vireonidae)

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Author Contributions

MAM: Wrote the paper, collected and analyzed song recordings, produced figures.

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Data Accessibility Statement: All of our data pertaining to song measurements, morphology, and associated R files are archived on the Dryad online database; all Vireonidae sequence data used in this study are present on GeneBank.

ABSTRACT **–** Acoustic signals show immense variation among passerines, and several hypotheses have been proposed to explain this diversity. In this study, we tested, for the first time, the relationships of song structure to phylogeny, habitat type, and morphology in the vireos and allies (Vireonidae). Every measure of song structure considered in this study had moderate and significant phylogenetic signal. Furthermore, two song-constraining morphological traits, bill shape and body mass, also exhibited significant phylogenetic signal. Song length showed the largest within-clade similarity; longer songs were highly conserved in part of the greenlet (*Hylophilus*) clade, whereas shorter songs characterized the remaining seven genera. We found no differences in song structure among vireonids living in different habitat types. However, vireonids with shorter, stouter bills and larger bodies sang songs with lower minimum and maximum peak frequency, compared with species with longer, thinner bills and smaller bodies. We conclude that Vireonidae song evolution is driven partially by phylogenetically conserved morphological traits. Our findings support the phylogenetic signal and morphological constraints hypotheses explaining structural diversity in avian acoustic signals.

KEY WORDS: habitat, evolution, phylogenetic comparative methods, phylogenetic signal, morphology, song evolution, sexual selection, vireos, Vireonidae.

Across the globe, passerines emit both simple and complex vocal displays in multiple contexts (Kroodsma 1991; Williams 2004). Specific functions of birdsong include species recognition (Falls and Brooks 1975; Kumar 2003), mate attraction (Eriksson and Wallin 1986; Searcy and Johnson 1996; Demko and Mennill 2018), territory defense (Krebs 1977; Searcy et al. 1998; Hyman 2003), and status signaling (Staicer et al. 2006; Liu and Kroodsma 2007). Although song serves these same functions in many passerines, song structure varies greatly among species (Brenowitz et al. 1997). Structural diversity in song is thought to serve as a precopulatory mechanism of reproductive isolation at species boundaries, which facilitates conspecific mating (Kreutzer and Vallet 1991; Grant

and Grant 1996), reduced hybridization (Qvarnström et al. 2006; Mason et al. 2017), and, ultimately, speciation (Slabbekoorn and Smith 2002). By exploring the processes shaping avian song structure, we can better understand the evolution of animal signals and the role those signals play in speciation and maintaining biological diversity.

Several non-mutually exclusive mechanisms have been proposed to explain structural variation in avian song. First, the sexual selection hypothesis (Darwin 1896; Andersson 1994; Catchpole 1980; Searcy 1986) views song as an acoustic equivalent of a peacock's "tail", with its underlying processes, such as mate choice, driving trait elaboration (Buchanan and Catchpole 1997; Byers and Kroodsma 2009). Despite being a widely accepted mechanism, the role of sexual selection in shaping acoustic traits is challenging to quantify because data on several proxies of sexual selection, including plumage dichromatism (Seddon et al., 2008; Kraaijeveld et al., 2011; but see Price, 2019), mating system (Møller and Cuervo 1998), and nesting data (Krakuer 2008; Balenger et al. 2009), are not readily available for all species. A second driver of song structure complexity is the number of singing, sympatric heterospecifics. More specifically, conspecifics living in depauperate songbird communities have fewer acoustic competitors, facilitating selection for elaborate song structure, whereas conspecifics living amongst diverse avifauna are more restricted in their vocal diversity because of increased competition for "acoustic space" (Naugler and Ratcliffe 1994; Espmark 1999). Anthropogenic noise can have a similar effect on avian songs, with birds living in noise polluted areas exhibiting significant shifts in song frequencies, as compared to conspecifics living in areas without anthropogenic noise (Slabbekoorn and Ripmeester 2008; Hanna et al. 2011). Third, song evolution can be affected by song learning, since copying mistakes by young birds can cause "cultural drift" (Podos et al. 2004; Koetz et al. 2007). In the current study, however, we focus on three non-mutually exclusive hypotheses shaping avian song structure: (1) phylogenetic history, (2) habitat structure, and (3) morphology.

Traits that exhibit high similarity amongst closely related species, as compared to species drawn at random from the same phylogenetic tree, are said to exhibit phylogenetic signal — a

pattern of trait distribution that is expected under a random walk model (Brownian motion) of trait evolution (Blomberg and Garland 2002; Blomberg et al. 2003; Münkemüller et al. 2012). With respect to birdsong, closely related species have had less time to diverge and could therefore sound similar, whereas distantly related species have had more time to diverge and could sound different. Evidence of phylogenetic signal in song structure has been found in oropendolas (Icteridae; Price and Lanyon 2002), kinglets and crests (Regulidae; Päckert et al. 2003), and wrens (Troglodytidae; Sosa-López et al. 2016). In contrast, some signals may diverge more rapidly among closely related species living in sympatry than among more distantly related species living in allopatry, giving rise to an inverse relationship between phylogenetic relatedness and trait divergence (Martin et al. 2010, 2015). Traits might also evolve rapidly to show homoplasy across the phylogeny, or even exhibit no change at all, leading to low levels of phylogenetic signal (Blomberg et al. 2003; Kamilar and Cooper 2013). Nonetheless, phylogenetic signal remains to be quantified in most avian song traits, and, thus, its prevalence in most avian families remains unclear.

Birdsong can travel long distances and the quality of these vocal displays can be impacted by the physical properties of their surroundings (Morton 1975; Wiley 1991; Barker 2008). Specific habitat-induced changes to songs include sound-reflecting surfaces distorting timing between elements via echoes and reverberations, as well as absorption and scattering of frequency components by vegetation (Wiley 1991; Naguib 2003; Padgham 2004). Thus, different habitats have different acoustic properties, meaning a habitat can shape song structure by determining which songs maintain their structure (and thus, communicative value) as they propagate through the environment. In forests, for example, selection may favour birds that sing songs with lower frequencies, whereas in open habitats, selection may favour birds that sing songs with higher frequencies. For example, high-frequency sounds have wavelengths that are shorter than the diameter of tree trunks and are thus distorted and scattered as they propagate through a forest, whereas low-frequency sounds have wavelengths longer than the diameter of tree trunks and thus pass these structures and reach their intended receivers with minimal reflection or distortion

(Naguib 2003). Similarly, denser habitats can select for songs with less frequency modulation, whereas open habitats can select for songs with more frequency modulation (Nemeth et al. 2001; Barker 2008). Lastly, the potential of vegetation to distort the onset and offset of individual song elements through reverberation may favour songs with fewer elements, and reduced overall song length in forested environments, as compared to open environments (Hunter and Krebs 1979; but see Ey and Fischer, 2009).

The evolution of avian song structure can also be constrained by morphology (Podos 2001). Opening and closing the bill contributes to a song's frequency modulation (Ryan and Brenowitz 1985; Palacios and Tubaro 2000), but species with larger bills cannot open and close their bills as fast as species with smaller bills while singing (Podos and Nowicki 2004; Ballentine 2006). Natural selection for the large bills needed to handle hard food items could thus lead to songs with little frequency modulation, whereas selection for the long, thin bills needed for capturing insects could lead to songs with rapid frequency modulation (Herrel et al. 2005; Huber and Podos 2006). Bill shape can also impact the rate of element delivery within songs, with larger bills uttering elements at a slower rate (Huber and Podos 2006; Derryberry et al. 2012), although the effects of bill morphology on other temporal traits, like song length, are not well established. The size of the syrinx also covaries with body size (Bowman 1979); the larger syrinx of a larger species should thus vibrate slower and produce sounds of lower frequency (Wallschläger 1980) than the smaller syrinx of a smaller species (Bowman 1979; Ryan and Brenowitz 1985; Nowicki and Marler 1988). Larger birds also experience greater respiratory constraints than smaller birds (Suthers 2001) and may thus produce shorter songs.

