Sensory ecology, morphology and behavior of Neotropical leaf-nosed bats

Leith B. Miller

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Sharlene E. Santana, Chair

Kate Stafford

Tom Daniel

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Abstract

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Leith B. Miller

Chair of the Supervisory Committee:
Sharlene E. Santana
Biology

Sensory systems perform fitness-relevant functions, and specialized sensory structures and behavior allow organisms to accomplish challenging tasks. Furthermore, echolocation is a highly complex sense, and in function, is influenced by morphology, phylogeny, behavior, and ecology. This dissertation seeks to deepen the knowledge of how elements of complex sensory systems evolved and how it is ecologically meaningful for an adaptive radiation of bats through coupling acoustics, ecological, morphological, and behavioral data in a phylogenetic context. In chapter 1, I quantify the echolocation calls of 21 genera and 35 species of Neotropical leaf-nosed bats (Phyllostomidae) to explore how dietary guild and body size contribute to echolocation call design and whether call parameters are adapted for prey detection, in a phylogenetic context. These analyses indicate that phyllostomids are more diverse in call design than previously thought and we suggest call structure may primarily be adapted for dealing with acoustic

constraints of foraging in a dense habitat, as all phyllostomids are narrow-spaced gleaners and then secondarily specialized in certain species or dietary guilds, whereas phyllostomids vary in diet. This study gives insight to what possible mechanisms shape an organism's sensory systems and how this influences species' ecology.

In chapter 2, I 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.

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. 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. Our results give insight into the morphological evolution of external sensory structures in bats, and highlight new links between morphological diversity and ecology.

In chapter 3, we use the short-tailed fruit bat (*Carollia*: Phyllostomidae) to investigate behavioral adaptations that allow bats to find ripe fruit effectively, and to test relative importance of different senses in varying foraging scenarios. Within Neotropical ecosystems, short-tailed fruit bats (*Carollia*: Phyllostomidae) are abundant nocturnal frugivores, relying primarily on plants of the genus *Piper* as a food resource. Previous research has demonstrated *Carollia* employ olfaction and echolocation to locate *Piper* fruit, but it is unknown how their sensory use and foraging decisions are influenced by the complex diversity of chemical cues that fruiting plants produce. Using wild *C. castanea* and their preferred food, *Piper scintillans*, we conducted behavioral experiments to test two main hypotheses: (1) foraging decisions in *C. castanea* are

primarily driven by ripe fruit scent and secondarily by vegetation scent, and (2) *C. castanea* re-weight their sensory inputs to account for available environmental cues, such that bats rely more heavily on echolocation in the absence of adequate scent cues. Our results suggest that *C. castanea* requires olfactory information and relies almost exclusively on ripe fruit scent to make foraging attempts. Ripe fruit scent is chemically distinct from vegetation scent in *P. scintillans*, with a greater abundance of β-caryophyllene, germacrene D and β-elemene, and a few unique compounds. Although variation in echolocation call parameters was independent of scent cue presence, bats emitted longer and more frequent echolocation calls in trials where no fruit scent was present. Altogether, these results highlight the adaptations, plasticity, and potential constraints in the sensory system of neotropical fruit bats.

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DEDICATION

To my mom, who has inspired a deep love and passion in me for the natural world and who continues to inspire curiosity in the world with everyone around her.

Chapter 1. SPECIES AND DIETARY GUILD CALL DESIGN IN NEOTROPICAL LEAF-NOSED BATS (PHYLLOSTOMIDAE)

1.1 ABSTRACT

Bats use echolocation to perform critical tasks, yet the factors influencing the design of echolocation calls across some of the most diverse bat families still remain unclear. For example, Neotropical leaf-nosed bats (Phyllostomidae) comprise the most ecologically diverse family of bats; however, their echolocation call diversity is poorly understood because they are underrepresented in acoustic studies. This is mainly because they echolocate at low intensities and forage in dense forests. In other bat species, echolocation call design has been found to reflect niche differentiation, morphological differences, and specialization in habitat use. Here, we present a data set of the echolocation calls of 21 genera and 35 species of Neotropical phyllostomid bats and use it to explore how phylogeny and body size contribute to echolocation call design, and whether and how call design and parameters match dietary diversity. Our results indicate that phyllostomids have more diverse call designs than previously thought, and that phylogeny and body size explain a portion of the diversity in echolocation call parameters among species. Given the presence of phylogenetic signal indicates that some degree of similarity in call traits across our species could potentially be due to phylogenetic relatedness rather than similarity of diet and body size is linked to some call parameters but may not constrain overall call structure in phyllostomid bats. Most species (61-77%) can be correctly assigned to their dietary guild based on call parameters, but there is overlap among species in call structure. Specifically, our results suggest call structure may be specialized to diet to some extent, and that

call parameters may be more reflective of functionally defined guilds than taxonomically defined guilds. Consequently, we suggest call structure may be primarily adapted for dealing with acoustic constraints of foraging in a dense habitat, as all phyllostomids are narrow-space gleaners that secondarily specialized in certain prey or dietary guilds. This study gives insight to the possible mechanisms shaping the diversity of sensory systems and their influence in ecological diversity.

1.2 INTRODUCTION

Echolocation is critical to bats and other animals to perform tasks such as spatial orientation, navigation, social communication, and foraging (Norris et al. 1961, Møhl et al. 2003, Schnitzler et al. 2003, Thomas and Jalili 2004, Brinkløv et al. 2013, Jung et al. 2014). Because the functions of echolocation are diverse, various factors have been associated with the diversity of echolocation call structure across animals, including phylogeny, social communication (including mate choice, individual recognition, and distress signaling), diet, and habitat (Nyby and Whitney 1978, Wilkinson and Wenrick Boughman 1998, Lammers et al. 2003, Russ et al. 2005, Voigt-Heucke et al. 2010, Jones and Siemers 2011, Schuchmann et al. 2012, Jung et al. 2014, Puechmaille et al. 2014). Among these factors, foraging ecology (e.g., foraging habitat and diet) seems to be a strong predictor of call structure in bats (Jones 1999). However, assessments of call structure differences across guilds are usually based on broadly defined foraging categories (i.e., aerial-hawking versus gleaning bats; Jones 1999) comparing ecologically distinct families. Furthermore, the call characteristics that are typically compared, such as the distinction between constant-frequency (CF) or frequency-modulated (FM) calls, represent coarse assessments of echolocation calls. Less is known about call structure differences on a fine-resolution scale within families of bats, particularly for families that are ecologically diverse and/or have calls that are difficult to record (e.g., 'whispering' or high-flying bats).

Phyllostomidae (Neotropical leaf-nosed bats) are an example of adaptive radiation (Dumont et al. 2012). Comprised of over 200 species, it is the most ecologically diverse bat family (Rex et al. 2010, Dumont et al. 2012). Phyllostomid dietary specializations include insectivory, sanguinivory, animalivory, nectarivory, omnivory and frugivory (Rex et al. 2010, Dumont et al. 2012). Previous studies have implicated craniodental morphology, biting behavior, and bite performance traits (e.g., foreshortened rostrum, unilateral molar bites, high bite force, etc.; Santana and Dumont 2009; Santana et al. 2010, 2012) as adaptations allowing species to access novel prey dietary adaptation in phyllostomids (Dumont et al. 2012). However, differences in traits for prey processing cannot solely explain the breadth of dietary diversity observed in phyllostomids. Sensory biases also play an important role in shaping the dietary ecology of these bats (Kürten and Schmidt 1982; Kalko and Condon 1998; Thies et al. 1998; von Helversen et al. 2000; Hodgkison et al. 2007; Müller et al. 2009; Safi and Siemers 2009; Santana et al. 2011; Jones et al. 2013; Gonzalez-Terrazas et al. 2016). Yet, much less is known about whether or how the phyllostomid echolocation system (e.g., call parameters, behavior, morphology of sensory structures) evolved in tandem with their dietary radiation.

Broadly, phyllostomid species are narrow-space foragers that primarily hunt in the forest understory or canopy (Wilson and Reeder 2005), thus their main echolocation task is short-range detection in a highly cluttered acoustic space (e.g., overcoming acoustic masking echoes from foliage and other obstacles, Schnitzler and Kalko 2001). Traditionally, they have been considered 'whispering' bats because they typically emit calls at lower intensities than species in other bat families (Griffin 1958), but research shows that some species may be capable of calling at louder intensities than previously thought (Brinkløv et al. 2009). Even so, knowledge about phyllostomid echolocation calls is still poor because of limitations associated with recording these low-intensity, high-frequency calls in the hot, humid and densely forested environments where most species live (Griffin 1971). Given these constraints, phyllostomid bats are underrepresented in acoustic comparative studies, and previous, largely qualitative, research has deemed phyllostomid calls as relatively uniform in structure across species (Brigham, Kalko, Jones, Parsons, & Limpens, 2002). Quantitative analyses of larger datasets, consistently collected in terms of recording environments, equipment used, and methods for call extraction, thus have the potential to reveal that phyllostomid calls are more variable than previously thought. Interestingly, phyllostomids seem to deviate from the allometric patterns in some call parameters that are exhibited by other taxa (e.g., bats; Hipposideridae, Rhinolophidae, Emballonuridae, Vespertilionidae, and Molossidae, Jones 1999; frogs, Ryan, 1985; birds, Martin, Doucet, Knox, & Mennill, 2011; Ryan & Brenowitz, 1985). This suggests that phylogeny and dietary ecology may contribute to echolocation call diversity in phyllostomids (Jacobs et al. 2007).

Echolocation call parameters have specific functions in shaping an acoustic field of view. Frequency is particularly important for encoding audible echo reflection (Møhl, 1988; Pye, 1993), range accuracy (Stamper et al. 2009), and for detecting targets against forest clutter (Bates et al. 2011). For the detection of a specific object, such as a prey item, acoustic theory predicts that spheres reflect weak echoes if their circumference is smaller than the wavelength of the impinging sound (Pye, 1993). Ensonification experiments further suggest that insects reflect sound in a similar way to spheres, and therefore bats must use high frequencies (short wavelengths) to obtain an audible echo from small insects (Møhl 1988, Safi and Siemers 2009). Previous work has demonstrated a relationship between emitted call frequency and prey size for some bat species (Vespertilionidae), corroborating the hypothesis that call frequency and prey size can be functionally linked (Houston et al. 2004). To date, it is unknown if this basic relationship exists for phyllostomids bats.

Here, we report an unprecedented data set spanning 21 genera, 35 species, and all dietary guilds of phyllostomid bats, which we use to 1) quantify and conduct comparative analyses of the structure of phyllostomid echolocation calls (both time and frequency-linked parameters), 2) test if phylogeny underlies diversity in call structure across species, 3) test whether call structure scales with body size, and 4) test if call structure reflects dietary guild. We also use this dataset to further link call parameters and dietary ecology by calculating and comparing estimates of minimum detectable prey sizes across species. Following the patterns for other families of bats (Jones 1999), we predict that call parameters (see Table 1 for definitions) will not scale with body size in Phyllostomidae. We also predict that species within the same dietary guild will have similar call parameters, independent of phylogenetic relatedness (see Table 2 for predictions). We predict that dietary guilds will have different levels of minimum detectable prey size. Specifically, insectivores will have the smallest detectable prey size (i.e., highest call frequency and shortest call wavelength). We predict that omnivores will have the largest detectable prey size (i.e., lowest call frequency and longest wavelength), as these bats forage for larger prey and use other senses besides echolocation for prey detection.

1.3 MATERIALS AND METHODS

1.3.1 *Acoustics*

We used mist nets to collect free-ranging bats in Palo Verde National Park, Guanacaste, Costa Rica and La Selva Biological Station, Sarapiquí, Costa Rica from 2015 to 2018, through the months of January – March, and July – December. We recorded release calls from 153 individuals spanning 21 genera and 35 species (Table 3) using a microphone condenser (UltraSoundGate 116) at 375 kHz sampling rate and 16-bit recording. 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 emitted as the individual flew away. We measured call parameters for 2-11 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, Glossophaga leachii,* and *Mesophylla macconnelli*), for which we only recorded one individual per species (Table 3). All collecting and handling procedures were approved by the University of Washington's Institutional Animal Care and Use Committee (protocol# 4307-01).

