



Functional Ecology

Morphological Diversity in the Sensory System of Phyllostomid Bats: Implications for Acoustic and Dietary Ecology

Leith B. Leiser-Miller* and Sharlene E. Santana

Department of Biology and Burke Museum of Natural History and Culture, University of Washington, Seattle, Washington 98195, USA

*Correspondence to: Leith B. Leiser-Miller. Department of Biology, University of Washington, 241 Life Sciences Building, Box 351800, Seattle, WA 98195. E-mail: leithmiller1@gmail.com

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DR LEITH LEISER-MILLER (Orcid ID : 0000-0003-4886-1494)

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ABSTRACT

1. Sensory systems perform fitness-relevant functions, and specialized sensory structures allow organisms to accomplish challenging tasks. However, broad comparative analyses of sensory morphologies and their performance are lacking for diverse mammalian radiations.
2. Neotropical leaf-nosed bats (Phyllostomidae) are one of the most ecologically diverse mammal groups; including a wide range of diets and foraging behaviors, and extreme morphological variation in external sensory structures used in echolocation (nose leaf and pinnae).
3. We coupled 3D geometric morphometrics and acoustic field recordings under a phylogenetic framework to investigate the mechanisms underlying the diversification of external sensory morphologies in phyllostomids, and explored the potential implications of sensory morphological diversity to functional outputs and dietary ecology.
4. We found that the nose leaf consists of two evolutionary modules, spear and horseshoe, suggesting that modularity enabled morphological and functional diversification of this structure.
5. We found a significant association between some aspects of nose leaf shape and maximum frequency and bandwidth of echolocation calls, but not between pinnae shape and echolocation call parameters. This may be explained by the

use of multiple sensory modes across phyllostomids and plasticity of some echolocation call parameters.

6. Species with different diets significantly differed in nose leaf shape, specifically in spear breadth, presence of a midrib, and cupping and anterior rotation of the horseshoe. This may relate to different levels of prey type specificity within each diet. Pinnae shape significantly differed between species that consume non-mobile, non-evasive prey (broad rounded, cupped pinnae) and mobile, evasive prey (flattened pinnae with a sharp tapering apex). This may reflect the use of different sound cues to detect prey.
7. Our results give insight into the morphological evolution of external sensory structures in bats, and highlight new links between morphological diversity and ecology.

Keywords: Phyllostomidae, echolocation, tropical bats, nose leaf, pinna, acoustics

INTRODUCTION

All animals use sensory cues to perform functions that impact their fitness, and many possess highly specialized sensory structures that allow them to accomplish challenging tasks (Catania, 1999, 2011; Schmitz & Wainwright, 2011). Prey detection is a critical use of the sensory system that can directly influence organismal fitness and ecology (Ferry-Graham, Bolnick, & Wainwright, 2002; Gracheva et al., 2011; Kalko &

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Condon, 1998; Persson, Andersson, Wahlström, & Eklöv, 1996; Placyk & Graves, 2002), and previous work has provided a solid foundation for understanding how the sensory structures of many predators have evolved to match the requirements of their dietary niches (e.g., Catania, 1999; Safi & Siemers, 2009). However, broad comparative analyses of morphology and function are still needed to understand sensory adaptations in some of the most diverse mammalian radiations, and how changes in the sensory system influence organismal ecology and evolution (Gracheva et al., 2011; Kalko & Condon, 1998). Here, we investigate several hypotheses regarding the source of morphological diversity and the links between morphological, functional and ecological diversity in the external sensory organs of the most tropically diverse family of mammals, the Neotropical leaf-nosed bats (Phyllostomidae).

Phyllostomids are an adaptive radiation comprised by over 200 species that vary greatly in diet, including insectivory, sanguivory, animalivory, nectarivory, omnivory and frugivory (Dumont et al., 2012; Rex, Czaczkas, Michener, Kunz, & Voigt, 2010). They are laryngeal echolocators, nasophonators, and possess a conspicuous leaf-shaped structure surrounding their nares –a nose leaf– that functions in the emission of echolocation calls (Bogdanowicz, Csada, & Fenton, 1997; Vanderelst et al., 2010). While the nose leaf acts as an acoustic reflector that focuses the echolocation sound beam (Wilson & Reeder, 2005; Hartley & Suthers, 1987; Linnenschmidt & Wiegrebe, 2016), the external ears (pinnae) act as acoustic horns that help detect returning echoes (Fuzessery, 1996; Obrist, Fenton, Eger, & Schlegel, 1993; Jen & Chen, 1988; Vanderelst et al., 2010).

Computational models and relatively limited experimental evidence has demonstrated changes in the morphology of the nose leaf and pinnae can drastically alter a bat's acoustic field of view (Feng, Gao, Lu, & Mu, 2012; Gao, Balakrishnan, He, Yan, & Müller, 2011; He, Pedersen, Gupta, Simmons, & Müller, 2015; Müller, 2015; Vanderelst et al., 2010; Zhuang & Müller, 2007; Zhuang, Wang, Li, Mao, & Wang, 2012). Phyllostomids exhibit great morphological diversity in nose leaf and pinnae, including insectivorous species with greatly elongated spears (e.g., *Gardnerycteris crenulatum*, Fig. 1D) and large pinnae (e.g., *Lophostoma silvicolum*, Fig. 1A), and nectarivorous or sanguivorous species with reduced or absent spears and small

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pinnae (e.g., *Glossophaga soricina*, Fig. 1C; *Desmodus rotundus*, Fig. 1B). This diversity makes phyllostomids an excellent system to investigate the ecomorphological diversity of the sensory system within an evolutionary context. To date, no studies have investigated the evolution of nose leaf morphologies in a broad comparative context, and only a few have explored how specific morphological components are related to sound beam spreading (Feng, Gao, Lu, & Müller, 2012; Gao, Balakrishnan, He, Yan, & Müller, 2011; Vanderelst et al., 2010; Zhuang & Müller, 2007) or the relationship between facial features and echolocation parameters using traditional morphometrics (Goudy-trainor & Freeman, 2002).

Morphological diversity may evolve through various mechanisms, including modularity (Goswami, 2006; Goswami & Polly, 2010). An anatomical module is defined by sets of highly correlated traits that have weak correlations with traits outside the module (Goswami & Polly, 2010). Modules can either increase or decrease morphological diversity via autonomy among modules or integration within modules, respectively (Goswami & Polly 2010). Modularity of morphological structures is a common feature across taxa (e.g., cichlid jaws, Hulsey, 2006; anoles, Sanger, Mahler, Abzhanov, & Losos, 2012; mammal skulls, Goswami, 2006), and the evolution of modules within a structure allows parts of the structure to specialize for different functions. Here, we investigate modularity as a potential mechanism underlying the morphological diversification of the nose leaf in phyllostomids. We specifically test the hypothesis that the nose leaf consists of two modules, spear and horseshoe (Table 1, Fig. 2D), and predict that this modularity underlies specialization of each of these parts on specific acoustic functions.