Vireonidae is a diverse avian family ideal for testing multiple hypotheses of song evolution. First, a recent multilocus phylogenetic study containing 50 of the 64 recognized species showed a monophyletic Vireonidae (Slager et al. 2014). The family comprises eight genera: *Pteruthius* and *Erpornis* are endemic to southern and eastern Asia, and the remaining six genera (*Vireo, Hylophilus, Pachysylvia, Tunchiornis, Cyclarhis,* and *Vireolanius*) are endemic to the New World, ranging from

Alaska to forests of the Caribbean, Bermuda, and South America (Slager et al. 2014, Brewer et al.

2019). Second, vireonids occupy diverse habitat, including shrublands, thickets, woodland edges,

and the interior of temperate and tropical forests (Barlow and James 1975; Kus 1998; Hudman and

Chandler 2002). Third, species vary dramatically in bill structure and body size (6-35 g; Brewer et al.

2019), and fourth, vireonids exhibit substantial variation in song structure, including duration, the

minimum and maximum song frequencies, and frequency modulation. Vireonid songs are also

unusual in that this family dispersed out of Asia (Slager et al. 2014) into the Western Hemisphere

independently from the Australasian-derived Passerida (Barker et al. 2004), yet some vireonids sing

songs more like New World passerines, such as Passerellidae and Troglodytidae, as opposed to the

less complex songs of their closer relatives in Corvoidea.

Our objective was to test for relationships of vireonid song structure to phylogenetic history,

habitat structure, and morphology. First, we tested for phylogenetic signal in Vireonidae song traits;

upon finding significant phylogenetic signal in vireonid songs, we measured phylogenetic signal in bill

shape and body size. This was important because phylogenetic history might also shape these

potential song-constraining traits, ultimately driving song trait distribution in Vireonidae. As with

phylogenetic signal in song structure, we predicted that more closely related species would have

greater similarity in bill shape and body size, as compared to more distantly related species. Having

detected phylogenetic signal in song traits, we then performed a series of phylogenetically informed

analyses to test for relationships between vireonid song structure and habitat type, bill shape, and

body size. We predicted that vireonids living in dense habitats would sing shorter songs, with lower

minimum and maximum frequencies, and less frequency modulation. In contrast, we predicted that

vireonids living in more open habitats would sing longer songs, with broader frequency ranges, and

more frequency modulation. Finally, we predicted inverse relationships between morphology and

song traits; specifically, species with deeper, larger bills would sing shorter songs with smaller

frequency ranges and less frequency modulation, as compared to species with smaller, thinner bills.

Similarly, we expected larger-bodied vireonids to sing shorter songs with narrower frequency ranges

and less frequency modulation, and smaller-bodied vireonids to sing longer songs, with broader

frequency ranges and more frequency modulation.

Material and Methods

PHYLOGENETIC INFERENCE

Slager et al. (2014) inferred the evolutionary relationships among 50 (78%) of the 64

currently recognized species in the Vireonidae (Brewer et al. 2019). Their phylogenetic analyses

based on the mitochondrial ND2 locus and three Z-linked nuclear loci showed that the North

American and South American Red-eyed Vireo (*Vireo olivaceous*) represent two divergent lineages,

suggesting two distinct species. Battey and Klicka (2017) further explored this relationship with

ddRAD sequencing and concluded that the two are not sister taxa, and do not exchange genes. They

recommended elevating the South American lineage to species status under its original name, Chivi

Vireo (*V. chivi*; Vieillot 1817), leaving the North American lineage as *V. olivaceous*; we thus treat *V.

chivi* and *V. olivaceous* as separate species, and follow the nomenclature of the American

Ornithological Society (A.O.S., Chesser 2019) for the other species. Therefore, we recognize 51

species on the phylogenetic tree of Slager et al. (2014).

We obtained the mitochondrial ND2 alignment from Slager et al. (2014). It contained the

most comprehensive taxonomic sampling for Vireonidae, including multiple individuals per species

(Fig. 1 in Slager et al. 2014). Sampling for the three Z-linked nuclear loci was poor (52%), and thus

these loci were not used in our study. Since the multispecies coalescent model cannot be used

reliably with a single gene dataset to infer a phylogenetic tree that will include one representative

per species, we selected the longest ND2 sequence per species, or randomly selected one when they

had equal lengths and completeness. Our resulting ND2 alignment contained 51 of 64 (80%)

vireonids; *Pteruthius*, *Erpornis*, and *Cyanocitta* were included as outgroups, as in Slager et al. (2014).

We used the Bayesian method implemented in BEAST v2.5.2 (Bouckaert et al. 2014), and the

parameters described in Slager et al. (2014) for the codon partitions, substitution site model, tree

prior, and clock model. We enforced the monophyly of the Vireonidae genera using a series of taxon

sets to obtain a tree with the same relationships as in Slager et al. (2014). We used a single run of a

Markov chain Monte Carlo (MCMC) chain length of 150 million generations on the CIPRES Science

Gateway (Miller at al 2010), sampling trees every 150 thousand generations, producing a file with

1000 trees, and a log file with the respective posterior probabilities (pp) per tree. We sampled all

other parameters every 15 thousand generations. We verified that the MCMC run reached

convergence by viewing the output in Tracer v1.7 (Rambaut et al. 2018), and that all effective

sample sizes exceeded 200. Following the removal of 25% burn-in, the remaining 750 sampled

posterior trees were summarized using Tree Annotator v.2.5.2 (Bouckaert et al. 2014) to generate a

maximum clade credibility tree (hereafter, "MCC Vireonidae tree") and calculate the pp as branch

support values. We visualized the Bayesian inference tree in FigTree v.1.4.2

(http://tree.bio.ed.ac.uk/software/figtree/).

HABITAT CLASSIFICATIONS

Many life-history and behavioural data are scant for vireonids outside of North America. Due

to this limitation, we utilized a three-way habitat categorization; this methodology was adopted in

previous studies (Tubaro and Segura 1995; Mason and Burns 2015). For each species, we used

habitat descriptions from the Handbook of the Birds of the World (Brewer et al. 2019) to group it

into one of three broad habitat categories, from closed to open: (1) woodlands, (2) open woodlands,

or (3) shrublands. Woodland habitat is forest with a dense interior and a dense understory (Morton

1975; Brewer et al. 2019). Open woodland is a forest with gaps in its interior, either from clear

cutting or tree fell, and little to no understory (Brewer et al. 2019). Shrubland is grassland covered

with extensive saplings and shrubs, interspersed with scattered larger trees and bushes (Brewer et

al. 2019). Morton (1975) described some tropical vireonids inhabiting edge or canopy habitats. He considered edge habitat to be intermediate between forest and grassland, and canopy habitats to be less open than edge habitat. Therefore, we categorized edge-dwelling species in open woodland habitat and canopy-dwelling vireonids in woodland habitat.