We analyzed release calls using Avisoft SASLabPro v. 5.2.12 (Avisoft Bioacoustics, Berlin, Germany). To optimize both frequency and temporal resolution, we set the frequency resolution parameters for the spectrogram at a fast Fourier transform (FFT; Brigham, 1988) length of 256, 100% frame size, with a flattop window. We also set the temporal resolution for the spectrogram with a window overlap of 93.75%. We then set the automatic measurements algorithm to take measurements of call duration, peak frequency, maximum frequency, minimum frequency, bandwidth, and number of harmonics at appropriate locations for each call within each file (Figure 1). We manually inspected each call classified by the automatic measurements algorithm to ensure accuracy in element detection. In the case that ultrasonic background noise above -20 dB was influencing measurements, we manually erased this noise from the spectrogram and recalculated measurements. To further reduce the influence of high-intensity, low-frequency sounds generated by background noise, we filtered all call files with a high-pass band filter set at 20 kHz, with the exception of the calls of *Phyllostomus hastatus*. This species had calls with a lower minimum frequency than other phyllostomids, so we set a high-pass band filter at 10 kHz. To determine valid calls in a recorded file, we set element separation at a hold time of 2 ms (i.e., within the range of call duration for phyllostomids, Kalko and Condon 1998; Thies et al. 1998; Brigham et al. 2002; Jennings et al. 2004; Weinbeer and Kalko 2007), with the exception of *Centurio senex*, for which we set a hold time of 10 ms because of the extended duration of this species' call.

We averaged call sequences per individual (a minimum of 5 calls per file) and calculated means and standard deviations of each measured parameter. We also report the range of each call parameter in the form of the maximum and minimum value recorded (Table 3). To estimate the minimum prey size detectable given an emitted frequency, we used the following equation (Yang 2010):

$$\lambda = v/f \tag{1.1}$$

Where λ is the wavelength, v is the wave velocity (speed of sound in air), and f is the frequency. For speed of sound, we used the speed 347 m/s for 25°C and 68% humidity, which reflects average annual environmental conditions of the sites where we recorded calls (sourced from worldweatheronline.com).

1.3.2 *Statistical Analyses*

First, we tested each call parameter for phylogenetic signal using a pruned version of the Rojas, Warsi, & Dávalos (2016) phylogeny. For duration, maximum frequency, minimum frequency, peak frequency, bandwidth, and harmonics, we calculated both Blomberg's K (Blomberg et al. 2003) and Pagel's lambda (Pagel 1992, Freckleton et al. 2004) in R version 5.3.0 (R Core Team 2018) using the function phylosig from the package 'phytools' version 0.6-60 (Revell 2012).

To test if call parameters are linked to body size, we ran phylogenetic generalized least squares (PGLS) regressions using the function pgls from the R package 'phytools' version 0.6-60 (Mao and Ryan 2013), using individual call parameters as a response variable, body size as a predictor variable, and a pruned version of the Rojas, Warsi, & Dávalos (2016) phyllostomids phylogeny. We sourced average forearm measurements from the literature as a proxy for body size (Timm and LaVal 1998).

To test if specific call parameters are associated with dietary guild, we grouped species into one of six taxonomic dietary categories (animalivores, insectivores, nectarivores, frugivores, omnivores, and sanguinivores; Giannini & Kalko, 2004). However, accumulating research suggests that many species of phyllostomids may not be as restricted to a specific dietary guild. Instead, many species may be opportunistic feeders with a broader trophic breadth than traditionally described (Giannini & Kalko, 2004). For this reason, we also tested for differences in call structure between two "functional" dietary guilds: predators of non-mobile, non-evasive prey (nectarivores, frugivores, and sanguinivores), and predators of mobile, evasive prey (omnivores, insectivores, and animalivores). To identify call parameters that differentiate echolocation calls between dietary guilds, we conducted a Linear Discriminant Analysis (LDA) using the function lda from the R package 'MASS' version 7.3- 49 (Hulsey et al. 2007). We then used the model derived from the LDA to test if echolocation call traits are predictors of a species' dietary guild. We used the function sim.char from the R package 'geiger' version 2.0.6.1 (Harmon et al. 2019), to simulate the evolution of call parameters on the phylogeny for each level of dietary assignment (i.e., taxonomic categories and functional guilds), and we tested if differences in call parameters are greater than expected under a Brownian motion model of trait evolution. Finally, to test for differences in minimum detectable prey size among taxonomical and functional dietary guilds, we performed phylogenetic ANOVAs using the function aov.phyl from the R package 'geiger' version 2.0.6.1 (Harmon et al. 2019).

1.4 RESULTS

1.4.1 *Phylogenetic signal and scaling of call parameters*

We found that duration, maximum frequency, minimum frequency, and peak frequency all contain exhibit a relatively high phylogenetic signal (Table 4). That is, more closely related species share more similarity in these call parameters (but note that they also have similar diet and foraging habitats, Figure 2). Conversely, bandwidth and number of harmonics exhibit a lower phylogenetic signal (Table 4).

We found a significant relationship between forearm length and maximum echolocation call frequency (PGLS; $p = 0.007$, $R^2 = 0.2$; Figure 3), but this proxy for body size was not a significant predictor of call duration, minimum frequency, peak frequency, number of harmonics, or sweep rate.

1.4.2 *Discrimination of call structure among dietary guilds*

The discriminant analysis for taxonomically defined guilds indicated that peak frequency has a strong positive loading and minimum frequency has a strong negative loading on the first discriminant axis (LD1, Figure 4A). This axis primarily separates frugivores and most nectarivores (i.e., *Glossophaga*) (-LD1) from most insectivores and omnivores (+LD1). Animalivores and sanguinivores fall between these groups along LD1 (Figure 4A). Maximum call frequency has a strong positive loading on the second discriminant axis (LD2), while peak frequency has a strong negative loading. There is considerable overlap among guilds along LD2 (Figure 4A). Interestingly, each taxonomically defined dietary category includes some species that are outliers with respect to their dietary guild along both LD1 and LD2; specifically, *Lampronycteris brachyotis,* an insectivore, shares more similarities with frugivores; *Hylonycteris underwoodi*, a nectarivore, shares more similarities with insectivores; *Phyllostomus hastatus*, an omnivore, is distinct from all species in call design; and the animalivores *Chrotopterus auritus, Vamyprum spectrum,* and *Trachops cirrhosus* are starkly different from each other and more similar to either insectivores or frugivores. Insectivores, with the exception of *L. brachyotis*, occupy two different areas of acoustic space, which is primarily driven by differences in peak and minimum frequency (Figure 4A). LDA predictions assigned 61.7% of species to the correct taxonomically defined dietary category *(*$P = 0.01$).

When functionally-defined guilds are considered, bandwidth has a strong positive loading on the first discriminant axis (+LD1) and a strong negative relationship with number of harmonics (-LD1, Figure 4B). This axis largely separates species feeding on non-mobile/non-evasive prey (+LD1) from species feeding on mobile/evasive prey (-LD1, Figure 4B). LDA predictions correctly assigned 77.1% of species to the correct functionally-defined dietary guild ($P = 0.02$).

1.4.3 *Minimum detectable prey size*

We calculated the minimum detectable prey size for each species using both peak call frequency and maximum call frequency. We found the largest minimum detectable prey size for omnivorous bats (Table 5; *Phyllostomus hastatus* and *Phyllostomus discolor*), and the smallest minimum detectable prey size for insectivorous bats (Table 5). In a phylogenetic ANOVA, we did not find significant differences among detectable prey size estimates among dietary guilds (Figure 5; $P > 0.05$), although omnivores are significantly different from all other dietary guilds when phylogeny is not taken into account ($P < 0.001$). Insectivores and frugivores show the largest variance in detectable prey size estimated from emitted peak frequency (Table 6) and animalivores and insectivores have the largest variance in minimum prey size for emitted maximum frequency (Table 6). For functionally-defined dietary guilds, we found that predators of non-mobile/non-evasive prey and predators of mobile/evasive prey significantly differ in minimum detectable prey size based on maximum frequency emitted (phylogenetic ANOVA: $P<0.05$), but not for prey size based on peak frequency emitted ($P > 0.05$; Figure 6). Predators of mobile/evasive prey show the largest variance in detectable prey size for both peak and max frequency (Table 6).

1.5 DISCUSSION

The limitations of the sensory system determine a species' perceptual field, therefore understanding the factors that shape interspecific differences in sensory systems can help elucidate the mechanisms that drive differences in ecology across taxa (Coombs et al. 1988, Weinbeer and Kalko 2007, Zimmermann et al. 2009, Safi and Siemers 2009, Denzinger and Schnitzler 2013, Valenta et al. 2013). Phyllostomid bats are narrow-space foragers (Wilson and Reeder 2005) and acoustically constrained by short-range detection in a highly cluttered acoustic space (Schnitzler and Kalko 2001). They represent an adaptive radiation in which species share foraging habitats, so they are a valuable system for evaluating how evolutionary relatedness, body size, and dietary ecology contribute to echolocation signal design, and potentially niche partitioning in sympatric species. In this study, we found phyllostomid echolocation call characteristics reflect dietary ecology to some extent, and that forces other than dietary specialization, such as phylogeny and body size, also drive call similarities and divergence among species.

We found a weak yet significant negative relationship between maximum call frequency and body size. Given that maximum frequency defines the upper limit of echolocation call capability, this result can be explained by a known relationship in which an increase in the linear size of structures associated with sound production results in lower produced call frequencies (Pye, 1979). However, we did not find any scaling relationship between any other call parameter and body size. Therefore, our results largely corroborate Jones' (1999) findings in that phyllostomids diverge from the allometric pattern found in other bat families. Other morphological features, such as vocal tract geometry (Hartley and Suthers 1988, Neuweiler 2000) or nose leaf morphology (Hartley and Suthers 1987; Vanderelst et al. 2010; Leiser-Miller and Santana, *in revision*) might be better predictors of emitted frequency than body size in phyllostomids, as the geometry of sound producing structures can also influence the frequency emitted (Hartley and Suthers 1988, Neuweiler 2000, Jakobsen et al. 2013b). For example, accessory nasal chambers along the vocal tract in Rhinolophidae absorb specific frequencies, resulting in a filtered emitted call (Neuweiler 2000). Moreover, given that phyllostomids use frequency modulated calls, and can exploit a wide range of frequencies, perhaps this relaxes constraint on call parameters and some of these (e.g., maximum frequency) may be more constrained by the physical limitations of sound production, while others (e.g., peak frequency) may be more plastic to match tasks associated with foraging habitat or prey detection (Jacobs et al. 2007).

Consistent with our predictions, both taxonomic and functional dietary guilds differ in some major parameters that define echolocation call structure. Call parameters were more effective at predicting functionally-defined dietary guilds than taxonomically-defined guilds; however, there was some overlap among guilds. This suggests that call structure may be specialized for diet to some extent, and that call parameters may be more reflective of specialization associated with specific foraging behaviors necessary to capture the different prey types. For instance, higher call frequencies reduce detection distance (e.g., species searching along leaf clutter for insects, *Micornycteris microtis*; Geipel et al. 2013) but allow perception of smaller prey (e.g., detection of small insects, fruits, or flowers). Conversely, lower frequencies allow for detection over longer ranges, but provide less resolution, which is only suitable for detecting larger prey(Neuweiler 2000, Brock Fenton et al. 2016). Based on our findings, these functional requirements and tradeoffs on echolocation parameters may be more influential than simple prey taxonomy in call evolution. Even so, some species have diverged in the call structure that would be predicted for their dietary guild. This interesting finding suggests that more detailed, quantitative studies of foraging behavior and diet are still needed to further elucidate the relationship between call structure and dietary ecology in phyllostomids.

Both peak frequency and minimum frequency are primary drivers of the call differences among dietary guilds. Omnivorous phyllostomids have the lowest minimum and peak frequency and are the most distinct from other guilds. In other bat families, peak frequency and minimum frequency are important for distinguishing among species (e.g., Fenton and Bell 1981; Vaughan et al. 1997; Hughes et al. 2011). According to our measurements, some phyllostomid species can also be distinguishable by the peak and minimum frequencies of their echolocation calls. This suggests that changes in most frequency-linked call parameters may reflect species-specific specialization for ecological niches; however, the total variation in call structure seen in phyllostomids cannot be fully explained by dietary niches as there is considerable overlap in calls among guilds.

Contrary to our predictions, time-linked parameters (i.e., duration) did not differ among any of the dietary guilds, suggesting time-linked parameters may be more plastic among species than frequency-linked parameters. This has been shown in other families of bats that use time-delayed information for localization of objects, as plasticity in time-linked parameters may help mediate acoustic masking (i.e., masking by echoes from foliage or objects; Denzinger and Schnitzler 2013) and navigate complex acoustic environments rapidly and with agility (Surlykke and Moss 2000, Schnitzler et al. 2003, Jones and Holderied 2007, Moss and Surlykke 2010).