Next, we test the overarching hypothesis that there is a functional link between morphological variation of the nose leaves and pinnae and echolocation call parameters across phyllostomids. High frequencies, necessary for detection of small and cryptic prey (e.g., katydids on leaves; Geipel et al., 2013; Simmons et al., 1975), need to be of high intensity to produce an audible echo for the bat –high frequencies attenuate rapidly, especially in the hot, humid habitats where phyllostomids live (Lawrence & Simmons, 1982). The angle in which sound reflects off a surface can greatly influence

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either the spread and concentration of sound (e.g., wider, less intense versus narrower, more intense sound beam; Herzfeld, 1938). Therefore, we predict most phyllostomids will have nose leaf morphologies that increase their ability to focus highest frequencies emitted, as particular shape components would be more efficient at directionally reflecting sound (Table 1; specific hypotheses and predictions based on Herzfeld, 1938; Wahlstrom, 1985; Zhuang & Müller, 2012). Across several families of bats, absolutely smaller pinnae typically have a more pointed apex and are not sensitive to detecting low frequencies (Obrist, Fenton, Eger, & Schlegel, 1993), whereas species with rounded, broad pinnae tend to cue on low frequency sounds (<15 kHz) for prey detection, such as prey mating calls (Bernal, Page, Ryan, Argo, & Wilson, 2009). Therefore, we also predict to find a link between overall pinna shape and the frequency of acoustic cues that may be used for prey detection by different phyllostomids species (see Table 1 for specific predictions).

Finally, we test the hypothesis that dietary ecology and foraging behavior have imposed selective pressures on the echolocation system of phyllostomids, including the nose leaf and pinnae, leading to morphological variation in these structures among species (Table 1). Phyllostomid bats rely on different sensory modalities depending on the type of prey they pursue. For example, insectivores use echolocation for navigation, and to detect, locate and classify prey (Geipel et al., 2013). In contrast, frugivores use echolocation for navigation, but rely heavily on olfaction to initially find ripe fruit (Bloss, 1999; Hodgkison et al., 2007; Laska, 1990; Reiger & Jakob, 1988). We predict that interspecific variation in the morphology of the nose leaf and pinnae is associated with specialization for dietary ecology in phyllostomids (Table 1).

METHODS

3D Imaging and shape analyses

We quantified the three-dimensional morphology of nose leaves and pinnae for 46 adult bats from 33 phyllostomids species that span the diversity in body size, nose leaf and pinnae morphologies, and dietary ecology within the family. The majority of specimens used (28 species) were collected by us in the field following approved methods (University of Washington IACUC protocol 4307-01), and the remainder (5 species)

were fluid-preserved museum specimens in which the nose leaf and pinnae were preserved in their natural position (supplemental Table 1). Based on information and classifications from the literature, we grouped species into six taxonomic dietary categories (animalivores, insectivores, nectarivores, frugivores, omnivores, and sanguinivores; Giannini & Kalko, 2004), and two functional dietary categories: predators of non-mobile/non-evasive prey (nectarivores, frugivores, omnivores and sanguinivores), and predators of mobile, evasive prey (insectivores, animalivores). While assignment of species to these broadly defined dietary categories may be an oversimplification of the breadth of their ecological roles (e.g., *Glossophaga soricina*; Clare et al., 2014), these classifications were necessary to overcome limitations due to sample sizes and the lack of quantitative dietary data that could inform more detailed analyses.

Unless the nose leaf and pinnae are adequately fixed during specimen preservation, this process can alter their shape (e.g., resulting in bent nose leaves). Furthermore, high-resolution imaging (such as μ CT scanning, below) of these structures yields better results if they are scanned in isolation from denser structures like the skull. Thus, we captured pinnae and nose leaf morphology by taking casts from freshly euthanized animals. To do so, we used a President Jet dispenser gun to apply President dental molding epoxy (Epo-tek 301) to the pinnae and nose leaf (Fig. 2A). We allowed casts to dry on the specimen for a minimum of five minutes before carefully removing them. Due to limitations of field conditions and primarily using freshly collected specimens, we were not able to assess the repeatability of this technique. However, individuals of a species cluster closely together in morphospace, which indicates that this casting method is adequate for capturing interspecific variation.

To increase the size and taxonomic scope of our dataset, we were also able to use several fluid-preserved specimens that were specifically preserved to avoid deformation of soft tissues and could be destructively sampled (i.e., nose leaf could be dissected out for μ CT scanning). This additional source of specimens did not seem to introduce errors in our quantification of morphology. We created 3D digital models of the nose leaf and pinnae by scanning either specimens or epoxy casts on a Skyscan 1174 μ CT scanner (Bruker MicroCT, Kontich, Belgium) at a 17- 30.1 μ m resolution,

depending on the size of the cast or specimen. We used NRecon (Microphotonics, Allentown, PA) to convert CT shadow images into image stacks (“slices”), and imported these into Mimics 17.0 (Materialise NV, Leuven, Belgium, 2014) to segment nose leaf and pinnae and produce 3D surface (*.stl) files (Fig. 2B). We imported raw stl files into Geomagic Studio 2014.1.0 (3D Systems, SC, USA, 2014) to remove scanning artifacts (e.g., debris in molds) from the models.

To quantify nose leaf and pinnae shape, we used 3D geometric morphometric analyses (Bookstein, 1997; Zelditch, Swiderski, Sheets, & Fink, 2004). These were based on single point landmarks and surface patches, all placed on 3D models using Stratovan Checkpoint© (Stratovan Corporation, Davis, CA). For the nose leaf, we placed: (1) single-point landmarks at the base of each nostril and the apex of the spear, (2) evenly-spaced semi-landmarks around the nose leaf perimeter, and (3) two “patches” of semi-landmarks in a grid across the surface of the spear and the surface of the horseshoe, respectively (Fig. 2D). To analyze shape changes of subcomponents of the nose leaf separately (i.e., spear and horseshoe), we added landmarks to ensure each subcomponent had a sufficient number of true landmarks. For the spear, we placed a single-point landmark at the apex of the spear, two landmarks at the point where the spear meets the horseshoe, and a patch of semi-landmarks over the anterior surface of the spear (Fig. 2D). Some species lack a spear, and therefore were not included in analyses of that structure. For the horseshoe, we placed a single-point landmark on each nostril and one patch over the surface of horseshoe.