BODY MEASUREMENTS

We quantified two morphological variables from vireonids: (1) bill shape and (2) body mass. We measured bills of specimens from the following collections: The Field Museum, American Museum of Natural History, Museum of Natural Science at Louisiana State University, and the Bell Museum at the University of Minnesota (Table S1). We used a digital caliper (Mitutoyo® model no. 573-721) to measure (± 0.01 mm) the following variables on the bill (Baldwin et al. 1931): (1) length of exposed culmen (LEC), (2) length of bill from the anterior margin of the naris to the tip (LNB), (3) width of bill at its base (WBB), (4) width of bill at the anterior margin of the naris (WBN), (5) depth of bill at its base (DBB), and (6) depth of bill at the anterior margin of the naris (DBN). When possible, three males from the same breeding population were measured. If three males that met these criteria were not available, we measured specimens that matched, in declining priority, subspecies, locality, and time of year. We measured female specimens only when three males that met our criteria were not available, but no more than one female specimen was measured per species. When fewer than three specimens meeting any of these criteria were available, we measured as many as were available. We did not measure specimens of the Chivi Vireo because it had not been elevated to species at the time of data collection; instead, we included data on three bill measurements (LEC, WBB, and DBB) for Chivi Vireo from Olson (1994). We estimated the three missing bill measurements by regressing the missing variables against their complements (e.g. LBN ~ LEC) and using the slope and intercept to estimate means for the missing variables. We collected body mass data from the VertNet Museum Database (http://vertnet.org/) and the Handbook of Birds of the

World (Brewer et al. 2019). For each species, we calculated mean values for each bill measurement and body mass and improved data normality by ln-transforming means prior to phylogenetic comparative analysis (Safi et al., 2005; Khaliq et al., 2014).

We performed a phylogenetic principal component analysis (pPCA) on the bill measurement data using the "BM" correlation structure and trait covariance matrix in the R package *phytools* (Revell 2009; Revell 2012). A pPCA takes phylogenetic relatedness into account, calculating summary axes that are concordant with a Brownian Motion model of evolution and thus satisfying the assumptions of our phylogenetic comparative analyses (Uyeda et al. 2015). Principal component analyses performed on a dataset comprising linear measurements will (nearly) always have the first pPC axis capture variance in size among the specimens measured (Pigot et al. 2016; Crouch and Ricklefs 2019).

SONG PROCESSING AND MEASUREMENTS

We obtained vireonid song recordings from the Macaulay Library (https://www.macaulaylibrary.org/) and xeno-canto (https://www.xeno-canto.org/). We used the recording quality rating systems of the two song libraries to select recordings with high signal-to-noise ratios. When possible, we collected 10 song recordings from across the geographic range of each species. For all species, we selected recordings separated in space by at least 2 km, and in time by at least 1 year to reduce the chance of resampling the same individual. For this study, we focused only on male songs for each species.

We viewed each recording as a waveform and spectrogram (Hamming window, FFT size = 512 samples, 87.5% overlap) using Raven Pro sound analysis software (v1.5; Charif et al. 2010), and annotated all high-quality songs (e.g., clear tracings and no overlap with other sounds on the spectrogram, and clear amplitude pulses on the waveform). We defined a song as a vocalization comprising one or more elements, and elements as a continuous trace on the spectrogram.

Elements of a single song were separated by < 0.5 s, and successive songs were always separated by ≥ 0.5 s of silence. We chose 0.5 s because it reflects the minimal length of silent periods that separate elements of separate songs for all vireonids we studied. We used a random number generator to select one annotated song per recording. Selected songs were exported as standalone clips with 0.3 s of silence before and after the song to act as a buffer, filtered with a 600-Hz high-pass filter, and normalized to a peak amplitude of -1 dB.

For each selected song, we visually identified and marked the start and end of each song element by inspecting the waveform and corresponding spectrogram. Previous song analyses have also measured the duration of silences among song elements (Nemeth and Winkler 2001; Sosa-López et al. 2014). but this was not possible for some of our species because they contained only a single element. We divided the song into 2-ms time bins using the "split border" function in Raven and recorded the peak frequency (i.e., the frequency where the greatest power occurs) of each bin within each element. From these raw data, we calculated four discrete song traits that were applicable to all species, regardless of song complexity: (1) song length (s), (2) minimum peak frequency (Hz), (3) maximum peak frequency (Hz), and (4) frequency modulation (Hz per second). Song length was defined as the interval from the onset of the first song element to the offset of the last. Minimum and maximum peak frequency were the 5th and 95th percentiles, respectively, of all peak frequency values from all 2-ms time bins within a song (excluding silent periods between elements). We used the 5th and 95th percentiles, rather than the minimum and maximum values, because they are less likely to be artifacts or outliers that misrepresent the acoustic space used by a given species (Podos et al. 2016; Fahmy and Wilson 2020). Finally, we calculated frequency modulation as the cumulative absolute change in peak frequency between all consecutive 2-ms time bins (excluding silent periods between elements), divided by the cumulative duration of all elements. Our measure of frequency modulation is similar to the measure of frequency excursion developed by Podos et al. (2016), except that it does not include changes in frequency that occur between the end of one element and the beginning of the next, since some of

our species produced songs with only one element. For this same reason, we did not analyze the duration of the silences among song elements, as done in previous studies (Nemeth and Winkler 2001; Sosa-López et al. 2014).

STATISTICAL ANALYSES

We measured phylogenetic signal in the four song traits, followed by the two morphological traits, using Pagel's lambda (λ) and Blomberg's K, as measured with the "phylosig" function in the "phytools" package (v.3.5.3; Revell 2012) in R. Pagel's λ and Blomberg's K measure the magnitude of phylogenetic signal of a continuous trait assumed to be evolving under Brownian motion (Pagel 1999; Blomberg, Garland et al. 2003). Pagel's λ transforms the branch lengths of the original phylogeny, such that the observed trait distribution on the tips matches the pattern expected under Brownian motion on the transformed phylogeny (Pagel 1999; Kamilar and Cooper 2013). Values for λ range from 0, where traits are evolving independently of the phylogeny (weak phylogenetic signal), to 1, where trait divergence is comparable to what would be expected under Brownian motion (strong phylogenetic signal). It is also possible for λ to be larger than 1, indicating traits are more similar than expected under a Brownian motion model (Kamilar and Cooper 2013). Blomberg's K calculates a ratio of observed versus expected trait variance in trait distributions within and between clades (Blomberg et al. 2003). Blomberg's K ranges from 0 (the null expectation) to infinity. Weak phylogenetic signal is detected when $K < 1$, indicating that closely related species are less similar to each other than would be expected under Brownian motion, showing greater trait variance within clades (Blomberg et al. 2003). Strong phylogenetic signal is detected when $K = 1$, which indicates that species' traits evolved under Brownian motion, showing greater trait variance among clades (Blomberg et al. 2003; Kamilar and Cooper 2013). When $K > 1$, closely related species are more similar to each other than would be expected by Brownian motion alone (Münkemüller et al. 2012).

We tested the probability of λ and K being significantly different from 0 by running a series of randomization tests under 10,000 simulations and comparing the resulting distribution of simulated values to the values generated from the MCC Vireonidae tree. We present the respective

P value for both λ and *K*; *P* values detect the presence of any significant, non-zero phylogenetic signal ($P < 0.05$), whereas λ and *K* express the magnitude of the phylogenetic signal. To account for phylogenetic uncertainty in the MCC Vireonidae tree, we calculated λ and *K* on each of the 100 trees in our distribution with the highest posterior probability to test the robustness of these findings. Preliminary analysis indicated that two species had to be removed from our phylogenetic trees. We removed Steller's Jay (*Cyanocitta stelleri*), an outgroup species used in the original Vireonidae phylogeny, because its vocalizations were complex calls, as opposed to the discrete songs seen in vireonids. Similarly, we removed the Brown-headed Greenlet (*Hylophilus brunneiceps*) from our analysis because its song length was an outlier (i.e., 13.5 sec) compared to the rest of the Vireonidae. Therefore, our final set of phylogenetic trees contained 50 species from the Vireonidae.