Acoustic detection of preferred prey size is constrained by wavelength and has only been studied in a few bat species (Houston et al. 2004). Houston et al. (2004) found that species emitting the highest frequencies (shortest wavelengths) fed on the smallest insects. However, the species that emitted the lowest frequencies (longest wavelengths) fed on insects that were smaller than predicted by wavelength alone. We calculated the minimum detectable prey size across phyllostomid species and found no significant differences among guilds when phylogeny is considered, but some guilds do exhibit greater variance than others in minimum detectable prey size (frugivores and insectivores, calculated using emitted peak frequency). Frugivory and insectivory are the dominant and most speciose dietary guilds within Phyllostomidae (Wilson and Reeder 2005), therefore a greater variance in detectable prey size may reflect both their phylogenetic (species) and ecological diversity. The variance in echolocation call design within guilds can further reflect dietary adaptation and niche partitioning through sensory biases. For instance, small differences in vespertilionid bats' (insectivores) echolocation call structure has been shown to contribute to niche differentiation within guilds (Siemers and Schnitzler 2004a, Siemers and Swift 2005). Further investigation is needed to determine if phyllostomid echolocation signals actually reflect fine-resolution differences in acoustic niches among species.

Phyllostomids have evolved other sensory specializations beyond echolocation, which they also use for prey detection. For example, *Desmodus rotundus* (sanguivore) uses infrared sensing pits to sense warm mammals (Jones et al. 2013) and *Trachops cirrhosus* and other animalivorous species use passive hearing to detect prey (Kalko et al. 1999). Many plant-eating and omnivorous species use olfaction and vision and rely on multimodal sensing approach for prey detection (Bell and Fenton 1986, Thies et al. 1998, Kalko and Condon 1998, Neuweiler 2000, Korine and Kalko 2005). Alternative or complementary sensory modalities are expected to relax selection on echolocation call specialization, but it is still poorly understood how multimodal sensing plays into unique foraging scenarios in phyllostomids. Even though these bats are diverse in their sensory abilities, there is growing experimental evidence that phyllostomid species across different dietary guilds use echolocation to find prey (Geipel, Jung, & Kalko, 2013; Gonzalez-Terrazas et al., 2016; Kalko & Condon, 1998; Thies et al., 1998). Therefore, the evolution of echolocation in the context of the phyllostomid dietary radiation likely involves a nuanced and complex interaction with the evolution of other sensory modalities.

All phyllostomid species forage and/or have to navigate dense clutter (Schnitzler and Kalko 2001, Wilson and Reeder 2005), and the extreme acoustic characteristics of this type of habitat may impose strong evolutionary pressures on echolocation call structure (Siemers and Schnitzler 2000, 2004b, Schnitzler and Kalko 2001, Broders et al. 2005, Denzinger and Schnitzler 2013). Schnitzler et al. (2003) argued that echolocation call structure first evolved for spatial orientation and secondarily for prey acquisition. Under this scenario, because species that forage in similar habitats must solve similar tasks, they are expected to share sensory system characteristics, particularly in the design of echolocation call signals (Schnitzler et al. 2003). Thus, habitat constraints likely explain why there is broad overlap in call design in many phyllostomids species.

Overall, we find that phyllostomids have more diverse echolocation calls than previously reported. Call structure may primarily be adapted for dealing with acoustic constraints of foraging in a dense habitat and is secondarily specialized to some extent for prey detection across dietary guilds or species. Phyllostomid bats are an ideal system to investigate how echolocation calls may be part of a suite of traits (including foraging mode) that evolved in tandem with dietary ecology and that ultimately led to niche differentiation among an ecologically diverse bat family. Further research on multimodal sensing, prey detection behavior, and greater knowledge of species' trophic role would help further elucidate the importance of echolocation call design in the Phyllostomidae adaptive radiation. We hope that the detailed information we present on the echolocation calls of a representative sample of phyllostomids is valuable for future studies aiming to understand the diversity and functionality of bat echolocation systems.

1.6 ACKNOWLEDGEMENTS

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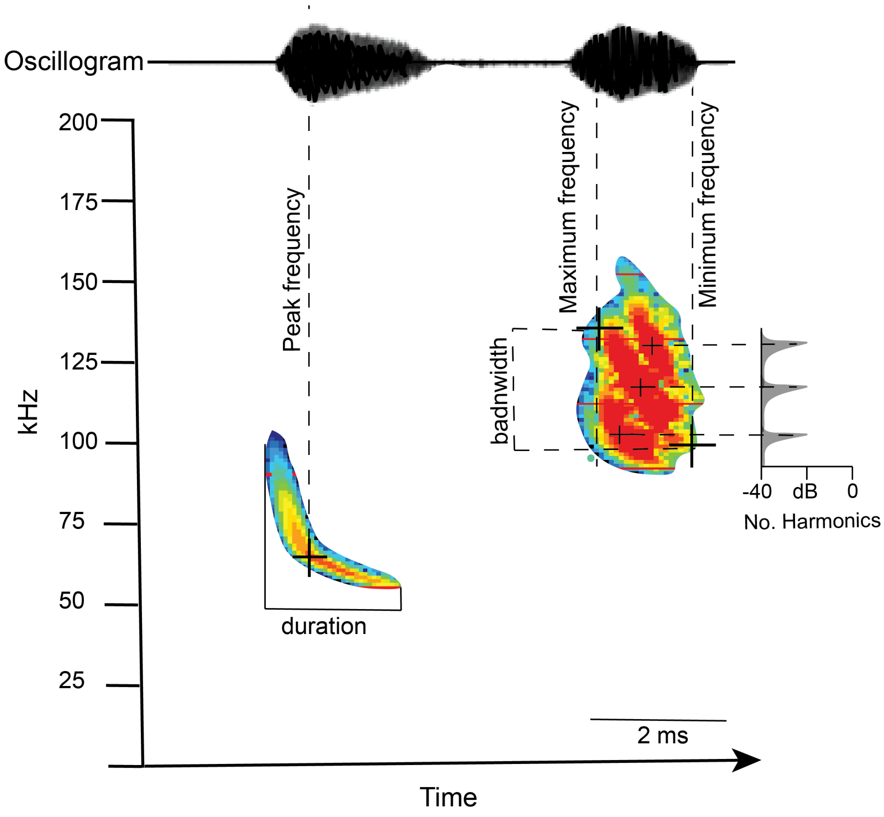

Figure 1.1. Schematic of spectrogram of *Hylonycteris underwoodi* (left) and *Platyrrhinus helleri* (right) illustrating measurement points of echolocation call parameters used in the analyses. Oscillogram (top) represents amplitude of calls. Duration of the call is calculated as the length of the call at an amplitude above -20dB relative to the maximum amplitude of the call, maximum frequency is taken at maximum amplitude at start of call, minimum frequency is taken at maximum amplitude at end of call, peak frequency is the maximum frequency over the entire call, bandwidth is calculated as the difference between the maximum and minimum frequency over the entire call, and number (No.) of harmonics is taken as the number of peaks with amplitude greater than -20dB relative to the maximum amplitude of the individual spectra

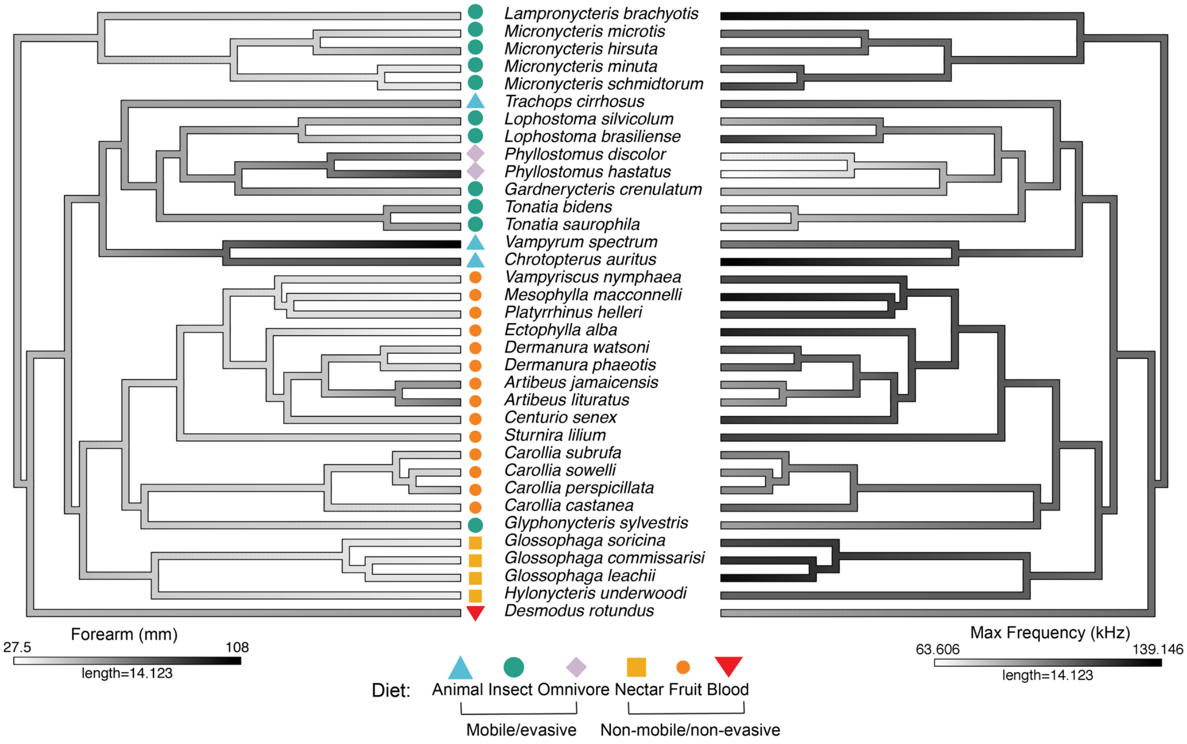

Figure 1.2. Forearm (left) and maximum frequency (right) reconstructed on the Phyllostomidae phylogeny using function in the ‘phytools’ package (Revell 2012) in R studio version 3.5.0 (R Core Team 2018). Taxonomic and functional dietary guilds denoted with symbols at tip ends