For pinnae, we placed two landmarks at the points where the pinna attaches to the head, and a patch of semi-landmarks across its surface (Fig. 2C). We exported landmark coordinates for each specimen as .csv files and computed species means for landmark coordinates in Excel. We then performed Procrustes superimposition analyses to scale, align and rotate landmark configurations (Rohlf, 1990), and obtain a set of variables describing the shape of the entire nose leaf, spear, horseshoe and pinnae across species. We used the package “geomorph” (Adams & Otárola-Castillo, 2013) in R v 99.903 (R Core Team, 2017) for geometric morphometric analyses.

Acoustics

Phyllostomid bats produce low-intensity calls (Brinkløv, Kalko, & Surlykke, 2009; Griffin, 1958) that are difficult to capture on passive recording devices. Consequently, call parameter data are sparse for most phyllostomid species. For this study, we collected 16-bit recordings of release calls using a microphone condenser (UltraSoundGate 116). Our sample included 101 individuals spanning 33 species. We held each bat in hand, placed a microphone approximately six inches from its face, and then released the bat away from environmental clutter while recording the calls it emitted as it flew away. Since bats had to be released to document their natural calls, we did not use these same individuals in morphological analyses. We measured call parameters for 3–7 individuals per species, with the exception of species that were rare or difficult to capture at our study localities (*Chrotopterus auritus*, *Glyphonycteris sylvestris*, *Phyllostomus hastatus*, *Sturnira lilium*), for which we were able to record 1 individual per species. We analyzed release calls using Avisoft SASLabPro v. 5.2.12 (Avisoft Bioacoustics, Berlin, Germany) to extract the following echolocation call parameters: minimum frequency (kHz), maximum frequency (kHz), peak frequency (kHz) (i.e., frequency with the highest amplitude), and total bandwidth (kHz) across the call. We averaged call sequences per individual (a minimum of 5) and calculated means and standard deviation of each parameter (supplemental Table 2). While release calls may not fully reflect the echolocation capabilities of the species, our own comparisons of release calls with foraging calls for one species (*Carollia castanea*) indicate that foraging call parameters fall well within the range of values recorded for release calls (Leiser-Miller, in press).

Statistical analyses

To test whether the nose leaf consists of two modules (spear and horseshoe; Fig. 2D), we used the function `phylo.modularity` (Geomorph package, Adams & Otárola-Castillo, 2013) to compute Covariance Ratio (CR) values for a two-module hypothesis based on the nose leaf landmark data, and estimate the p-value for this relationship over 1,000 random permutations. The CR ratio indicates the degree of covariation among landmarks within possible modules; values from 0 to 1 indicate less covariation *between* modules than *within* each module, supporting the modularity hypothesis, CR values

greater than 1 describe greater covariation between modules than within modules, supporting the null hypothesis of no modules (Adams, 2016).

To identify major axes of shape variation across sensory structures, we conducted phylogenetic Principal Component Analyses (pPCA), using the Rojas, Warsi, & Dávalos (2016) phylogeny, on the Procrustes (shape) coordinates for each structure/substructure using the R package “phytools” (Revell, 2012). We assessed the significance of pPCA axes via Horn’s parallel analysis from the ‘paran’ function in R (Dinno, 2015). Nose leaf and pinna shape axes were not correlated with size (forearm length; Supplementary Table 5), and therefore size was not considered in subsequent analyses. To identify if shapes of external sensory structures are correlated with call parameters, we ran separate phylogenetic generalized least squares (PGLS) regressions under Brownian motion of acoustic parameters across the call (minimum frequency, maximum frequency, peak frequency, and total bandwidth) against significant pPCs shape scores (see Results; nose leaf pPC 1-5; pinnae pPC 1-4). Finally, we ran phylogenetic ANOVAs and post-hoc analyses to test for an association between diet category and nose leaf and pinnae shape, respectively. We used significant pPCs axes as response variables, and dietary category as the predicting factor.

RESULTS

Modularity of the nose leaf

Our Covariance Ratio analyses indicate that landmarks within the spear and the horseshoe covary more within each of these subcomponents than between them (CR: 0.882, $P < 0.001$). This strongly suggests that the spear and the horseshoe constitute separate modules within the nose leaf.

Morphological diversity of sensory structures

For the complete nose leaf, we identified five significant axes (pPCs) representing 68.3% of the total shape variation. Principal components 3-5 combined explain less than one-third of the total shape variation, and very subtle morphological differences. The same is true for other structures examined, thus we focus on describing the shape

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differences for pPC1-2 for all structures. Nose leaf pPC1 (32.4% of total shape variation) is positively correlated with shapes that are flattened in the coronal plane and have a reduced ventral edge of the horseshoe, and negatively correlated with shapes that have distinct midribs and a horseshoe that is rotated anteriorly (see extremes on Fig. 3A). Lower values of nose leaf pPC2 (13.2% of total shape variation) describe spear shapes that are wider, taper sharply towards the apex and have a slight anterior rotation of the outer edges, and a more pronounced horseshoe that protrudes from the rostrum. In contrast, higher values along nose leaf pPC2 describe shapes characterized by narrow spears that taper smoothly towards the apex.

Because the nose leaf consists of two modules and some species lack a spear, we also examined the morphological trends within each of the modules across species. For the spear, we identified five significant pPCs representing 75.5% of total shape variation (Fig. 3B). Spear pPC1 (30.5% of total shape variation) is positively correlated with a slightly convex surface with expanded edges of the spear, resulting in a more rounded spear. Lower values of pPC1 describe more triangularly-shaped spears with a midrib. For the horseshoe, we identified five significant pPCs representing 63.7% of the total shape variation. Horseshoe pPC1 (28.6% of total shape variation) is positively correlated with the posterior inflection of the septum between the nostrils, anterior shifting of the ventral edge of the horseshoe, and the inseting of the nostrils relative to the rest of the horseshoe (Fig. 3C). Horseshoe pPC2 (12.3% of total shape variation) is positively correlated with a squarer horseshoe that is more flattened on the face versus a rounded horseshoe surrounding the nostrils and protruding from the face.