We used phylogenetic ANOVAs to test whether vireonids living in the three habitat types differ in the four song traits, using the "phylANOVA" function in the *phytools* package in R (Revell 2012). This function conducts post-hoc pairwise comparisons of means between groups, based on methods described by Garland et al. (1993). To take phylogenetic relatedness into account, we performed the phylogenetic ANOVAs using our MCC Vireonidae tree, and report the global *F* statistic and *P* value for each song trait. To account for phylogenetic uncertainty, we ran each habitat phylogenetic ANOVA on the 100 best posterior trees.

We used the *caper* package (Orne et al., 2013) in R to fit multiple, simple phylogenetic generalized least squares models (i.e., pgls) to test for relationships between song structure and morphology. The four song structure traits were included as dependent variables in separate models. Bill shape (pPC2 scores) and body mass (ln-transformed) were included as predictor variables, and the MCC Vireonidae tree was included to account for shared evolutionary histories among species. We visually inspected the distributions of the residuals of each model using the plot function in R. Any species whose studentized residuals were ≥ 3 were treated as outliers and removed, as recommended by Garland et al. (1992) and Jones and Purvis (1997). Removing these individuals did not change the results with respect to statistical significance of any of our models. To

account for phylogenetic uncertainty, we ran all pgls models on the 100 best posterior trees. All statistical analyses were run using R v.3.5.2 (R Development Core Team, R Foundation of Statistical Computing, Vienna, Austria).

Results

VIREONIDAE PHYLOGENETIC TREE

Our Bayesian analysis of the ND2 alignment for Vireonidae achieved convergence after 150,000,000 generations with ESS values > 2,000 for nearly all parameters in the model. Species-level relationships recovered in our MCC Vireonidae tree were largely concordant with those of Slager et al (2014), especially within the genera *Pteruthius*, *Vireolanius, Hylophilus,* and *Pachysylvia*. Species-level relationships within *Vireo*, the largest genus, differed slightly from the Slager et al. (2014) ND2 topology, but the posterior probabilities in our MCC Vireonidae tree (smallest value: 0.47 – largest value: 1; Fig S1) were similar to the posterior values on the same nodes in Slager's phylogeny (Fig. 1 in Slager et al., 2014). We also successfully recovered the major clades within *Vireo*, as described in Slager et al. (2014).

PHYLOGENETIC SIGNAL IN SONG AND MORPHOLOGICAL TRAITS

We analyzed songs from 359 individuals from 51 species (Table S2). Song structure among the 50 vireonids included in our analyses was diverse: song length (mean $\pm$ SD: 1.1 $\pm$ 1.0 s; range: 0.16–5.5 s), minimum peak frequency (2603 $\pm$ 546 Hz; 1803–5254 Hz), maximum peak frequency (4207 $\pm$ 960 Hz; 2518–6718 Hz), and frequency modulation (18544 $\pm$ 8827 Hz/s; range: 814–37610 Hz/s; Fig 1). Songs among the eight genera have noteworthy differences. *Pteruthius* produce simple songs that either have a long, rapid, monotonous single element delivery, or, short songs composed of two mournful elements (Fig 1). *Erpornis* sings a rapid, high-pitched trill that is ether ascending or descending (Fig 1). *Hylophilus* produce the longest songs in Vireonidae; these species sing repetitive

songs comprising rapid or slow whistles and trills. *Tunchiornis* songs are pure, slowly modulated whistles. *Vireolanius* songs are simple in structure; songs range from an emphatic, monosyllabic element that begins with an ascending wine, then subtly descends into a prominent whistle, or, songs that are steady repeats of 3-4 elements of melodic quality (Fig 1). *Vireo* possess the greatest structural diversity in the family. Their songs range from monotonous trills, to steady repeats of a single element, to short and long songs of highly modulated, buzzy, and whistling elements (Fig 1). *Pachysylvia* songs are all short, containing melodic, highly modulated elements, uttered steadily (Fig 1). Lastly, *Cyclarhis* produce whistled songs of 4-6 slowly modulated elements uttered steadily.

Vireonids showed significant phylogenetic signal in all four song traits. Our metrics of λ and K suggest strong and moderate phylogenetic signal, respectively. Song length exhibited the strongest phylogenetic signal, with longer songs being largely conserved within the *Hylophilus* genus (λ = 1.01 $P < 0.0001$; $K = 0.62$, $P = 0.0056$; Fig 2A). We found weaker, albeit significant, phylogenetic signal in minimum peak frequency ($\lambda = 0.88$, $P = 0.0001$; $K = 0.58$, $P = 0.0066$; Fig 2B), maximum peak frequency ($\lambda = 0.90$, $P = 0.0002$; $K = 0.45$, $P = 0.0009$; Fig 2C), and frequency modulation ($\lambda = 0.85$, $P = 0.0001$; $K = 0.51$, $P = 0.0004$; Fig 2D). Our phylogenetic signal analysis on the 100 best Vireonidae trees, using our four song traits, corroborated the MCC Vireonidae tree results (Table1). In addition to song traits, bill shape and body size also varied among vireonids (see details in "morphology and song traits," below) and exhibited moderate to strong phylogenetic signal. More specifically, we found significant phylogenetic signal in bill shape ($\lambda = 0.83$, $P < 0.0001$; $K = 0.59$, $P = 0.0004$; Fig 3A) and body mass ($\lambda = 0.94$, $P < 0.0001$, $K = 0.71$, $P = 0.0004$; Fig 3B). Phylogenetic signal analyses on the 100 best Vireonidae trees, using these two morphological traits, corroborated the MCC Vireonidae tree findings (Table 1).

HABITAT AND SONG TRAITS

We grouped vireonids into three broad habitat types: woodlands (n = 30), shrublands (8), and open woodlands (12). There were no significant relationships between habitat type and any of

the song variables. Using phylogenetic ANOVAs, we found that vireonids from the different habitat classes did not differ in song length (F = 5.31, P = 0.079), maximum peak frequency (F = 0.71, P = 0.701), minimum peak frequency (F = 1.11, P = 0.575), or frequency modulation (F = 5.50, P = 0.075). None of our habitat phylogenetic ANOVA models on the 100 best trees yielded significant results, corroborating the MCC Vireonidae tree results.

MORPHOLOGY AND SONG TRAITS

We measured 161 museum specimens representing 58 species of vireonids, with all but 10 species represented by three specimens. This dataset was pared down to contain the 50 species considered in our ND2 phylogeny reconstructed for this study, and then used in the pPCA. The analysis produced six pPC axes, of which the first two explained 89.2% of the variance in the data (Table S3). The first axis, pPC1, explained 72.5% of the variance. The six bill variables loaded positively onto pPC1, suggesting that this axis represents the overall size of the specimens. Indeed, pPC1 explained 63% of the variation in ln-transformed body mass (linear regression: β = 0.95, Adj R^2 = 0.63, P < 0.0001; Fig. S2). Thus, we used ln-transformed body mass as a proxy for body size in this study and did not analyze pPC1 scores. In general, the larger-bodied species comprised *Cyclarhis* and *Vireolanius*, whereas the smaller-bodied species comprised *Vireo* and *Pachysylvia.*

The second pPC axis accounted for 16.7% of the variance, which is greater than the remaining four pPC axes' contributions combined. The eigenvector of this axis was positively loaded by both length measurements, negatively loaded by both depth measurements, and only weakly loaded by the width measurements (positively for WBB, negatively for WBN; Table S3). Species such as Rufous-browed Peppershrike (*Cyclarhis gujanensis*) had negative pPC2 scores and relatively short and deep bills, whereas species such as Lesser Greenlet (*Pachysylvia decurtata*) had positive scores and relatively long and shallow bills. We therefore used pPC2 scores (hereafter, "bill shape") to represent bill morphology.