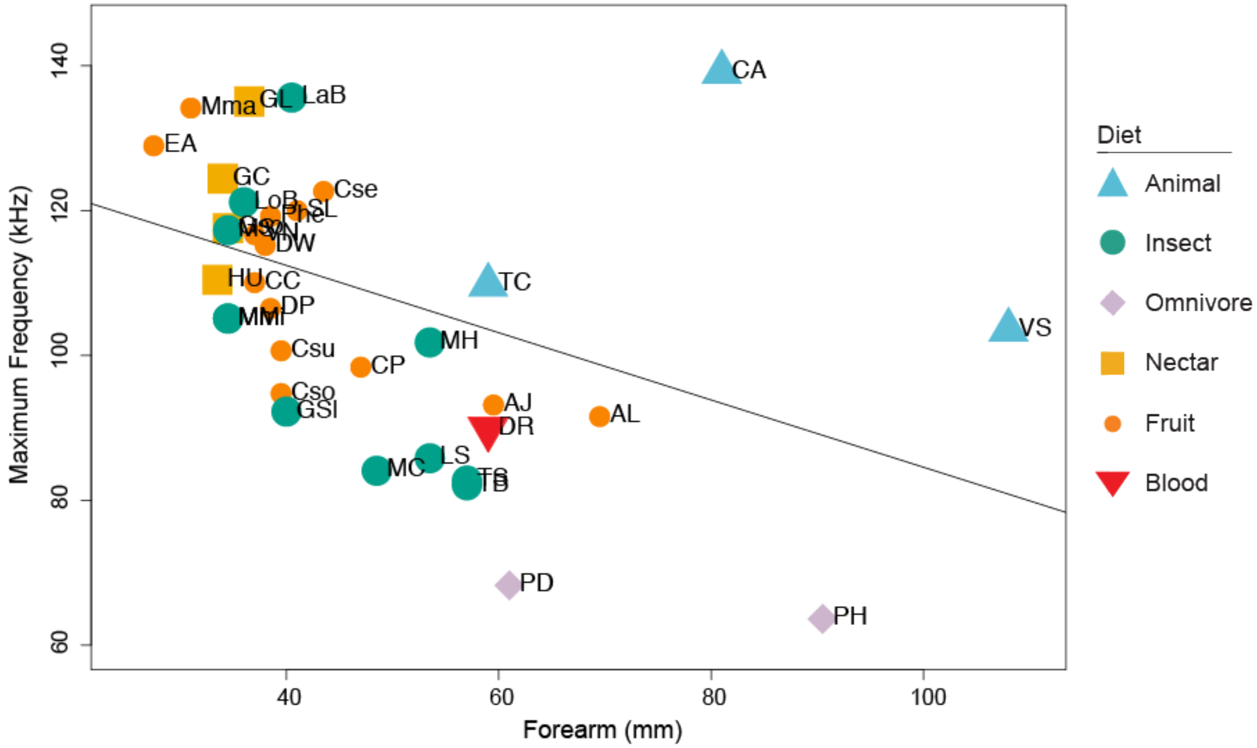

Figure 1.3. Phylogenetic generalized least squares regression of maximum frequency and forearm length, y= -0.4766 +129.24x, R^2=0.2. Species codes are listed as AJ-*Artibeus jamaicensis,* AL -*Artibeus lituratus,* CC- *Carollia castanea,* CP- *Carollia perspicillata,* Cso -*Carollia sowelli,* Csu -*Carollia subrufa,* Cse – *Centurio senex,* CA -*Chrotopterus auritus,* DP-*Dermanura phaeotis,* DW- *Dermanure watsonii,* DR -*Desmodus rotundus,* EA-*Ectophylla alba,* GC-*Glossophaga commissarisi,* GL-*Glossophaga longirostirs,* Gso-*Glossophaga soricina,* GSl-*Glyphonycteris sylvestris,* HU-*Hylonycteris underwoodi,* LaB-*Lampronycteris brachyotis,* LoB-*Lophostoma Brasiliense,* LS-*Lophostoma silvicolum,* Mma-*Mesophylla macconnelli,* MH-*Micronycteris hirsute,* MM-*Micronycteris microtis,* Mmi-*Micronycteris minuta,* MS-*Micronycteris schmidtorum,* MC-*Gardnerycteris crenulatum,* PD-*Phyllostomus discolor,* PH-*Phyllostums hastatus,* Phe-*Platyrrhinus helleri,* SL-*Sturnira lilium,* TB-*Tonatia bidens,* TS-*Tonatia saurophila,* TC-*Trachops cirrhosis,* VN-*Vampyriscus nymphaea,* VS-*Vampyrum spectrum*

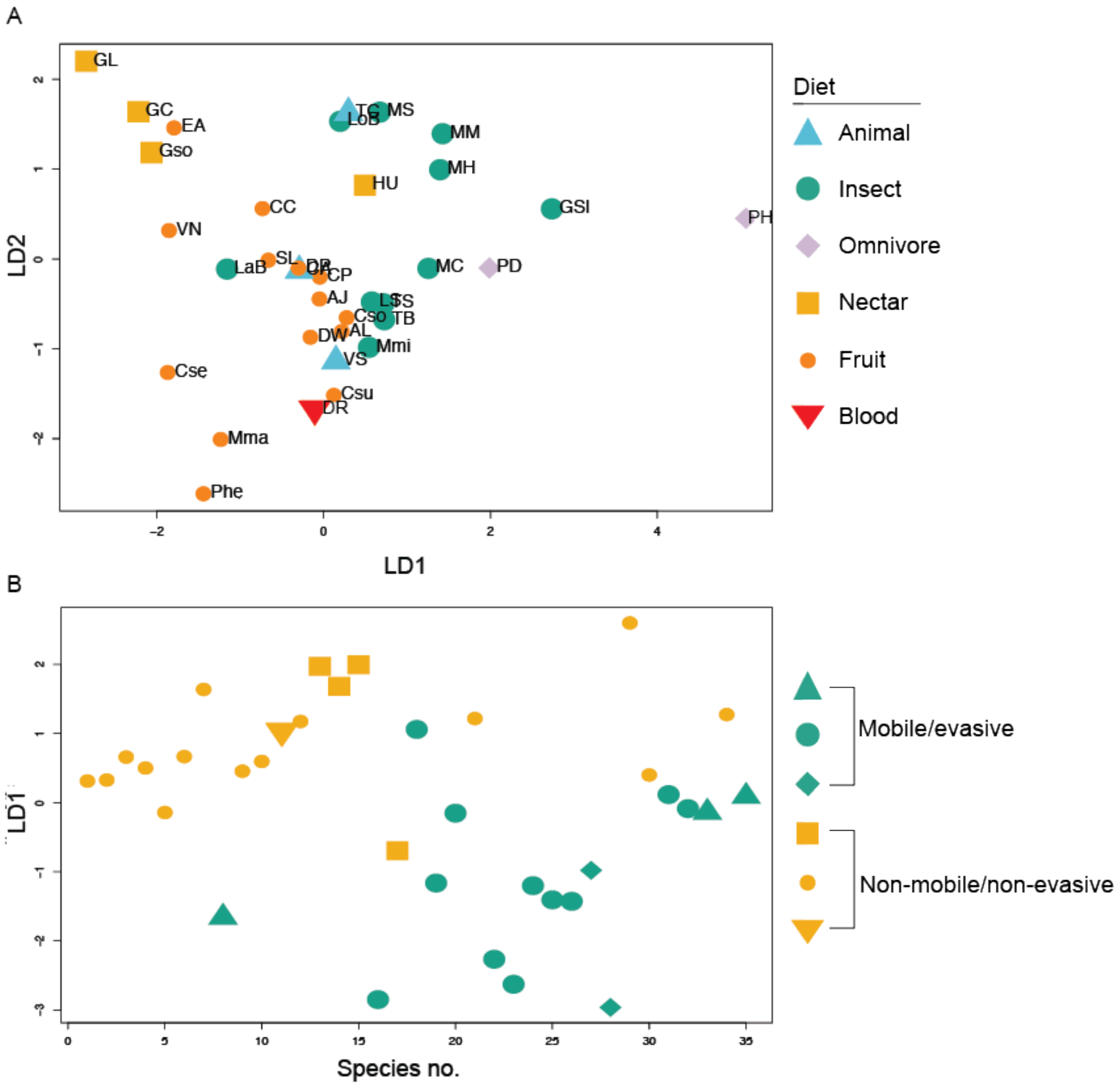

Figure 1.4. Linear discriminant analysis (LDA) of A) taxonomically defined dietary guilds and B) functionally defined dietary guilds. Species codes are listed as AJ-*Artibeus jamaicensis*, AL -*Artibeus lituratus*, CC- *Carollia castanea*, CP- *Carollia perspicillata*, Cso -*Carollia sowelli*, Csu -*Carollia subrufa*, Cse – *Centurio senex*, CA -*Chrotopterus auritus*, DP-*Dermanura phaeotis*, DW- *Dermanure watsonii*, DR -*Desmodus rotundus*, EA-*Ectophylla alba*, GC-*Glossophaga commissarisi*, GL-*Glossophaga longirostirs*, Gso-*Glossophaga soricina*, GSl-*Glyphonycteris sylvestris*, HU-*Hylonycteris underwoodi*, LaB-*Lampronycteris brachyotis*, LoB-*Lophostoma Brasiliense*, LS-*Lophostoma silvicolum*, Mma-*Mesophylla macconnelli*, MH-*Micronycteris hirsute*, MM-*Micronycteris microtis*, Mmi-*Micronycteris minuta*, MS-*Micronycteris*

schmidtorum, MC-*Gardnerycteris crenulatum,* PD-*Phyllostomus discolor,* PH-*Phyllostums hastatus,* Phe-*Platyrrhinus helleri,* SL-*Sturnira lilium,* TB-*Tonatia bidens,* TS-*Tonatia saurophila,* TC-*Trachops cirrhosis,* VN-*Vampyriscus nymphaea,* VS-*Vampyrum spectrum*

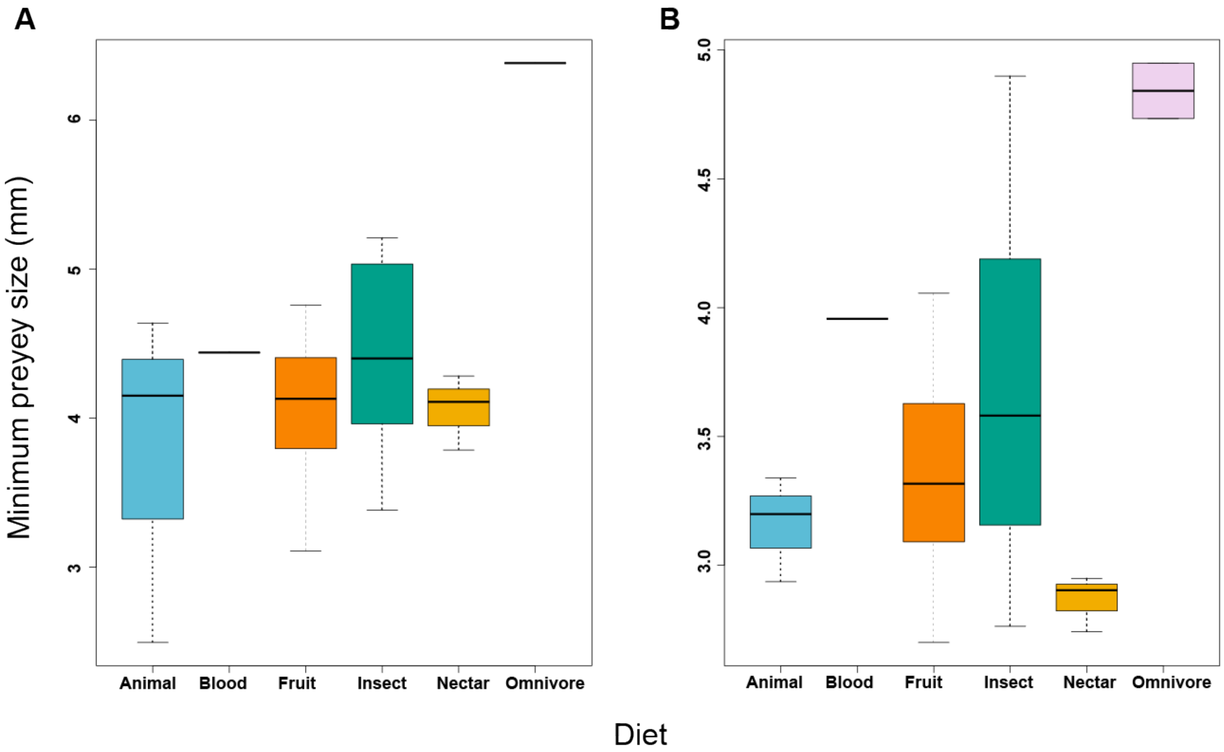

Figure 1.5. Minimum detectable prey size across taxonomically defined dietary guilds for A) peak frequency and B) max frequency

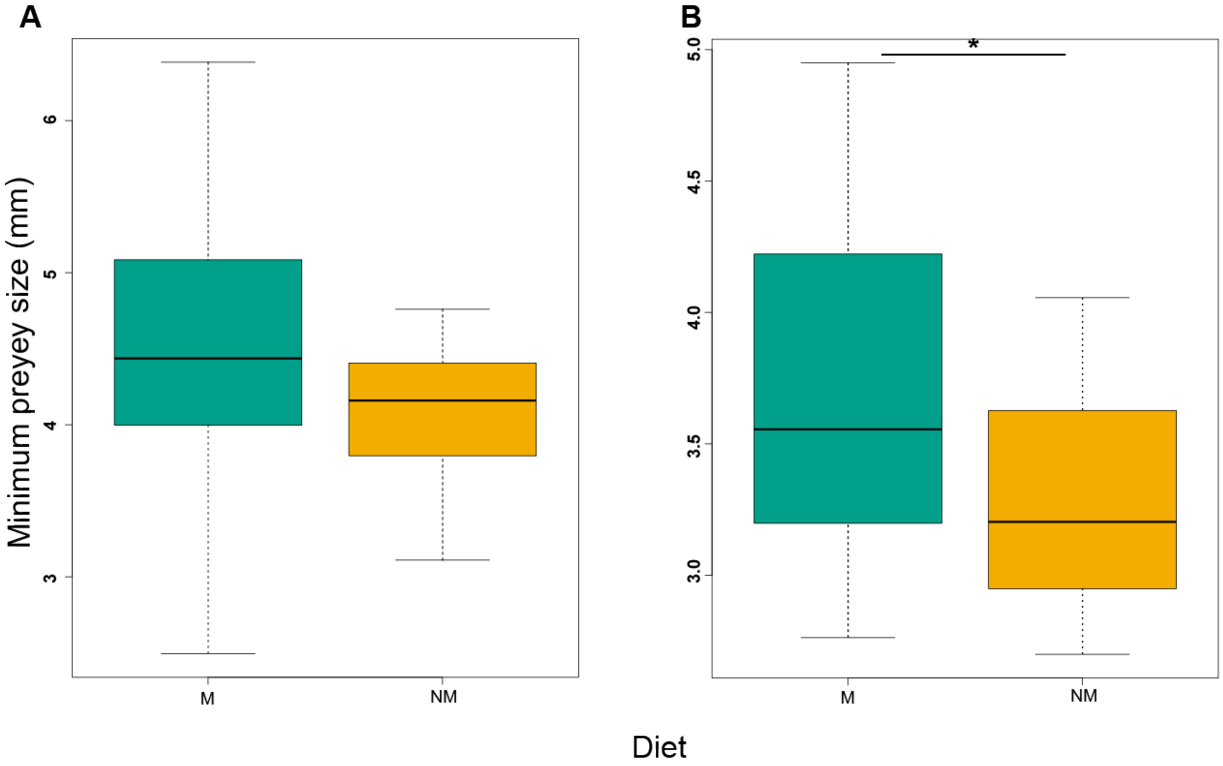

Figure 1.6. Minimum detectable prey size across functionally defined dietary guilds (NM- non-mobile/non-evasive prey and M – mobile/evasive prey) for A) peak frequency and B) max frequency

Table 1.1. Definition and functional significance of call parameters. Within each call parameter group (Par. groups) are the specific, call parameters (Call specific) measured in this study, along with their function, predictor traits, and associated citations.