For the pinnae, four significant axes explained 66.4% of the total shape variation. Pinnae pPC1 (35.1% of total shape variation) is positively correlated with shapes that are planar and negatively correlated with broad and rounded shapes that are deeply cupped (Fig. 4). Pinnae pPC2 (13.6% of the total shape variation) is positively correlated with a slightly tapered, posteriorly rotated, pointed apex and an anteriorly located pinna fold, and negatively correlated with inward curling of the pinna's margin, specifically at the base of the pinna.

Links among morphology, call parameters, and diet

Nose leaf. PGLS regressions revealed a significant relationship between pPC1 and maximum frequency and bandwidth (Table 2; supplemental Fig. 1); species with a flattened nose leaf in the coronal plane and a reduced ventral edge of the horseshoe have lower maximum frequency and bandwidth in their echolocation calls. Phylogenetic ANOVAs also revealed significant differences in nose leaf shape for the whole nose leaf (pPC2 and pPC4) and spear (pPC2) among dietary categories (Table 4, all pairwise comparisons are each dietary category against all dietary categories). *Post-hoc* tests revealed that whole nose leaf shape was significantly different between frugivores (pPC2) and omnivores (pPC4) when compared to all other dietary groups, and spear shape (pPC2) between frugivores and omnivores and all other dietary categories (supplemental Table 3). For the horseshoe, we found no significant differences across all dietary groups (Table 4).

Pinnae. We found no significant relationships between pinnae shape and echolocation call parameters (Table 3), and no significant differences in pinnae shape among taxonomically-defined dietary categories (Table 4). However, we did find that pinnae shape pPC1 was significantly different between bat species that pursue non-mobile, non-evasive prey and species that pursue mobile, evasive prey (supplemental Table 4). These differences are defined by two distinct morphologies: pinnae that are broad with a rounded apex (-pPC1), versus those with a sharply pointed apex (+pPC1) (Fig. 4).

DISCUSSION

Focusing on the extraordinary adaptive radiation of the family Phyllostomidae, we used modern tools and analyses to explore some of the potential intrinsic and extrinsic factors shaping the diversity of sensory structures in bats. We investigated whether and how an evolutionary mechanism (modularity; Olson & Miller, 1999) may underlie extreme diversity of sensory structures, and explored if functional outputs and ecological demands explain morphological differences among species.

Modularity is a common phenomenon that facilitates adaptive responses to conflicting selective pressures and impacts the adaptive evolution of novel phenotypes, for example by allowing modules to specialize on different functions (Hulsey, 2006,

Sanger, Mahler, Abzhanov, & Losos, 2011; Goswami, 2006; Goswami & Polly 2010). Consistent with our hypothesis, we identified two modules, the spear and the horseshoe. These results suggest decoupling these two parts may contribute to their morphological and functional variation across phyllostomids. For example, across different dietary categories, species range from having no spear (e.g., *Desmodus rotundus*, sanguinivore; *Brachyphylla nana*, nectarivore) to an extremely large one (e.g., *Gardnerycteris crenulatum*, insectivore; *Chrotopterus auritus*, animalivore) independently of horseshoe shape. Some species that have evolutionarily lost the spear module have further sensory specialization of the horseshoe module (e.g., *D. rotundus* have enlarged nostrils and infrared sensing pits that aid in prey detection; Gracheva et al., 2011; Jones, Teeling, & Rossiter, 2013). Similarly, species with spears of similar shape may exhibit widely different horseshoes (e.g., *Lophostoma silvicolum* and *Micronycteris microtis*, Fig. 1). Thus, modularity potentially allows phyllostomids to evolve nose leaf morphologies specialized for a particular dietary niche or foraging strategy. Further comparative studies across other nasophonating bat families (e.g., Rhinolophidae, Hipposideridae, Megadermatidae) would be informative to determine whether and how nose leaf modularity constrains or promotes nose leaf diversity at a larger macroevolutionary scale.

We proposed that the detection of particular prey items influenced the morphological evolution of the nose leaf and pinnae across phyllostomids, via selection on the function of these structures. A few studies have investigated the three-dimensional morphology of the bat sensory system and its relationship with functional outputs (Feng, Gao, Lu, & Müller, 2012; Gao, Balakrishnan, He, Yan, & Müller, 2011; He, Pedersen, Gupta, Simmons & Müller, 2015; Vanderelst et al., 2010). These have found a significant link between nose leaf and pinnae morphology and echolocation call frequency in other groups of nasophonating bats (Rhinolophidae and Hipposideridae; Robinson 1996; Huihua, Shiyu, Mingxue, & Jiang 2003). Here, we found a relationship between one axis of nose leaf shape and two echolocation call parameters across the species, maximum frequency and bandwidth, but not between any other shape axes or with pinnae shape. This raises the question, why is there a weak relationship between nose leaf shape and echolocation call parameters in phyllostomids?

In comparison to other nasophonators, most phyllostomids (e.g., frugivores and nectarivores) rely on multiple sensory modalities (e.g., olfaction and echolocation) for prey detection (Kalko & Condon, 1998; Korine & Kalko, 2005; Sánchez et al., 2006; Thies, Kalko, & Schnitzler, 1998). It is highly likely that sensory mode specialization and plasticity varies across species, complicating the relationship between external sensory morphology and echolocation parameters. Modeling studies have found that nose leaf and pinnae function differs across morphologically-similar species with different sensory specializations (Obrist, Fenton, Eger, & Schlegel, 1993; Vanderelst et al., 2010). For example, *Phyllostomus discolor* and *Micronycteris microtis* share similar gross morphologies, but the nose leaf shape of *M. microtis*, which relies heavily on echolocation for prey detection, focuses the energy to a different extent than *P. discolor*, which also relies on vision and olfaction for the same task. Phyllostomids also use diverse foraging behaviors (Bloss, 1999; Korine & Kalko, 2005; Kunz, Braun de Torrez, Bauer, Lobova, & Fleming, 2011; Weinbeer & Meyer, 2006), which can also alter the bat's perceptual field and reconcile physical differences of the sensory system. For example, *M. microtis* flies closely along the vegetation inspecting small areas to detect motionless prey and relies on high-resolution acoustic information about texture differences between vegetation and prey items (Geipel et al., 2013). Conversely, *Phyllostomus* often forages in groups and detects patches of fruits or flowers at longer ranges (Kwiecinski, 2006). Additionally, it has been suggested that some phyllostomids may not be strict nasal emitters and may also echolocate orally (Gessinger, 2016; B. Fenton pers. comm.), albeit this has not been experimentally tested.