Song length was not related to bill shape (PGLS: β = 0.87, SE = 0.91, Adj R^2 = -0.002, $F_{1,41}$ =

0.93, P = 0.3410, Fig. 4A) or to body size (β = -0.22, SE = 0.51, Adj R^2 = -0.02, $F_{1,41}$ = 0.18, Fig. 4B P =

0.6722). All 100 of the alternative trees produced comparable, non-significant results.

Minimum peak frequency and bill shape were positively related; songs of vireonids with

shorter, deeper bills had lower minimum peak frequencies, compared with those with longer,

thinner bills (β = 1267.49, SE = 464.11, Adj R^2 = 0.12, $F_{1,48}$ = 7.46, P = 0.0088, Fig. 1, 4C. There was a

significant negative correlation between minimum peak frequency and body mass (β = -697.15, SE =

255.53, Adj R^2 = 0.12, $F_{1,47}$ = 7.44, P = 0.0089, Fig 4D). A total of 97 of the 100 alternative trees

yielded significant results for bill shape, and all 100 alternative trees yielded statistically significant

results for body mass.

Songs of vireonids with shorter, deeper bills had lower maximum peak frequencies,

compared to those with longer, thinner bills (β = 2666.03, SE = 778.48, Adj R^2 = 0.18, $F_{1,48}$ = 11.73, P

= 0.0012, Fig. 1, 4E. There was a significant negative relationship between maximum peak frequency

and body mass (β = -1601.65, SE = 426.15, Adj R^2 = 0.21, $F_{1,48}$ = 14.13, P = 0.0005, Fig 4F). All 100

alternative trees yielded significant results for both bill shape and body mass).

In contrast, frequency modulation was not correlated with bill shape (β = 8288.3, SE =

7885.4, Adj R^2 = 0.002, $F_{1,48}$ = 1.11, P = 0.2985, Fig. 4G) or body mass (β = -7413.1, SE = 4185.0, Adj

R^2 = 0.04, $F_{1,48}$ = 3.14, P = 0.0829, Fig. 4H). None of the alternative trees yielded significant results for

bill shape, though four trees yielded significant results for body mass.

Discussion

We tested non-mutually exclusive hypotheses of how the temporal and

frequency components of Vireonidae songs evolve. We first showed that phylogenetic history

predicted song length, minimum peak frequency, maximum peak frequency, and frequency

modulation, as well as bill shape and body mass, which can affect song structure. Our phylogenetic

comparative approach then explored relationships between habitat and morphology and each of these four song traits.

PHYLOGENETIC SIGNAL IN SONG AND MORPHOLOGICAL TRAITS

All four vireonid song traits possessed significant phylogenetic signal. Pagel's λ indicated that song traits evolved very similarly to Brownian motion, whereas *K* suggested these traits are moderately more divergent between species than expected under Brownian motion. Across all four song traits, sister taxa showed considerable trait similarity, explaining the presence of significant phylogenetic signal (Fig 2). However, the entire phylogeny revealed that small and large song trait values occurred in all 8 genera, indicating distantly related vireonids produce songs of similar structure, which likely diminished the strength of *K*. Our single temporal trait, song length, showed the strongest phylogenetic signal; longer songs were highly conserved in the *Hylophilus* clade, whereas shorter songs dominated the remaining genera (Fig 2A). Taken together, our metrics show that vireonid song structure has moderate and significant phylogenetic signal.

Our results are congruent with previous research that found significant, albeit lower, phylogenetic signal strength in passerine song traits. Such patterns were found in the song structure of antbirds (Thamnophilidae; Gómez et al. 2010) and leaf warblers (Phylloscopidae; Mahler and Gil 2009; Tietze et al. 2015). In addition, our finding that a temporal trait had stronger phylogenetic signal than frequency traits was in accordance with earlier bird studies (Price and Lanyon 2002, Tietze et al. 2015). One possible explanation for the moderate phylogenetic signal is that some traits are innate, and others are learned (Beecher and Brenowitz 2005; Mason et al. 2017). Temporal components, such as element length and syntax, are intimately linked to phylogenetic history in several avian families **(**Päckert et al. 2003, Cardoso and Mato 2007; Tietze et al. 2008). In contrast, frequency components may be more prone to rapid structural changes through copying mistakes and improvisation (Payne 1981; Price 1998; Olofsson and Servedio 2008). Nonetheless, our study provides compelling evidence that phylogenetic history contributes to song diversification in Vireonidae and other avian families.

Vireonid morphology also showed significant phylogenetic signal. Like the four song traits, Pagel's λ indicated that bill shape and body size largely conform to Brownian motion. Similarly, both morphological traits exhibited significant K values, yet still less than 1, indicating that closely related species are less similar to each other than would be expected under Brownian motion. Blomberg et al.'s (2003) fundamental paper on the K metric concluded that behavioural traits are more labile than morphological traits. However, our vireonid study did not detect this pattern, as the behavioural (i.e., song) traits and morphological (i.e., bill shape and body size) traits exhibited similar K values. *Cyclarhis* and *Vireolanius* comprised species with the shortest, deepest bills, and largest bodies, whereas longer, thinner bills, and smaller bodies were more prevalent in *Vireo* and *Pachysylvia* (Fig 3A,B). Thus, the presence of phylogenetic signal in traits that constrain birdsong has important implications in vireonid song evolution (see details in "morphology and song traits," below).

Additional avian families with significant phylogenetic signal in bill traits and body size include Hummingbirds (Trochilidae; Puga‑Caballero in press), Leaf Warblers (Tietze et al. 2015), and several South American species (Tobias et al. 2010; Barcelo et al. 2012). This trait evolution could extend to other morphological traits not considered here, including wing length, tarsus length, and tail length. Structural similarity between closely related species is the hallmark of evolution by common decent (Gregory 2008), and the resulting speciation allows birds to interact with different niches and prey items, reducing interspecific competition, as noted in Hawaiian Honeycreepers (Fringillidae) and Darwin's Finches (Losos and Ricklefs 2009).