Par. groups	**Call specific**	**Function**	**Predictor traits**	**Citation**
Harmonics		Distinguish clutter echoes from target echoes.	unknown	Simmons et al. 1975; Bates et al. 2011
Frequency		Influences acoustic field of view (i.e., sonar beam width), influences resolution of acoustic image.		Neuweiler 2000; Bates et al. 2011; Brock Fenton et al. 2016
	Minimum frequency	**Low values**: increase range detection, increase beam width, decrease resolution.	unknown	Neuweiler 2000; Bates et al. 2011; Brock Fenton et al. 2016
	Maximum frequency	**High values**: decrease range detection, decrease beam width, increase resolution and target discrimination.	unknown	Neuweiler 2000; Bates et al. 2011; Brock Fenton et al. 2016
	Peak frequency	Reflects with the highest energy, most critical for determining field of view.	body size	Jones 1999; Bates et al. 2011
Bandwidth		**High values**: better temporal resolution and accuracy in range detection.	unknown	Simmons et al. 1975; Denzinger and Schnitzler 2013; Brock Fenton et al. 2016
	Narrow-frequency band	**High values:** encode information about small frequency changes produced by fluttering insects. **Low values:** better for detecting larger objects at longer distances.	foraging habitat	Brock Fenton et al. 2016
	Broad-frequency band	**High values:** reduced masking effects for foraging within clutter, particularly insects, improves lower resolution limit.	foraging habitat, diet	Siemers and Schnitzler 2004; Boonman and Ostwald 2007; Denzinger and Schnitzler 2013
Duration		**Low values:** optimize the resolution of target distance and range accuracy; increases the signal overlap-free window zone (i.e., no echo interference). **High values:** decreases the acoustic overlap-free window, making is difficult to distinguish outgoing from incoming call information and clutter echoes from target echoes.	foraging habitat	Simmons et al. 1975; Denzinger and Schnitzler 2013; Brock Fenton et al. 2016

Table 1.2. Hypotheses and predictions for specific call parameters (Call specific) within each call parameter group (Par. groups), as well as predicted dietary guild to exhibit each prediction.

Par. groups	**Call specific**	**Prediction**	**Dietary guild (taxonomic/functional)**
Harmonics		Species that forage on cryptic prey in dense clutter will have a higher number of harmonics.	Insectivore/mobile-evasive
Frequency			
	Minimum frequency	Species that forage over long distances and larger prey will have lower values of minimum frequency.	Sanguinivore, omnivore/non-mobile-non-evasive
	Maximum frequency	Species that forage on highly cryptic prey such as insects on leaves or vertebrates will have higher values of maximum frequency.	Insectivore, animalivore /mobile-evasive
	Peak frequency	Peak frequency will be similar within and different between dietary guilds, as each guild experiences a different set of foraging challenges.	-
Bandwidth			
	Narrow-frequency band	Species that forage over longer distances and on larger prey items, prey items separated from leaves (i.e., fruits and flowers) will exhibit lower values of bandwidth.	Omnivore, sanguinivore, frugivore, nectarivore/ non-mobile-non-evasive
	Broad-frequency band	Species that forage for prey in dense clutter and where prey is hidden in clutter will exhibit higher values of bandwidth.	Insectivore/ non-mobile-non-evasive
Duration		Species that heavily rely on echolocation to detect and locate prey on leaf background will have shorter duration of calls.	Insectivore/mobile-evasive

Table 1.3. Call parameter means, standard deviations (SD) and range (minimum - maximum values, listed beneath) for duration (dur.), maximum frequency (maxF), minimum frequency (minF), peak frequency (pF), bandwidth (bw), and harmonincs (harm.) across 35 species of phyllostomid bats

Species	**dur.(ms)**	**maxF (kHz)**	**minF (kHz)**	**pF (kHz)**	**bw (kHz)**	**harm.**
Artibeus jamaicensis	0.78 ±0.42	93.1 ±3.7	63.2 ±6.1	77.1 ±4.4	39.3 ±12.6	1.54 ±0.27
	(0.042-1.5)	(83.4-96.0)	(53.1-71.7)	(69.1-82.5)	(25.6-58.5)	(1.10-1.90)
Artibeus lituratus	0.84 ±0.15	91.5±3.49	57.7 ±4.23	72.9±1.7	34.8±5.8	1.33 ±0.33
	(0.61-0.95)	(88.3-95.2)	(52.4 -61.6)	(71.8-75.5)	(27.3-40.6)	(1.0-1.80)
Carollia castanea	0.44 ±0.09	110.0 ±4.29	56.0 ±5.27	80.7 ±4.2	58.1 ±9.7	1.35 ±0.345
	(0.31-0.54)	(115.7-104.4)	(47.9 -64.1)	(73.5-86.4)	(39.7-68.0)	(1.00-1.80)
Carollia perspicillata	0.65 ±0.30	98.4 ±11.6	56.8 ± 11.9	77.7 ±7.3	48.8 ±15.2	1.47 ± 0.23
	(0.29-1.11)	(84.8-124.2)	(29.7-74.8)	(68.1-88.4)	(29.9-87.7)	(1.2-1.86)
Carollia sowelli	0.57 ±0.24	94.7 ±14.7	64.5 ±17.1	78.8 ±15.4	33.8 ±5.4	1.41 ±0.13
	(0.28-1.00)	(85.0-137.2)	(41.6-107.1)	(65.4-121.8)	(25.1-44.8)	(1.10-1.60)
Carollia subrufa	0.52 ±0.12	100.6 ±1.6	64.6 ±6.8	85.5 ±2.9	37.5 ±1.1	1.29 ±0.14
	(0.42-0.66)	(49.3-102.9)	(55.2-70.6)	(82.4-89.5)	(36.4-38.6)	(1.10-1.40)
Centurio senex	1.04 ±0.22	122.6 ±12.5	67.8 ±3.1	86.7 ±4.1	34.5 ±7.3	1.05 ±0.08
	(0.93-1.37)	(106.1-141.3)	(63.7-71.4)	(83.5-94.0)	(25.7-42.3)	(1.00-1.21)
Chrotopterus auritus	0.28	139.1	96.3	118.2	42.1	2.47
	-	-	-	-	-	-
Dermanura phaeotis	0.53 ±0.08	105.0 ±8.9	59.8 ±5.5	81.3 ±7.9	50.2 ±9.7	1.48 ±0.36
	(0.43-0.70)	(84.9-114.2)	(49.7-68.2)	(72.4-90.1)	(38.6-70.4)	(1.05-2.36)
Dermanura watsoni	0.45 ± 0.10	115.1 ± 4.8	63.1 ±10.4	91.9 ±7.1	50.7 ±12.1	1.48 ± 0.17
	(0.28-0.57)	(109.1-120.4)	(55.3-80.2)	(78.5-99.8)	(30.6-64.8)	(1.33-1.73)
Desmodus rotundus	0.74 ±0.18	89.8 ±7.7	64.4 ±1.7	78.1 ±3.7	30.7 ±1.8	1.18 ±0.03
	(0.61-0.95)	(81.1-96.2)	(62.4-65.8)	(73.9-80.9)	(29.1-32.7)	(1.13-1.21)
Ectophylla alba	0.63 ±0.11	128.9 ±10.4	49.1 ±8.8	81.1 ±10.7	71.4 ±15.4	1.27 ± 0.16
	(0.46-0.74)	(118.4-144.5)	(36.6-59.1)	(69.7-93.7)	(56.1-95.3)	(1.07-1.5)
Glossophaga commissarisi	0.86 ±0.51	124.4 ±24.1	49.5 ±16.7	81.0 ±7.1	81.3 ±30.7	1.54 ±0.39
	(0.33-1.48)	(91.8-149.9)	(34.2-71.9)	(72.3-89.8)	(43.0-114.3)	(1.23-1.91)
Glossophaga leachii	0.45	135.0	61.3	91.6	84.1	1.60
	-	-	-	-	-	-

Glossophaga soricina	0.58 ±0.27 (0.31-0.95)	117.5 ±6.1 (111.9-124.4)	54.5 ±19.5 (32.8-78.6)	82.6 ±12.3 (67.9-97.3)	75.2 ±17.0 (60.1-97.4)	1.38 ±0.12 (1.21-1.50)
Glyphonycteris sylvestris	1.49 -	92.2 -	51.0 -	70.8 -	41.1 -	2.80 -
Hylonycteris underwoodii	0.36 ±0.14 (0.26-0.46)	110.4 ±19.8 (96.3-124.5)	49.4 ±3.3 (47.0-51.8)	78.2 ±14.7 (67.8-88.6)	56.1 ±30.3 (34.7-77.6)	1.49 ±0.51 (1.12-1.85)
Lampronycteris brachyotis	0.53 ±0.12 (0.38-0.60)	135.6 ±10.5 (116.8-161.9)	66.0 ±16.9 (42.0-89.0)	102.5 ±10.0 (80.4-116.6)	68.4 ±15.3 (39.6-103.8)	1.80 ±0.49 (1.10-2.53)
Lophostoma brasiliense	0.44 ±0.81 (0.36-0.58)	121.2 ±7.4 (113.1-133.4)	59.1 ±11.5 (45.9-73.8)	88.3 ± 12.9 (70.6-100.6)	64.3 ±10.9 (48.0-77.9)	2.08 ±0.15 (1.86-2.28)
Lophostoma silvicolum	0.79 ±0.17 (0.67-0.92)	85.7 ±15.3 (74.9-96.6)	56.8 ±0.56 (56.4-57.2)	69.5 ±8.2 (63.7-75.3)	33.6 ±12.8 (24.5-42.7)	1.40 ±0.49 (1.05-1.76)
Mesophylla macconnelli	0.63 -	134.2 -	84.0 -	111.6 -	43.0 -	1.69 -
Micronycteris hirsuta	0.40 ±0.13 (0.22-0.54)	101.7 ±9.1 (89.2-108.7)	57.5 ±7.9 (50.1-68.6)	76.7 ±6.6 (70.9-85.5)	41.4 ±10.9 (30.6-51.5)	1.83 ±0.22 (1.53-2.07)
Micronycteris microtis	0.35 ±.073 (0.29-0.43)	105.1 ±8.3 (96.0-112.4)	59.7 ±9.8 (48.8-67.6)	79.4 ±2.4 (77.1-81.9)	9.15 ±20.33 (39.1-55.2)	2.02 ±0.98 (1.45-3.15)
Micronycteris schmidtorum	0.34 -	117.2 -	52.2 -	83.7 -	65.2 -	1.96 -
Mimon crenulatum	0.53 ±0.26 (0.38-1.32)	84.0 ±3.7 (80.1-93.3)	56.8 ±2.2 (53.6-61.9)	66.5 ±1.8 (63.4-69.3)	24.6 ±4.6 (18.8-35.6)	1.31 ±0.15 (1.09-1.50)
Phyllostomus discolor	0.83 ±0.21 (0.58-1.16)	68.2 ±9.8 (56.8-79.5)	41.1 ±4.0 (35.8-46.0)	54.3 ±8.5 (45.8-64.7)	33.6 ±4.0 (28.0-38.2)	1.39 ±0.21 (1.08-1.63)
Phyllostomus hastatus	0.74 -	63.6 -	21.3 -	54.3 -	56.3 -	2.3 -
Platyrrhinus helleri	0.74 ±0.16 (0.62-0.86)	119.2 ±1.1 (118.3-120.0)	75.6 ±1.8 (74.3-76.9)	102.4 ±0.02 (102.4-102.5)	45.3 ±8.8 (39.0-51.6)	1.34 ±0.22 (1.18-1.50)
Sturnira lilium	0.54 ±0.12 (0.45-0.69)	119.9 ±16.2 (101.2-131.0)	62.4 ±14.9 (47.2-77.2)	89.1 ±1.1 (88.4-90.4)	53.0 ±25.2 (24.8-73.4)	1.51 ±0.18 (1.38-1.72)