Additional factors in the sound production system of phyllostomids likely influence the relationship between external sensory morphology and call parameters, including the morphology of the vocal tract, nasal cavity, and the size of sensory structures. Accessory folds, along the vocal tract, can influence the frequencies emitted (Neuweiler, 2000) and the possibility that some phyllostomids may not be strict nasophonators further complicates this relationship. Phyllostomids also vary greatly in the size of their nose leaves, both relative to body size and relative to the size of the pinnae. For example, some insectivorous and nectarivorous species share a similar nose leaf shape but differ greatly in its size (e.g., 10mm vs. 5mm in height). The size of

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this structure may be an important factor linking phyllostomid echolocation and ecology, as the size of a surface can influence how sound is reflected, and the size of a reflector must be greater than the size of the wavelength being reflected. Therefore, the size of the nose leaf could have evolved in tandem with the minimum echolocation call frequency (longest wavelength in call).

Supporting our hypothesis, we found differences in sensory morphologies across dietary categories. Presumably, differences in nose leaf shape among dietary categories could be driven by pressures on echolocation performance, specifically how sound is reflected and directed. Insectivores and frugivores are the most divergent in whole nose leaf shape, whereas frugivores and nectarivores differ the most when spear shape alone is compared. Additionally, not all axes of nose leaf shape variation are significantly different across dietary categories, suggesting that some shape changes may be more functionally important for some foraging strategies than others.

Frugivorous and some animalivorous species have a wider spear with more anteriorly rotated outer edges, and a more pronounced horseshoe that protrudes from the rostrum (lower pPC1 in Fig. 3), a morphology that provides a more curved reflecting surface. Curved surfaces (e.g., parabolic surfaces) reflect sound in a very concentrated area, increasing its intensity (Wahlstrom, 1985) and directionality (e.g., reducing spread). Curved morphologies specialized for sound concentration are found in diverse taxa and in structures that aid in both sound emission and reception, such as the parabolic skulls of odontocetes that help focus emitted sound (Huggenberger, André, & Oelschläger, 2014; Norris & Harvey, 1900), the antlers of the bull moose that help focus received sound toward their pinnae (Bubenik & Bubenik, 2008), and the spherical facial disks of owls aid in sound localization (Coles & Guppy, 1988). In contrast, nectarivorous and some insectivorous species have narrower and more flattened spears that taper smoothly towards the apex (higher pPC2 in Fig. 3B). Insectivores also have a slightly more pronounced horseshoe, which is almost non-existent in nectarivores. Planar (more flattened) surfaces reflect sound off-axis and at the same angle of incidence (Herzfeld, 1938), possibly increasing the call beam width. Overall, these differences in nose leaf shape likely influence the bats' perceptual fields and their foraging ecology.

Pinnae shape did not differ significantly among species grouped into taxonomic

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dietary categories (e.g., insectivore, frugivore, nectarivore), but it did when species were grouped into functional diet categories that represented major challenges of prey capture (mobile, evasive prey versus non-mobile, non-evasive prey). Specifically, we found that species that use passive acoustic cues for prey detection (e.g., *Chrotopterus auritus*) have broader and large pinnae. This is similar to the pattern observed in other mammal taxa with sensitive hearing (e.g., Serval cats; Smithers, 1978). Broader and large pinnae presumably perform better at detecting the low frequency, low intensity sounds produced by potential prey rustling in the understory or in water (Arlettaz, Jones, & Racey, 2001; Halfwerk, Jones, Taylor, Ryan, & Page, 2014), and low frequency mating calls produced by potential prey, such as túngara frogs (calling at 6-11 kHz; Bernal, Page, Ryan, Argo, & Wilson, 2009).

Finally, it is important to note that external sensory structures are dynamically controlled, and phyllostomids seem to vary at least in the attachments of the muscles that control their pinnae (Storch, 1968). Furthermore, some *Micronycteris* species have an interauricular band that connects the pinnae (Timm & LaVal, 1998) and *Lophostoma* species can curl their pinnae posteriorly (Williams & Genoways, 2007). Differences in the musculature and, subsequently, how bats move sensory structures may be more important than their static shape. Presently, very little is understood about how different species control the movement of their nose leaves and pinnae, with the exception of Old-world leaf nosed bats and horseshoe bats (Feng et al., 2012; He et al., 2015; Müller, 2015). Future studies should incorporate behavioral and functional experiments within a comparative framework to assess the role and performance of the nose leaf and pinnae during foraging tasks.

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AUTHORS CONTRIBUTIONS

LBLM and SES conceived the ideas and designed methodology; LBLM and SES collected the data; LBLM analyzed the data; LBLM and SES wrote the manuscript. Both authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Raw data is archived on Dryad Digital Repository (<https://doi.org/10.5061/dryad.95x69p8g9>), and .stl files will be available upon request.

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Figure legends

Figure 1: Phylogeny of phyllostomid species in our dataset. Branches do not contain information about scale and are colored based on diet (green: insectivore, blue: animalivore, pink: omnivore, orange: frugivore, yellow: nectarivore, red: sanguinivore). Species starting at the root and moving clockwise: *Macrotus waterhousii*, *Lamproncycteris brachyotis*, *Micronycteris microtis*, *Micronycteris hirsuta*, *Micronycteris minuta*, *Trachops cirrhosus*, *Lophostoma*

silvicolum (A), *Phyllostomus discolor*, *Phyllostomus hastatus*, *Gardnerycteris crenulatum* (D), *Chrotopterus auritus*, *Platyrrhinus helleri*, *Artibeus phaeotis*, *Artibeus jamaicensis*, *Artibeus lituratus*, *Phyllops falcatus*, *Sturnira lilium*, *Carollia perspicillata*, *Glyphonycteris sylvestris*, *Brachyphylla nana*, *Phyllonycteris poeyi*, *Erophylla bombifrons*, *Glossophaga soricina* (C), *Glossophaga commissarisi*, *Glossophaga longirostris*, *Monophyllus redmani*, *Lichonycteris obscura*, *Hylonycteris underwoodii*, *Desmodus rotundus* (B), and *Diphylla ecaudata*. Photo credit: David Villalobos-Chaves, Brock Fenton, José G. Martínez-Fonseca, Sharlene Santana, Joaquín Ugarte, and Laurel Yohe.

Figure 2: Epoxy molding technique on freshly collected specimen (A), μ CT scan slice with molding space highlighted in blue for 3D reconstruction (B), reconstructed 3D model of nose leaf and pinna with landmarks (L, orange) and semilandmarks (blue) labeled (C), and modularity hypothesis tested (D), where the spear (yellow) and horseshoe (green) constitute separate modules, with landmarks (L) labeled (D).