HABITAT TYPE AND SONG TRAITS

The acoustic adaptation hypothesis predicts that temporal and frequency components of avian songs are dependent on the size and density of sound-reflecting surfaces in the habitat of the singer (Morton 1975; Boncoraglio and Saino 2007). However, variation in the four song traits considered here was not associated with habitat type. Despite the principles of sound propagation

providing a strong basis for the acoustic adaptation hypothesis, results from previous studies are mixed (Boncoraglio and Saino 2007). Relationships between habitat and song traits have been found in the Little Greenbul (*Eurillas virens*; Slabbekoorn and Smith 2002), Malagasy Paradise Flycatcher (*Terpsiphone mutata*; Van Dongen and Mulder 2006), and White-crowned Sparrow (*Zonotrichia leucophrys*; Derryberry 2009), but not in the Indigo Bunting (*Passerina cyanea*; Hylton and Godard 2001), some corvid species (Corvidae; Laiolo and Rolando 2003), and several Australian songbirds (Blumstein and Turner 2005). Even when some studies found significant habitat effects on song traits, some of their results deviated from the predicted direction of the acoustic adaptation hypothesis. Mason and Burns (2014) found that habitat explained only three out of 10 Tanager (Thraupidae) songs traits, and, of these, only one (i.e., frequency modulation being higher in non-forested habitats) followed the directional predictions of the acoustic adaptation hypothesis. One explanation for conflicting results is that broad habitat categories do not capture microhabitat use of singing birds (MacArthur 1958). For example, woodland-dwelling songbirds may vocalize from the tops of tree crowns with less tree density, whereas shrubland songbirds may sing within dense thickets. Broader habitat types might also be problematic with habitat generalists. For example, the Rufous-browed Peppershrike breeds in both open (e.g., gardens and savannahs) and closed habitats (e.g., montane forests) (Brewer et al. 2019); we classified this species as an open woodland bird in our study. Tubaro and Segura (1995) compared the song structure of Rufous-browed Peppershrikes in three broad habitat categories and found that peppershrikes in relatively open habitats produced lower frequency songs, again contradicting the predictions of the acoustic adaptation hypothesis. These conflicting results could be resolved by quantifying and correlating microhabitat use of individual birds (e.g., song perches) with song traits.

MORPHOLOGY AND SONG TRAITS

We found significant positive correlations between bill shape and some frequency components of vireonid songs. Species with deeper, shorter bills sang songs with significantly lower

minimum and maximum peak frequency, compared to vireonids with longer, thinner bills. (Fig 1, 3A, 3B). A positive correlation between bill morphology and avian song traits also occurred in woodcreepers (Furnariidae; Palacios and Tubaro 2000), Darwin's finches (Podos 2001), and true finches (Fringillidae; Giraudeau et al. 2014; Porter and Smith 2019). Birds with larger bills cannot manipulate their gape size with the height necessary for higher frequencies (Podos 2001). Hoese et al. (2000) compared song frequency components in sparrows with temporarily immobilized bills to those with unhindered bills and found that songs produced from immobilized bills suffered frequency constraints. Previous research proposed that selection may drive the diversification of bill shapes and sizes across species for optimal capture and consumption of different food items, and that these changes in bill size may, in turn, affect song traits (Herrel et al. 2009). For example, vireonids with shorter, deeper bills, like the Rufous-browed Peppershrike and Chestnut-sided Shrike-Vireo (*Vireolanius melitophrys*), consume larger, hard food items, including beetles, small frogs, lizards, large (~ 70 mm) caterpillars, and Hawthorne apples (Schaldach 1963; Barlow and James 1975; Brewer et al. 2019), and they sing songs with lower frequencies. In contrast, vireonids with longer, thinner bills, such as the Blue-headed Vireo (*Vireo solitarius*) and the Philadelphia Vireo (*Vireo philadelphicus*), consume small insects (Brewer et al. 2019), and they sing songs with higher frequencies. Contrary to our predictions, we found no effect of bill shape on frequency modulation and song length. Several tropical vireonids in our study, particularly species in *Hylophilus* and *Tunchiornis*, had longer, thinner bills, yet, their songs were short, low-modulated whistles, suggesting alternative selective pressures are shaping frequency modulation in vireonid songs.

We found significant negative relationships between body size and some frequency traits. Larger-bodied vireonids produced songs with lower minimum and maximum peak frequencies, compared to smaller-bodied vireonids. Similar correlations were found in several birds in Europe(Wallschläger 1980) and the Neotropics (Martin et al. 2011; Derryberry et al. 2018). Bigger-bodied birds have larger syrinxes, which produce lower frequencies. Despite this, we found no relationship between body size and frequency modulation. Some tiny (~ 10 g) species of *Hylophilus*

in our study, like Grey-chested Greenlet (*Hylophilus semicinereus*) and Tepui Greenlet (*Vireo sclateri*), produced songs with similar frequency modulation found in the larger-bodied (~ 30 g) vireonids, such as Yellow-browed Shrike-Vireo (*Vireolanius eximius*) and Green Shrike-Vireo (*Vireolanius pulchellus*). As with bill shape, body size, alone, may not explain all frequency components of bird song. Our study also found no significant relationship between body size and song length. Relationships between avian body size and temporal song traits are mixed. Body size did not explain temporal features of songs in bush warblers (Cettiidae; Wei et al. 2017) or blue cardinals (Cardinalidae; García and Tubaro 2018), whereas larger thraupids sang slower paced songs than their smaller-bodied counterparts (Mason and Burns, 2015). However, due to vireonid song complexity, we did not take temporal measurements at the individual element level. Therefore, relationships between body size and temporal song traits in the Vireonidae require further investigation.

ADDITIONAL FACTORS SHAPING SONG TRAITS

Sexual selection is arguably the most accepted driver of song structure diversity in birds (Darwin 1896; Searcy and Andersson 1986, Mikula et al. 2020). One mechanism of sexual selection is female choice, where females prefer males with certain song characteristics, which males may learn or inherit (Catchpole 1987). For example, males with larger song repertoires and more complex songs acquire mates faster than males with smaller repertoires and less complex songs (Yasukawa et al. 1980; Buchanan and Catchpole 1997; but see Byers and Kroodsma 2009), and song performance (reviewed in Wilson et al. 2014). More recent research, however, suggests sexual selection in birdsong evolution is more complicated than just female choice, since female song was shown to be phylogenetically widespread and ancestral in present-day songbirds, and has been frequently lost during passerine evolution, including in vireos and greenlets (Odom et al. 2014). Understanding the degree to which song traits conform to phylogenetic history requires the consideration of other factors and evolutionary processes, in addition to sexual selection.

The songs young passerines hear during their song learning phase can also influence song structure (Beecher and Brenowitz, 2005, Phan et al., 2006). Although this phase marks the acquisition of conspecific song, deviations can occur when learning songbirds make mistakes, such as reproducing heterospecific signals (James 1981). Noteworthy examples among vireonids include White-eyed Vireo (*Vireo griseus*) songs containing Downy Woodpecker (*Picoides pubescens*) calls, and Red-eyed Vireos reproducing Olive-sided Flycatcher (*Contopus cooperi*) songs (Adkisson and Conner 1978; James 1976; James 1981; James 1984). Copying mistakes and improvisation during song learning can lead to additional signal diversity, a process known as cultural drift (Podos 2004). Within the context of our study, habitat and morphology could each strengthen cultural evolutionary song shifts: different habitats may transmit different frequencies better and thus affect which frequency components are likely to

be learned, whereas bill shape variation may make perfect imitation of some songs impossible. Songbirds can also adjust song frequency in response to ambient noise, which reflects song plasticity within individuals rather than fixed population differences (Hu and Cardoso 2010; de Magalhães Tolentino et al., 2018). The behavioural strategies used by singing birds may also influence song structure evolution. For example, birds that regularly communicate over short distances, such as duetting male-female tropical pairs (Langmore 1998; Logue and Hall 2014), may utilize a broader acoustic space because their proximity may minimize signal degradation. In contrast, temperate species that communicate over longer distances, and rarely as duets, may experience greater signal degradation (Fotheringham et al. 1997; Benedict 2008), which could restrict the range of frequencies that could be incorporated into their signals. Similarly, territory size data could provide additional insight into the evolution of song structure. The risk of habitat-induced signal degradation could be higher for species holding large territories, and thus favour songs with low frequencies that degrade less over distance. An interesting possibility, however, is that degradation of long-range signals could benefit receivers by providing reliable information about the signaler's location (Naguib and Wiley 2001). Unfortunately, such natural history is not readily

available for many species, including tropical vireonids. This information would lead to the formulation and testing of more rigorous hypotheses on habitat use and the evolution of birdsong structure.