Tonatia bidens	0.62 ±0.15	82.0 ±6.4	53.6 ±5.8	68.2 ±5.4	35.6 ±6.7	1.25 ±0.08
	(0.52-0.83)	(75.5-88.4)	(49.9-60.3)	(62.0-72.5)	(28.6-41.9)	(1.20-1.35)
Tonatia saurophila	0.86±0.28	82.7 ±10.0	53.5 ±3.8	66.6 ±5.8	34.2 ±5.4	1.39 ±0.25
	(0.71-1.29)	(73.5-97.0)	(48.3-57.7)	(58.6-72.7)	(29.5-41.3)	(1.17-167)
Trachops cirrhosus	0.58 ±0.23	109.7 ±11.4	40.5 ±13.9	74.7 ±12.8	74.2 ±20.9	1.77 ±0.44
	(0.33-0.99)	(92.8-122.6)	(24.9-63.2)	(58.7-93.4)	(39.3-97.0)	(1.07-2.29)
Vampyriscus nymphaea	0.57±0.13	116.6 ±14.5	72.6 ±3.0	91.4 ±11.9	55.5 ±7.4	1.57 ±0.24
	(0.48-0.67)	(106.3-126.9)	(70.5-74.8)	(82.9-99.8)	(50.3-60.8)	(1.40-1.74)
Vampyrum spectrum	0.52 ±0.08	103.5 ±2.8	64.0 ±1.5	83.0 ±0.7	32.8 ±16.0	1.23 ±0.32
	-	-	-	-	-	-

Table 1.4. Phylogenetic signal for call parameters; Pagel's lambda (λ) and Blomberg's K

Parameter	**λ**	**K**
Duration	0.74	1.02
Max. frequency	1.10	1.02
Min. frequency	0.92	0.75
Peak frequency	1.11	1.02
Bandwidth	0.54	0.61
Harmonics	7.35e-05	0.64

Table 1.5. Summary of species, diet and calculated minimum detectable prey size based on peak frequency, pF (mm) and maximum frequency emitted, mF (mm)

Species	**Diet**	**pF (mm)<**	**mF (mm)<**
Artibeus_jamaicensis	fruit	4.52	3.89
Artibeus_lituratus	fruit	4.76	4.06
Carollia_castanea	fruit	4.30	3.24
Carollia_perspicillata	fruit	4.47	3.54
Carollia_sowelli	fruit	4.41	3.81
Carollia_subrufa	fruit	4.05	3.47
Centurio_senex	fruit	4.00	3.63
Chrotopterus_auritus	animal	2.49	2.94
Artibeus_phaeotis	fruit	4.21	3.40
Artibeus_watsoni	fruit	3.77	3.17
Desmodus_rotundus	blood	4.44	3.96
Ectophylla_alba	fruit	4.28	2.90
Glossophaga_commissarisi	nectar	4.28	2.90
Glossophaga_leachii	nectar	3.79	2.74
Glossophaga_soricina	nectar	4.11	2.95
Glyphonycteris_sylvestris	insect	3.76	4.90
Hylonycteris_underwoodi	nectar	4.44	3.38
Lampronycteris_brachyotis	insect	3.38	2.76
Lophostoma_brasiliense	insect	3.93	2.90
Lophostoma_silvicolum	insect	4.99	4.02
Mesophylla_macconnelli	fruit	3.11	2.70
Micronycteris_hirsuta	insect	4.52	3.56
Micronycteris_microtis	insect	4.37	3.35
Micronycteris_minuta	insect	4.00	3.61
Micronycteris_schmidtorum	insect	4.14	2.96
Gardnerycteris_crenulatum	insect	5.21	4.33
Phyllostomus_discolor	omn	6.38	4.95
Phyllostomus_hastatus	omn	6.38	4.73
Platyrrhinus_helleri	fruit	3.39	2.95
Sturnira_lilium	fruit	3.89	3.09
Tonatia_bidens	insect	5.08	4.16
Tonatia_saurophila	insect	5.21	4.22
Trachops_cirrhosus	animal	4.64	3.20
Vampyriscus_nymphaea	fruit	3.80	3.17
Vampyrum_spectrum	animal	4.15	3.34

Table 1.6. Group means, standard deviation (SD), and variance for minimum detectable prey size for taxonomic and functional dietary guilds, calculated from peak frequency (pF) and maximum frequency (mF).

Prey size variable	**Diet**	**Mean ± SD**	**Variance**
Min.(pF)	animal	3.76 ± 1.12	1.26
	blood	4.44 ± NA	NA
	fruit	4.06 ± 0.45	0.20
	insect	4.41 ± 0.60	0.36
	nectar	4.05 ± 0.25	0.06
	omnivore	6.38 ± 0.00*	8.8e-07
Min.(mF)	animal	3.15 ± 0.20	0.04
	blood	3.95 ± NA	NA
	fruit	3.35 ± 0.39	0.15
	insect	3.67 ± 0.65	0.43
	nectar	2.86 ± 0.10	0.01
	omnivore	4.84 ± 0.15	0.02
Min.(pF)	non-mobile/non-evasive	4.08 ± 0.41	0.17
	mobile/evasive	4.53 ± 0.97	0.95
Min.(mF)	non-mobile/non-evasive	3.30 ± 0.42	0.18
	mobile/evasive	3.72 ± 0.72	0.52

* 0.00094 -- full value for omnivore

Chapter 2. MORPHOLOGICAL DIVERSITY IN THE SENSORY SYSTEM OF PHYLLOSTOMID BATS: IMPLICATIONS FOR ACOUSTIC AND DIETARY ECOLOGY

2.1 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.

2.2 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 and Wainwright 2011). Prey detection is a critical use of the sensory system that can directly influence organismal fitness and ecology (Persson et al. 1996, Kalko and Condon 1998, Ferry-Graham et al. 2002, Placyk and Graves 2002, Gracheva et al. 2011), 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 (Kalko and Condon 1998, Gracheva et al. 2011). Here, we investigate a source of morphological diversity (modularity) in the external sensory organs of the most trophically diverse family of mammals, the Neotropical leaf-nosed bats (Phyllostomidae), and explore whether this diversity is linked to echolocation call parameters and dietary ecology.

Phyllostomids are an adaptive radiation comprised by over 200 species that vary greatly in diet, including insectivory, sanguinivory, animalivory, nectarivory, omnivory and frugivory (Rex et al. 2010, Dumont et al. 2012). They are laryngeal echolocators, nasophonators, and possess a conspicuous leaf-shaped structure surrounding their nares –a nose leaf– that comprises the major external sensory structure 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 and Suthers 1987, Linnenschmidt and 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). For example, removing particular nose leaf components can drastically influence the spread of bats' calls, and curling the pinnae can change a bat's ability to localize sound (Zhuang and Müller, 2007; Vanderelst et al., 2010). Phyllostomids exhibit great morphological diversity in nose leaf and pinnae (Fig. 1) and thus represent a natural experiment 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 (Feng, Gao, Lu, & Müller, 2012; Gao, Balakrishnan, He, Yan, & Müller, 2011; Vanderelst et al., 2010; Zhuang & Müller, 2007) have explored how specific morphological components are related to sound beam spreading.

Morphological diversity may arise through various mechanisms, including modularity (Goswami 2006, Goswami and Polly 2010). An anatomical module is defined by sets of highly correlated traits that have weak correlations with traits outside the module (Goswami and 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 et al. 2012); mammal skulls (Goswami 2006), and the evolution of modules within a structure allows parts of the structure to specialize for different functions. While modularity has been primarily tested in osteological components involved in feeding, less attention has been paid to modularity of the components of sensory systems. Here, we investigate modularity as potential mechanism underlying the morphological diversification of the nose leaf in phyllostomids. We hypothesize that the nose leaf consists of at least two modules, spear and horseshoe (Fig. 2), which would allow for specialization of each of these parts on specific acoustic functions. We expect to find greater covariation among landmarks within each module than between modules, which would indicate independent variation of the spear and the horseshoe across species and over evolutionary time. Alternatively, finding a single module within the nose leaf would indicate that all parts of the nose leaf change together over evolutionary time.

Next, we test if there is a link between morphological variation of the nose leaves and pinnae and echolocation call parameters across phyllostomids. High frequencies, necessary for small prey detection, 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 and Simmons 1982). High frequency sounds are crucial for insectivorous phyllostomids because they allow distinguishing cryptic prey from a leaf background and determining the texture of edible prey (e.g., katydids; Geipel et al., 2013; Simmons et al., 1975). Therefore, we predict most phyllostomids will have nose leaf morphologies that increase their ability to focus higher frequencies emitted, and that certain shape components of nose leaf morphology will be associated with specific echolocation call parameters. The angle in which sound reflects off a surface can greatly influence either the spread of sound (e.g., wider, less intense sound beam) or the concentration of sound (e.g., narrower, more intense sound beam; Herzfeld, 1938). Thus, we predict that an anteriorly rotated, relatively broad spear and an anteriorly rotated horseshoe will be correlated with higher values of peak frequency, maximum frequency and bandwidth, as we expect these shape components to be more efficient at directionally reflecting sound (Herzfeld, 1938; Wahlstrom, 1985; Zhuang & Müller, 2012). Conversely, a nose leaf that is flattened in the coronal plane and a reduced horseshoe would be correlated with lower values of peak frequency and minimum frequency. For pinnae, we expect to find a link between overall shape and the frequency of acoustic cues that may be used for prey detection. Across several families of bats, absolutely smaller pinnae are not sensitive to detecting low frequencies (<15 kHz). Typically, smaller pinnae have a more pointed apex and this results in less surface area compared to rounded, broad morphologies (Obrist, Fenton, Eger, & Schlegel, 1993). Thus, we predict that pointed, narrower pinnae will be correlated with the peak frequency emitted. Alternatively, broader, rounder pinnae might not be associated with frequencies or bandwidth emitted as these species may be cueing in on low frequency sounds (<15 kHz), such as prey mating calls (Bernal et al. 2009).

Finally, we investigated whether interspecific variation in the morphology of the nose leaf and pinnae is associated with dietary ecology in phyllostomids. Phyllostomid bats rely on different sensory modalities depending on the task they are performing, and 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 (Reiger and Jakob 1988, Laska 1990, Bloss 1999, Hodgkison et al. 2007). We hypothesize that different dietary ecologies and foraging behaviors have imposed different selective pressures on the echolocation system of phyllostomids, including the nose leaf and pinnae, leading to morphological variation in these structures among dietary categories. More specifically, we predict that species within dietary categories that rely heavily on precise echolocation information to find and capture more elusive prey (i.e., insectivory) will exhibit more specialized nose leaf and pinnae morphologies that enable them to better focus echolocation calls and receive a wider array of sound frequencies. In contrast, we predict that frugivores and nectarivores, which rely on other senses such as olfaction, will exhibit sensory morphologies that are less functionally tied to specific echolocation call parameters.

2.3 METHODS

2.3.1 *3D Imaging and shape analyses*

We quantified the three-dimensional morphology of nose leaves and pinnae for 47 adult bats from 31 phyllostomids species that span the diversity in body size, nose leaf and pinnae morphologies, and dietary ecology within the family. The majority of species used (26) were collected in the field following approved methods (IACUC protocol 4307-01), and the remainder (5) were fluid-preserved museum specimens (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).

Specimen preservation often alters the shape of soft tissue structures (e.g., bent nose leaves). To mitigate this problem, we captured pinnae and nose leaf morphology by taking casts from freshly euthanized animals. 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. We used both this casting method and select fluid-preserved specimens that did not have deformed soft tissues to compile our dataset. We created 3D digital models of the nose leaf and pinnae by scanning either specimens or epoxy casts on a Skyscan 1174 micro-CT scanner (Bruker MicroCT, Kontich, Belgium) at a 17- 30.1 μm 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. 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 et al. 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 modified landmark placement 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). 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 single-point 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 and Otárola-Castillo 2013) in R v 99.903 (R Core Team, 2017) for geometric morphometric analyses.

2.3.2 *Acoustics*

Phyllostomid bats produce low-intensity calls (Griffin 1958, Brinkløv et al. 2009) 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.

2.3.3 *Statistical analyses*

To test whether the nose leaf consists of two modules (spear and horseshoe; Fig. 2D), we computed Covariance Ratio (CR) values for a two-module hypothesis using the nose leaf landmark data, and estimated the p-value for this relationship via 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). Then, 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.