Figure 3: Morphospace (phylogenetic Principal Components; pPC) plots for nose leaf (A), spear (B), horseshoe (C), and pinna (D). Landmark clouds with arrows show shape changes along pPC1 (right) and pPC2 (left). Areas of change are outlined in red dashed lines.

Figure 4: Morphospace (phylogenetic Principal Components; pPC) plots for pinnae. Landmark clouds with arrows show shape changes along pPC1 (right) and pPC2 (left). Areas of change are outlined in red dashed lines.

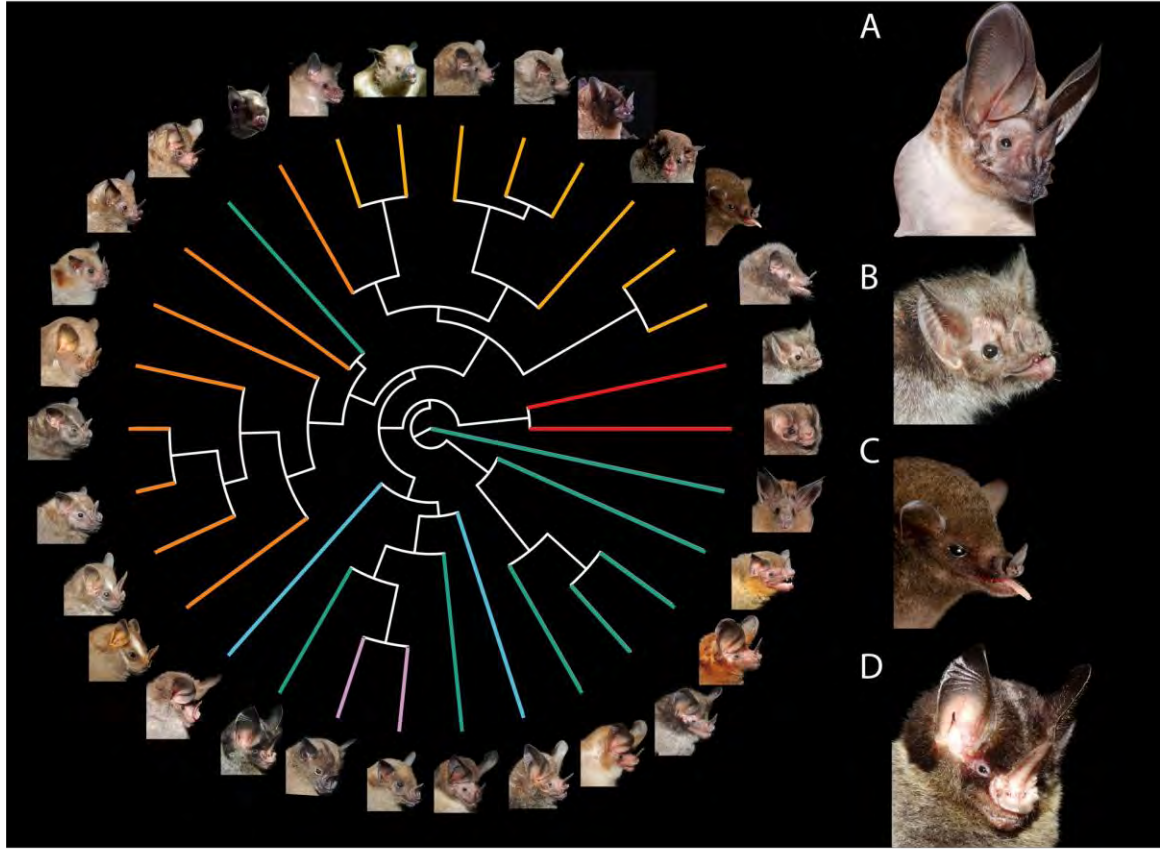


Figure 1.