Whether birds are migratory or non-migratory is also hypothesized to affect song structure. Songs of migratory species are expected to be under stronger selection because migrants have less time on breeding grounds for mate acquisition and breeding, compared to resident birds (Catchpole 1982). Consequently, both song repertoire and song complexity are often greater in migratory birds, compared to residents (Read and Weary 1992; Collins et al. 2008). Mountjoy and Leger (2001) explored this relationship in *Vireos* and found that migratory species sang more complex songs (i.e., repertoire size) than non-migratory species, although migration distance was not related to song complexity. In addition to *Vireo*, which are predominately temperate migrants, our study included several genera containing year-round tropical residents: *Erpornis*, *Pteruthius*, *Cychlarhis*, *Vireolanius*, *Hylophilus*, *Tunchiornis*, and *Pachysylvia*. Whether a significant relationship between migratory status and song complexity and other aspects of song structure remains will require further investigation. We could not test this because our song selection criteria did not discriminate between migratory and non-migratory individuals from temperate vireonid populations.

CONCLUSIONS

Taken together, our results support the hypotheses that shared phylogenetic history and morphology shape song structure in Vireonidae, and these two factors are not mutually exclusive. It is plausible that vireonid song structure has significant phylogenetic signal because bill shape and body mass, factors shown to constrain song frequencies, also adhere to the phylogeny. Within the context of evolutionary biology, this rich song diversity could serve as a behavioural mechanism that reduces hybridization and promotes vireonid speciation. Future studies can use playback experiments to assess how receivers respond to divergent songs within the Vireonidae, and whether the structural diversity of songs traits functions as a behavioural mechanism for reproductive

isolation. If song structure is an effective pre-mating barrier, one would predict stronger responses towards similar sounding stimuli, and weaker responses to dissimilar songs. The present study advances our understanding of how evolutionary processes shape signals that are critical for reproduction, reproductive isolation, and, ultimately, speciation.

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TABLES

Table 1. Phylogenetic signal of four song traits and two morphological traits calculated from the Vireonidae MCC tree and the 100 trees with the greatest posterior probability. Minimum and maximum values correspond to the smallest and largest Pagel's λ and Blomberg's K values detected across the 100 trees from the posterior, respectively.

	λ	λ min	λ max	λ P min	λ P max	K	K min	K max	K P min	K P max
Song Length	1.01	0.89	1.03	< 0.0001	0.0009	0.62	0.49	0.68	0.0027	0.0124
Max Peak Frequency	0.90	0.81	0.96	< 0.0001	0.0007	0.45	0.37	0.51	0.0002	0.0035
Minimum Peak Frequency	0.88	0.85	0.92	< 0.0001	0.0006	0.58	0.43	0.66	0.0004	0.0035
Frequency Modulation	0.85	0.70	0.94	< 0.0001	0.0011	0.51	0.42	0.60	0.0001	0.0011
Bill Shape (pPC2)	0.83	0.80	0.90	<0.0001	<0.0001	0.59	0.46	0.69	0.0001	0.0020
ln (Body Mass)	0.94	0.93	0.97	<0.0001	<0.0001	0.71	0.57	0.83	0.0001	0.0008