2.4 RESULTS

2.4.1 *Modularity in 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.

2.4.2 *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 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 insetting 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.

2.4.3 *Links among morphology, call structure and diet*

Nose leaf. PGLS regressions revealed a significant relationship between pPC1 and maximum frequency and bandwidth (Table 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 and spear (pPC2 and pPC4, and pPC2, respectively) among dietary categories (Table 3). *Post-hoc* tests revealed that whole nose leaf shape was significantly different between frugivores and all other dietary groups, and spear shape between frugivores and omnivores and all other dietary categories (supplemental Table 2). For the horseshoe, we found no significant differences across all dietary groups (Table 3). *Post-hoc* test revealed a difference in pPC3 between omnivores and all other dietary groups and in pPC5 between frugivores and nectarivores and all other diets ($p= 0.02$, $p=0.04$, supplemental Table 2). However, these axes represent less than 13% of the total shape variation.

Pinnae. We found no significant relationships between pinnae shape and echolocation call parameters (Table 2). We found no significant differences in pinnae shape among all taxonomically-defined dietary categories (Table 3), but *post-hoc* tests reveal sanguinivores and omnivores are different in pinnae shape (pPC1) compared to all other dietary groups (p = 0.03, p = 0.04; supplemental Table 2). Sanguinivores and omnivores both possess highly tapered pinnae, that can be posteriorly recurved. We also 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 (p = 0.006, supplemental Table 3). 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. 3D).

2.5 DISCUSSION

A major goal of functional ecology is to link morphological diversity to ecology (Bickel & Losos, 2002; Santana, Dumont, & Davis 2009; Santana, Grosse, & Dumont, 2012; Schluter, 1993), but only a few studies have focused on the ecomorphology of sensory systems (e.g., Coombs, Janssen, & Webb, 1988; Pankhurst, 1989; van der Meer, Anker, & Barel, 1995). Using an ecological radiation (Phyllostomidae), we investigated whether and how an evolutionary mechanism (modularity; Olson & Miller, 1999) may underlie extreme diversity of sensory structures, explored if morphological differences in external sensory structures are related to differences in functional outputs, and tested if morphological differences match expectations related to different ecological demands. Altogether, these analyses explore some of the potential intrinsic and extrinsic factors shaping the diversity of sensory structures in bats.

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 in different functions (Hulsey, 2006, Sanger, Mahler, Abzhanov,

& Losos, 2011; Goswami, 2006; Goswami & Polly 2010). Consistent with our hypothesis of multiple modules in the nose leaf, 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., *Mimon 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, many phyllostomids (e.g., frugivores and nectarivores) rely on multiple sensory modalities (e.g., olfaction and echolocation) for prey detection (Thies et al. 1998, Kalko and Condon 1998, Korine and Kalko 2005, Sánchez et al. 2006). 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 relies on vision and olfaction for the same task. Phyllostomids also use diverse foraging behaviors (Bloss 1999, Korine and Kalko 2005, Weinbeer and Meyer 2006, Kunz et al. 2011), 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). *Phyllostomus* often forages in groups and detects patches of fruits or flowers that can be detected at longer ranges (Kwiecinski 2006).

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). 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 (10mm vs. 5mm in height). The size of the structure may be an important factor linking phyllostomid echolocation and ecology, as the size of a surface can influences 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 evolve 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, these 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. However, 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 odontecetes that help focus emitted sound (Norris and Harvey 1900, Huggenberger et al. 2014), the antlers of the bull moose that help focus received sound toward their pinnae (Bubenik and Bubenik 2008), and the spherical facial disks of owls aid in sound localization (Coles and 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, differences in nose leaf shape likely influence the bat's perceptual field, and the relationship between nose leaf morphology and foraging ecology.

Pinnae shape did not differ significantly among species grouped into taxonomic dietary categories (e.g., insectivore, frugivore, nectarivore), but it did when species were grouped into functional diet categories that represented 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 the 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 and LaVal 1998) and *Lophostoma* species can curl their pinnae posteriorly (Williams and 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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2.8 AUTHOR 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. All authors contributed critically to the drafts and gave final approval for publication.

2.9 DATA ACCESSIBILITY

Upon acceptance of this manuscript we intend to deposit all raw data in Dryad Digital Repository and .stl files will be available upon request.

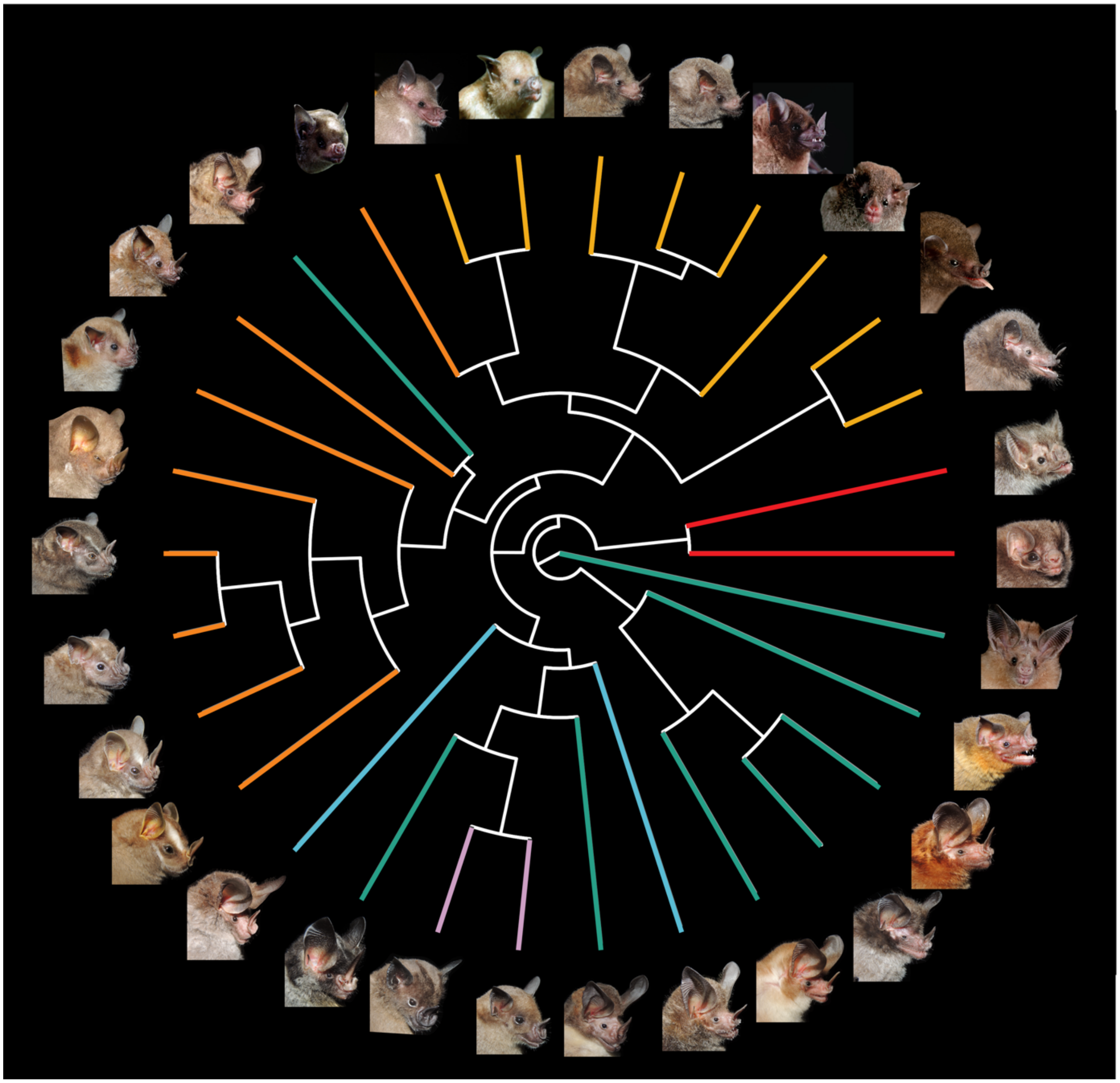

Figure 2.1. Phylogeny of phyllostomid species in our dataset. Branches do not contain information about scale and are colored based on diet. Species starting at the root and moving clock-wise: *Macrotus waterhousii, Lampronycteris brachyotis, Micronycteris microtis, Micronycteris hirsuta, Micronycteris minuta, Trachops cirrhousus, Lophostoma silvicolum, Phyllostomus discolor, Phyllostomus hastatus, Gardnerycteris crenulatum, 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, Glossophaga commissarisi, Glossophaga longirostris, Monophyllus redmani, Lichonycteris obscura, Hylonycteris*

underwoodii, Desmodus rotundus, and *Diphylla ecaudata*. Photo credit: David Villalobos-Chaves, Brock Fenton, José G. Martínez-Fonseca, Sharlene Santana, Joaquín Ugarte, and Laurel Yohe

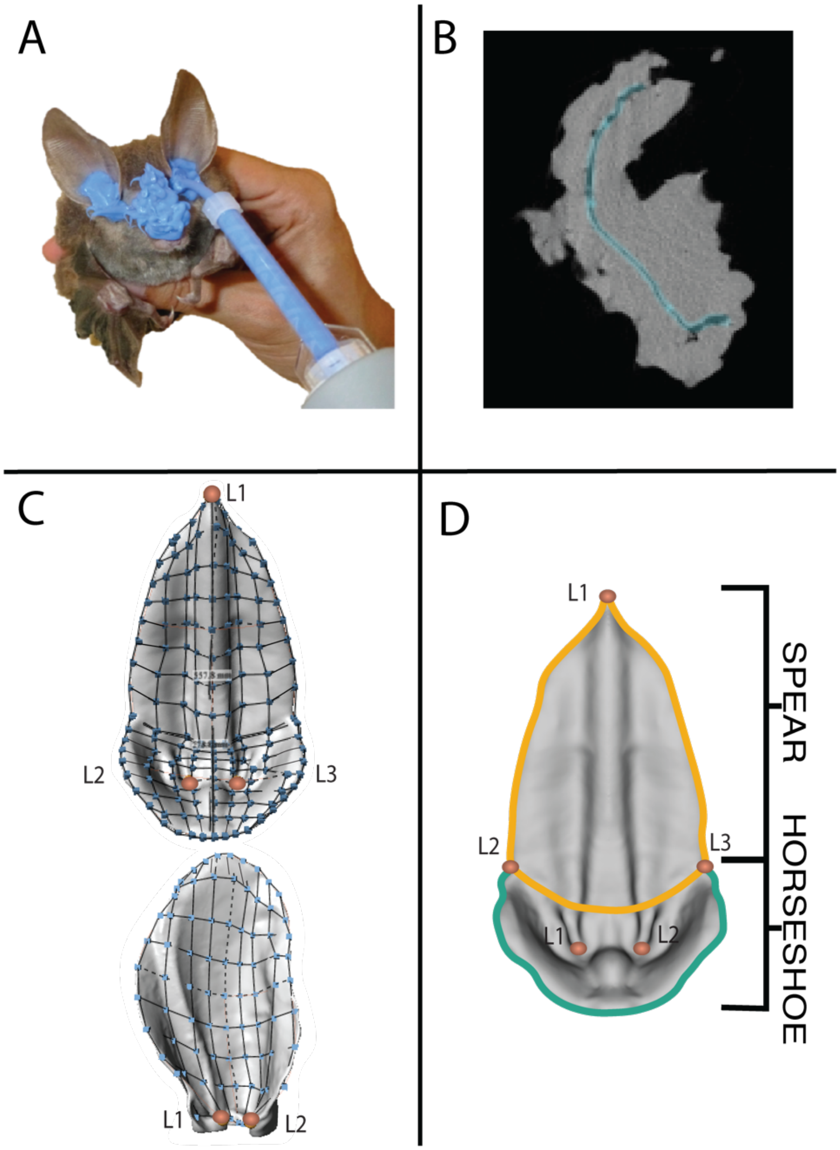

Figure 2.2. Epoxy molding technique on freshly collected specimen (A), microCT scan slice with molding space highlighted in blue for 3D reconstruction (B), reconstructed 3D model of nose leaf and pinna with landmarks (L, orange) and semilandmarks (blue) labeled (C), and modularity hypothesis tested (D), where the spear (yellow) and horseshoe (green) constitute separate modules, with landmarks (L) labeled (D)