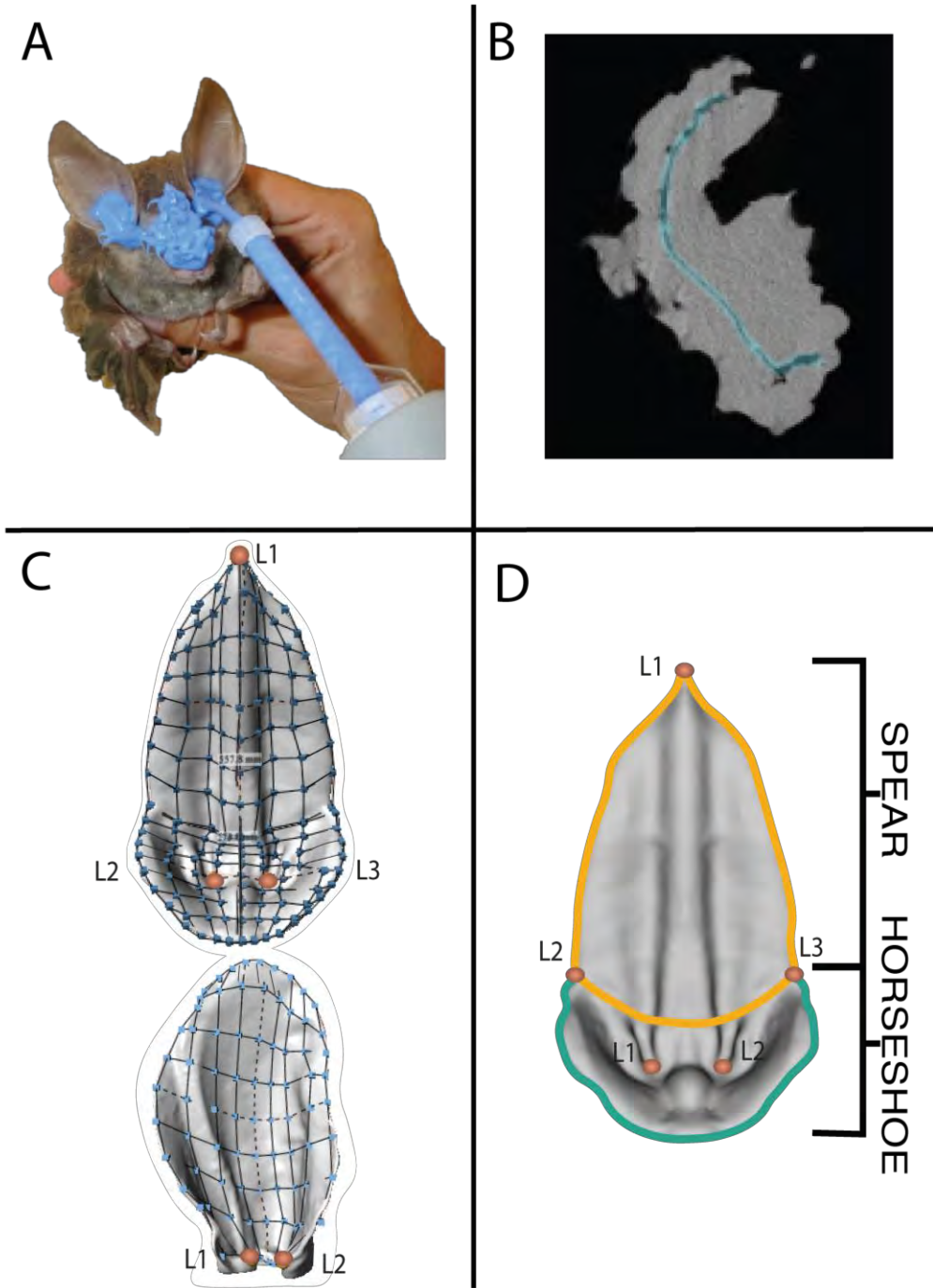


Figure 2.

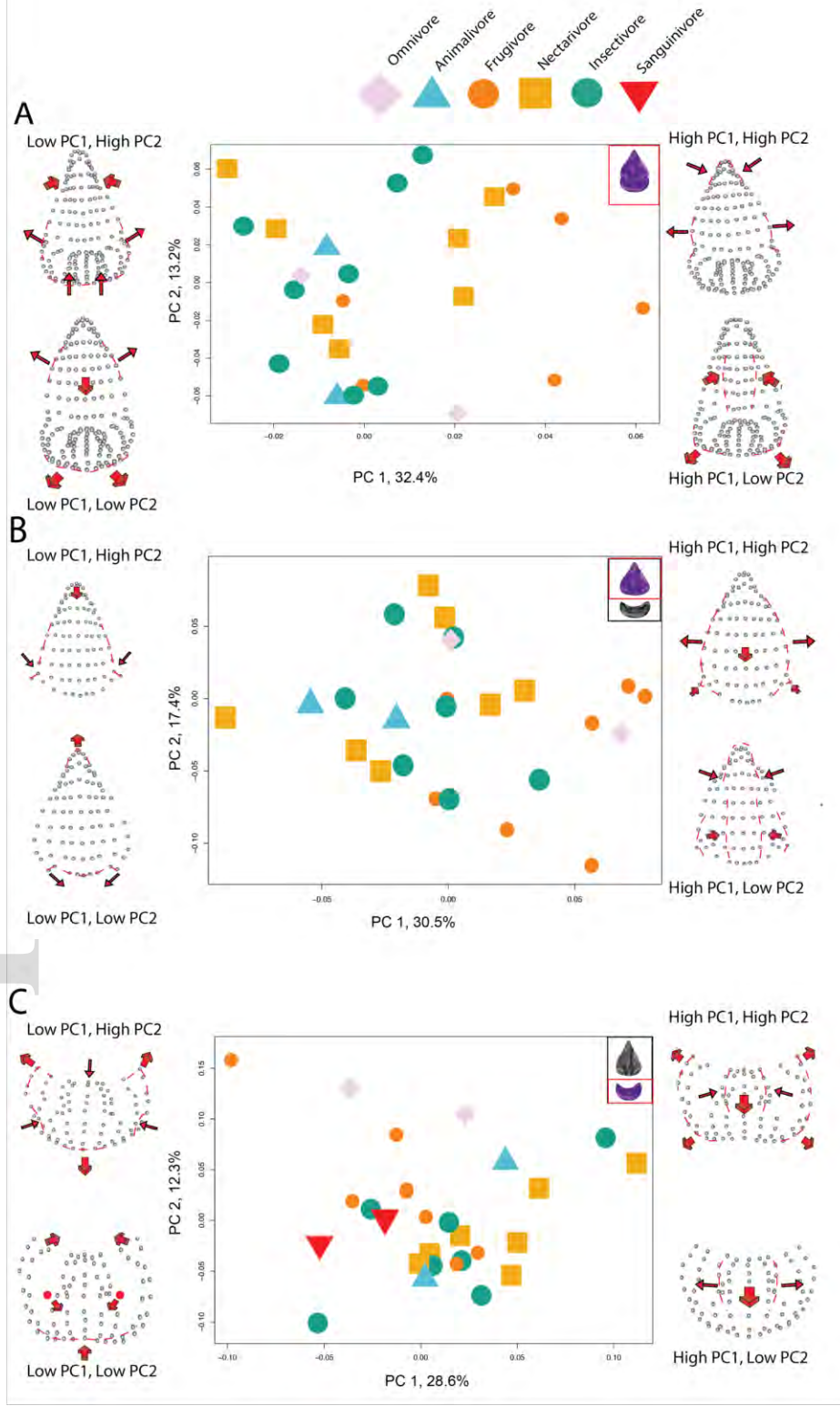


Figure 3.

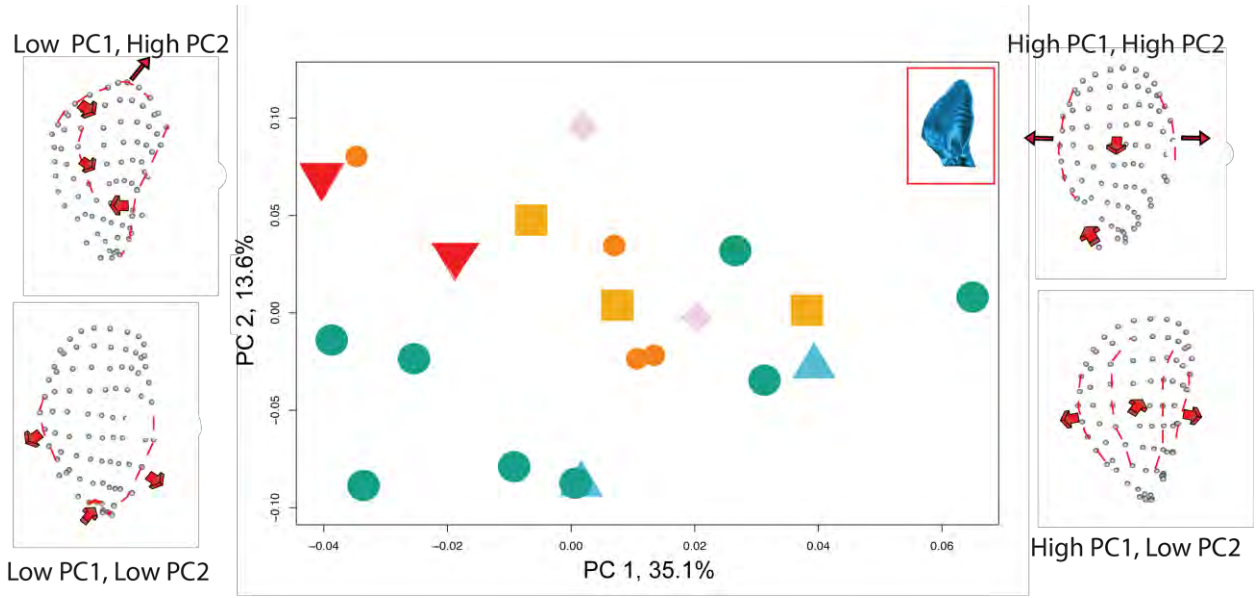


Figure 4.