FIGURE CAPTIONS

Figure 1. Sonograms and illustrations of vireonid species and their relative positions on the MCC Vireonidae tree. The colours of the outlines of spectrograms and illustrations match portions of the tree where species are located. Dashed branches denote clades whose representatives were not depicted with sonograms and illustrations. Species were selected to highlight relationships between bill shape and song structure across the family. Vireonids with shorter, deeper bills produce songs of lower minimum and maximum peak frequency, compared with vireonids with longer, thinner bills. Time on sonogram x-axis is variable to accommodate differences in song lengths. Illustrations reproduced with permission of Lynx Edicions; Wilson, D.E., Lacher, T.E., Jr and Mittermeier, R.A. eds. (2017). Handbook of the Mammals of the World. Vol. 7. Rodents II. Lynx Edicions, Barcelona
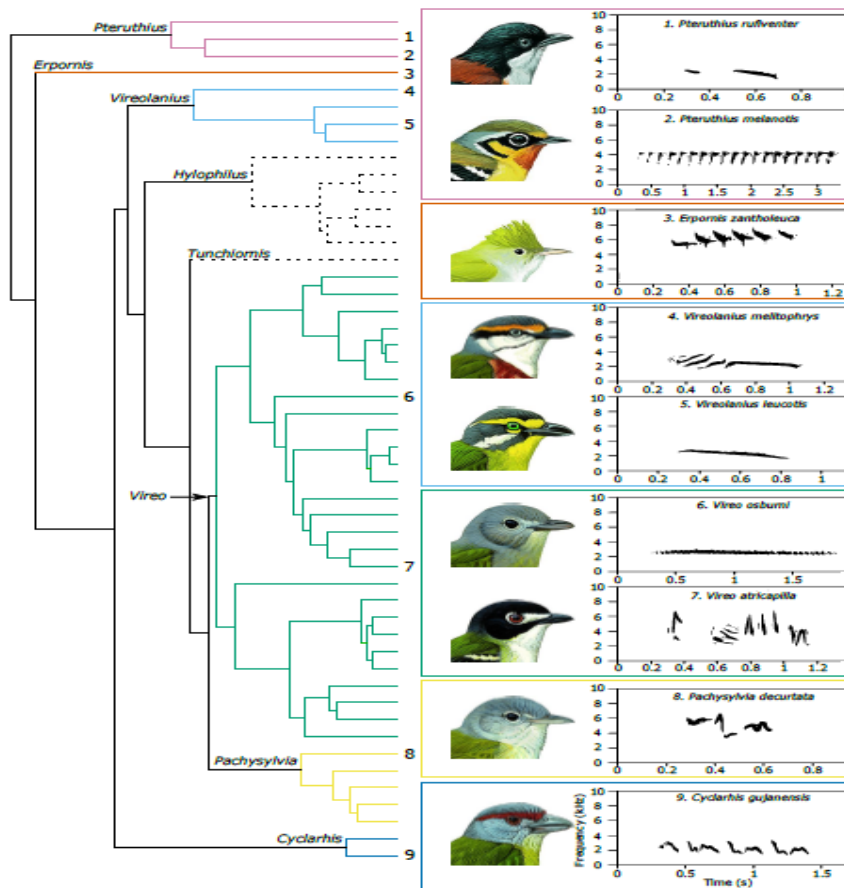
Figure 2. Song trait distribution on the MCC Vireonidae tree: (A) song length (s), (B) minimum peak frequency (Hz), (C) maximum peak frequency (Hz), and (D) frequency modulation (Hz/s). Song traits were mapped onto phylogenies using the plotBranchbyTrait function in the *phytools* package in R. Colder colours correspond to smaller trait values and warmer colours to larger trait values. Song length showed the strongest song trait conservatism (i.e., phylogenetic signal); longer songs were conserved in the *Hylophilus* clade. The remaining three song traits showed less trait conservatism, with small and large values appearing throughout vireonid genera. The respective magnitude of phylogenetic signal (i.e., Blomberg's *K* and Pagel's *λ*) is shown for each song trait.
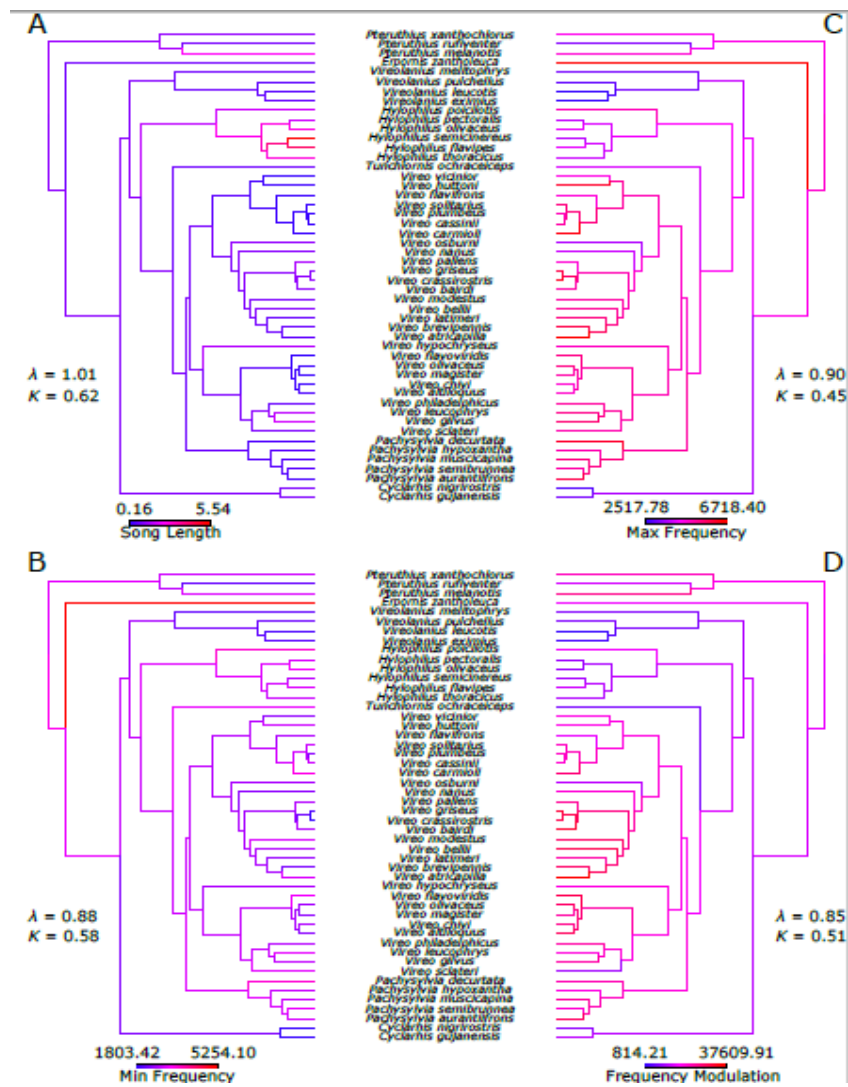
Figure 3. Morphology trait distribution on the MCC Vireonidae tree: (A) bill shape (pPC2 scores) and (B) ln(body mass). Both traits were mapped onto phylogenies using the plotBranchbyTrait function in the *phytools* package in R. Colder colours correspond to the smaller trait values and warmer colours to larger trait values. Shorter, deeper bills were largely confined to the *Cyclarhis* and *Vireolanius* clades, whereas *Erpornis*, *Hylophilus*, *Tunchiornis*, *Vireo*, and *Pachysylvia* had longer, thinner bills; *Pteruthius* species were intermediate between the two bill extremes. Similarly, *Cyclarhis* and *Vireolanius* contained larger bodies than the remaining six genera. The respective magnitude of phylogenetic signal (i.e., Blomberg's K and Pagel's λ) is shown for both morphological traits.
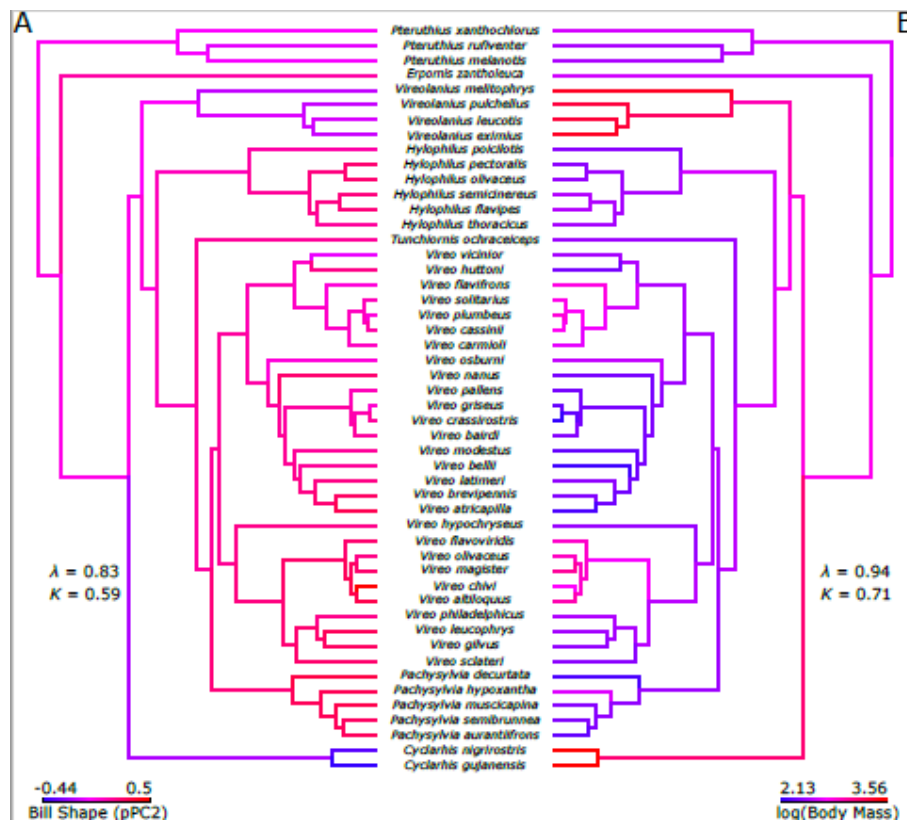
Figure 4. Relationships of structure and morphology. Panels in the left column show the relationships between song structure and bill shape (pPC2 scores), whereas those in the right column show the relationships between song structure and body size (ln-body mass). Song structure include song length (A, B), minimum peak frequency (C, D), maximum peak frequency (E, F), frequency modulation (G, H). Illustrations of Black-billed Peppershrike (*Cyclarhis nigrirostris*) and White-bellied Erpornis (*Erpornis zantholeuca*) depict vireonids with shorter, deeper bills, and longer, thinner bills, respectively. Similarly, Black-eared Shrike-babbler (*Pteruthius melanotis*) and Chestnut-sided Shrike-Vireo (*Vireolanius melitophrys)* depict smaller and larger-bodied vireonids, respectively. Both minimum and maximum frequency showed a significant, positive relationship with vireonid bill shape. In contrast, these same frequency traits showed a significant, negative relationship with body size. Illustrations reproduced with permission of Lynx Edicions; Wilson, D.E., Lacher, T.E., Jr and Mittermeier, R.A. eds. (2017). Handbook of the Mammals of the World. Vol. 7. Rodents II. Lynx Edicions, Barcelona.