Table 1. Summary of the hypotheses and predictions tested in this study.

	Structure	Hypotheses	Prediction
Modularity	Nose leaf	The nose leaf consists of two modules, the spear and the horseshoe.	Greater covariation among landmarks within each module than between modules.
Call parameters	Nose leaf (whole)	Nose leaf morphologies increase the ability to focus high frequencies emitted.	1) An anteriorly rotated, relatively broad spear and an anteriorly rotated horseshoe will be correlated with higher peak frequency, maximum frequency and bandwidth. 2) A flattened nose leaf in the coronal plane and a reduced horseshoe will be correlated with lower peak frequency and minimum frequency.
	Pinnae	Pinna shape reflects acoustic cues used for prey detection.	Pointed, narrower pinnae will be correlated with peak frequency emitted; broader, rounder pinnae might not be associated with frequencies or bandwidth emitted.

Diet	Nose leaf and pinnae	Dietary ecologies and foraging behaviors have imposed selective pressures on the echolocation system of phyllostomids, leading to morphological variation in nose leaf and pinnae among species.	<p>1) Species that rely heavily on precise echolocation information to find and capture more elusive prey (i.e., insectivores) will exhibit specialized nose leaf and pinnae morphologies that enable them to better focus echolocation calls and receive a wider array of sound frequencies.</p> <p>2) Frugivores and nectarivores, which rely on other senses such as olfaction for food detection, will exhibit sensory morphologies that are weakly associated to diet and specific echolocation call parameters.</p>
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Table 2. Results from Phylogenetic Generalized Least Squares regressions of nose leaf shape variables (pPCs) on acoustic call parameters. Bold values indicate statistically significant regressions (at $\alpha < 0.05$)

Nose leaf shape pPC	Call parameter	B	SE	t	P
1	Peak frequency	-0.524	0.708	-0.74	0.47
	Min. frequency	-0.117	0.776	-1.51	0.15
	Max. frequency	-0.255	0.100	2.54	0.02
	Bandwidth	-0.138	0.589	-2.35	0.03
2	Peak frequency	.0144	0.118	0.12	0.90
	Min. frequency	-0.855	0.133	-0.64	0.53
	Max. frequency	-0.154	0.187	-.089	0.39
	Bandwidth	-0.790	0.108	-0.74	0.47
3	Peak frequency	0.951	0.131	0.73	0.48
	Min. frequency	0.241	0.142	1.70	0.12
	Max. frequency	0.234	0.208	1.13	0.27
	Bandwidth	-0.650	0.124	-0.05	0.96
4	Peak frequency	-0.759	0.156	-0.49	0.63
	Min. frequency	0.314	0.180	0.02	0.99
	Max. frequency	-0.491	0.254	-0.19	0.85
	Bandwidth	-0.522	0.146	-0.36	0.72
5	Peak frequency	-0.160	0.195	-0.82	0.42
	Min. frequency	-0.407	0.206	-1.97	0.06
	Max. frequency	-0.344	0.311	-1.11	0.28
	Bandwidth	0.632	0.184	0.34	0.73

Table 3. Results from Phylogenetic Generalized Least Squares regression of pinna shape variables (pPCs) on acoustic call parameters.

Pinna shape pPC	Call parameter	B	SE	t	P
1	Peak frequency	-0.128	0.636	-0.20	0.84
	Min. frequency	-0.493	0.501	-0.98	0.34
	Max. frequency	-0.044	0.906	-0.00049	0.99
	Bandwidth	0.493	0.785	0.63	0.54
2	Peak frequency	0.168	0.129	-0.91	0.38
	Min. frequency	-0.114	0.921	0.34	0.74
	Max. frequency	0.143	0.183	0.078	0.94
	Bandwidth	0.256	0.161	0.16	0.88
3	Peak frequency	-0.101	0.111	-0.91	0.38
	Min. frequency	0.313	0.921	0.34	0.74
	Max. frequency	0.672	0.161	-0.42	0.68
	Bandwidth	-0.983	0.140	-0.70	0.49
4	Peak frequency	-0.227	0.134	-1.70	0.11
	Min. frequency	-0.151	0.112	-1.35	0.20
	Max. frequency	-0.355	0.187	-1.89	0.08
	Bandwidth	-0.203	0.176	-1.16	0.26

Table 4. Phylogenetic ANOVA results comparing nose leaf, spear, horseshoe, and pinna shape across dietary categories: animalivore, insectivore, frugivore, nectarivore, sanguinivore, and omnivore. Bold values indicate statistically significant regressions (at $\alpha \leq 0.05$)

Structure	Shape PC	df	SS	MS	F	P
Nose leaf	1	4	0.0070	0.0018	0.96	0.45
	2	4	0.0048	0.0012	2.8	0.05
	3	4	0.0018	0.00045	1.02	0.42
	4	4	0.0057	0.0014	3.4	0.03
	5	4	0.0008	0.00020	0.41	0.80
Spear	1	4	0.010	0.0025	1.2	0.36
	2	4	0.017	0.0043	3.7	0.03
	3	4	0.0028	0.00070	0.84	0.51
	4	4	0.0015	0.00037	0.44	0.78
	5	4	0.0050	0.0012	2.5	0.76
Horseshoe	1	5	0.035	0.00711	2.3	0.08
	2	5	0.0092	0.0019	1.5	0.24
	3	5	0.010	0.0020	2.3	0.08
	4	5	0.0033	0.00066	1.8	0.16
	5	5	0.0033	0.00067	1.5	0.23
Pinna	1	5	0.027	0.0055	2.6	0.07
	2	5	0.0028	0.00056	0.63	0.68
	3	5	0.0049	0.00097	1.2	0.36