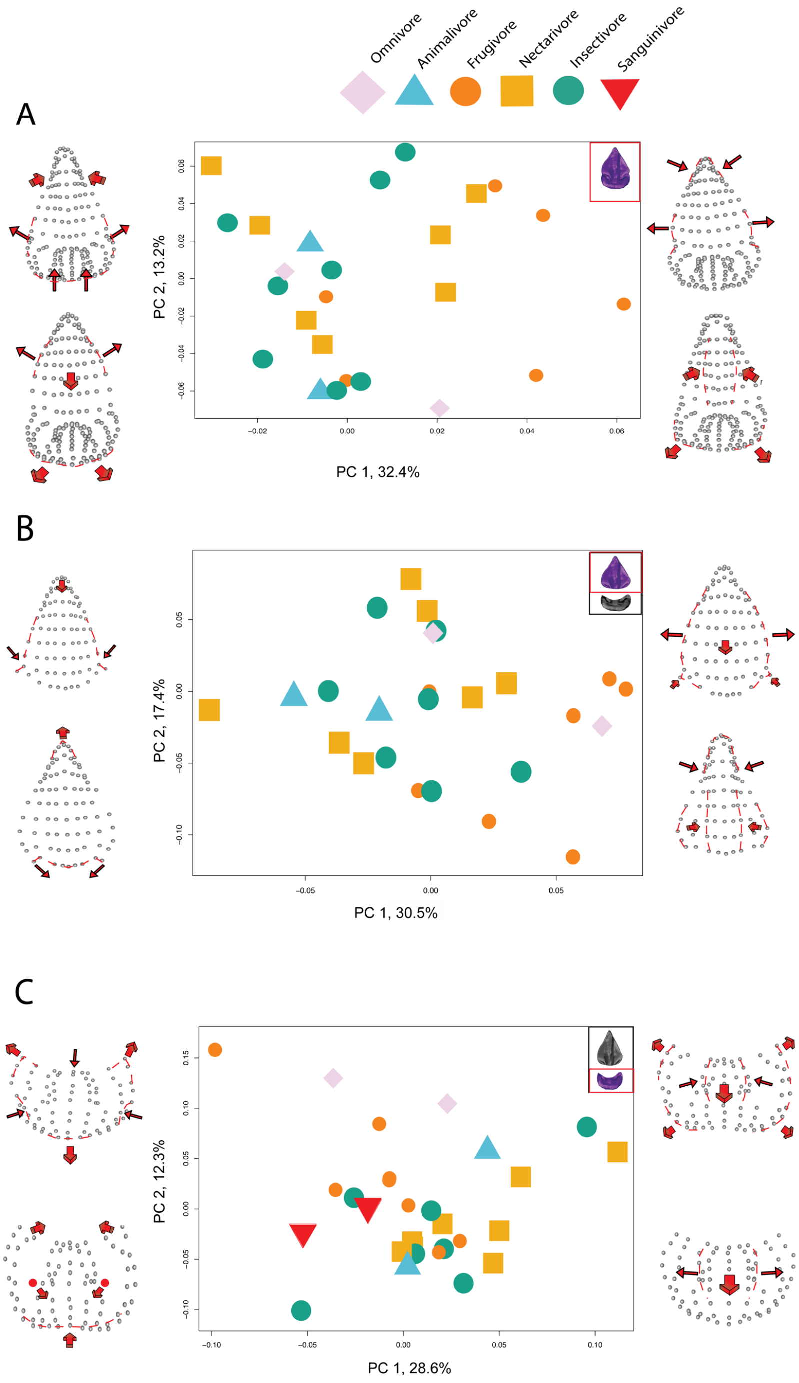

Figure 2.3. Morphospace (pPC) plots for nose leaf (A), spear (B), horseshoe (C), and pinna (D). Landmark clouds with arrows show shape changes along pPC1 (right) and pPC2 (left). Areas of change are outlined in red dashed lines

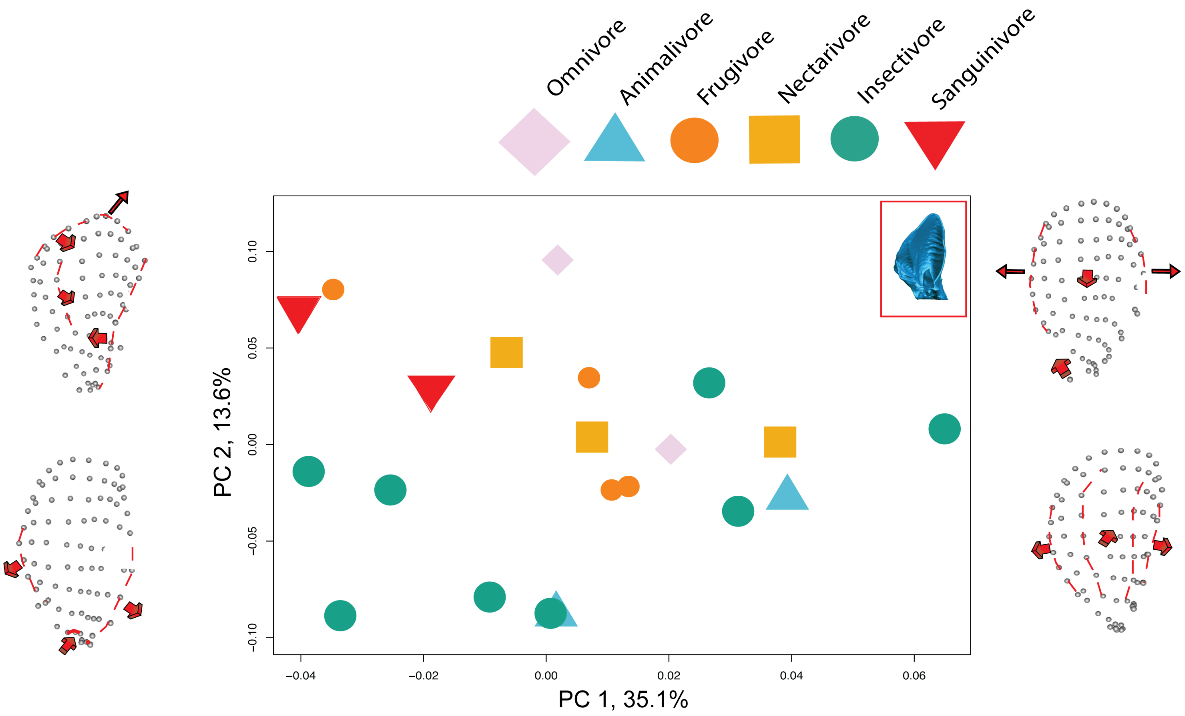

Figure 2.4. Morphospace (pPC) plots for pinnae. Landmark clouds with arrows show shape changes along pPC1 (right) and pPC2 (left). Areas of change are outlined in red dashed lines

Table 2.7. Phylogenetic Generalized Least Squares regression of nose leaf shape variables (PCs) on acoustic call parameters.

Nose leaf shape PC	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	0.063
	Max. frequency	-0.344	0.311	-1.11	0.28
	Bandwidth	0.632	0.184	0.34	0.73

Table 2.8. Phylogenetic Generalized Least Squares regression of pinna shape variables (PCs) on acoustic call parameters.

Pinna shape PC	**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	0.077
	Bandwidth	-0.203	0.176	-1.16	0.26

Table 2.9. Phylogenetic ANOVA results comparing nose leaf, spear, horseshoe, and pinna shape across dietary categories: animalivore, insectivore, frugivore, nectarivore, sanguinivore, and omnivore.

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	**0.052***
	3	4	0.0018	0.00045	1.02	0.42
	4	4	0.0057	0.0014	3.4	**0.027***
	5	4	0.00080	0.00020	0.41	0.802
Spear	1	4	0.010	0.0025	1.2	0.36
	2	4	0.017	0.0043	3.7	**0.026***
	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	0.075
	2	5	0.0092	0.0019	1.5	0.24
	3	5	0.010	0.0020	2.3	0.078
	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	0.069
	2	5	0.0028	0.00056	0.63	0.68
	3	5	0.0049	0.00097	1.2	0.36
	4	5	0.0066	0.0013	1.05	0.43

Chapter 3. A FRUITFUL ENDEAVOR: SCENT CUES AND ECHOLOCATION BEHAVIOR USED BY *CAROLLIA CASTANEA* TO FIND FRUIT

3.1 ABSTRACT

Frugivores have evolved sensory and behavioral adaptations that allow them to find ripe fruit effectively, but the relative importance of different senses in varying foraging scenarios is poorly known. Within Neotropical ecosystems, short-tailed fruit bats (*Carollia*: Phyllostomidae) are abundant nocturnal frugivores, relying primarily on plants of the genus *Piper* as a food resource. Previous research has demonstrated *Carollia* employ olfaction and echolocation to locate *Piper* fruit, but it is unknown how their sensory use and foraging decisions are influenced by the complex diversity of chemical cues that fruiting plants produce. Using wild *C. castanea* and their preferred food, *Piper scintillans*, we conducted behavioral experiments to test two main hypotheses: (1) foraging decisions in *C. castanea* are primarily driven by ripe fruit scent and secondarily by vegetation scent, and (2) *C. castanea* re-weight their sensory inputs to account for available environmental cues, such that bats rely more heavily on echolocation in the absence of adequate scent cues. Our results suggest that *C. castanea* requires olfactory information and relies almost exclusively on ripe fruit scent to make foraging attempts. Ripe fruit scent is chemically distinct from vegetation scent in *P. scintillans*, with a greater abundance of β-caryophyllene, germacrene D and β-elemene, and a few unique compounds. Although variation in echolocation call parameters was independent of scent cue presence, bats emitted longer and more frequent echolocation calls in trials where no fruit scent was present. Altogether, these

results highlight the adaptations, plasticity, and potential constraints in the sensory system of neotropical fruit bats.

3.2 INTRODUCTION

Animals rely on multiple sensory modalities to perform even the simplest ecological tasks (Burkhardt 1989, Siemers and Schnitzler 2000, Holland et al. 2005, Knaden and Graham 2016). One of the main goals of behavioral and sensory ecology lies in understanding the ability of different species to employ and modulate their sensory modes in the context of different environmental cues, and how the resulting behavioral decisions ultimately affect their ecology and evolution. Frugivores use various cues, including components of fruit color (Burkhardt 1989; Osorio & Vorobyev 2008, Melin et al. 2008, Hiramatsu et al. 2008; Valenta et al. 2013), shape (Helversen & Helversen 1998; Kalko & Condon 1998), and scent (Valenta et al. 2013, Sánchez et al. 2006) to find and select ripe fruit, and exhibit corresponding sensory specializations in their visual, auditory and/or olfactory systems to target those cues (Catania 1999, Muller et al. 2007, Vanderelst et al. 2010). Little is known, however, about what scenarios facilitate or constrain sensory system use and modulation during fruit location, selection, and acquisition in vertebrate frugivores. Insight into these processes would provide mechanistic understanding of the behaviors underlying their foraging ecology.

Frugivorous and omnivorous Neotropical leaf-nosed bats (Phyllostomidae) use three major senses, echolocation, olfaction and vision, for navigation and foraging (e.g., Bloss 1999; Laska 1990; Reiger et al. 1988; Schnitzler et al. 2003; Winter et al. 2003), which makes these organisms an exceptional system for investigating the relative role of different sensory modes during ecologically important tasks. Although there has been recent work on phyllostomid vision, including comparisons of their short- and long-wave opsins(Muller et al. 2007, Müller et al. 2009), the importance of vision in ripe fruit location and selection has not been experimentally tested in phyllostomids. By contrast, experimental evidence strongly suggests that omnivorous phyllostomids rely primarily on echolocation to locate fruits (Kalko and Condon 1998), and specialized frugivores employ either olfaction or a combination of olfaction and echolocation to locate ripe fruit (Kalko and Schnitzler 1993, Korine and Kalko 2005, Hodgkison et al. 2007, 2013).

The relative importance of different sensory modes for fruit detection may depend on which plant cues can be readily perceived within a specific environmental context. To date, it is unknown whether and how frugivorous phyllostomids integrate their primary sensory modes (echolocation and olfaction) conditional on which plant cues are present. While plant scents can travel over short or long distances (Riffell et al. 2014), they are rarely directional and may be difficult to detect in saturated scent environments such as rainforests. Conversely, echolocation allows for highly precise prey detection (Schnitzler and Kalko 2001; Brinkløv et al. 2009; Jakobsen et al. 2013), but phyllostomids emit short, high frequency and low intensity calls (Thies et al. 1998; Korine and Kalko 2005). The echoes from these calls provide information about size, shape, texture, range and position of an object in space relative to the bat (Simmons et al. 1975, 1983, Simmons and Stein 1980, Neuweiler 1989, Schmidt et al. 2000, Schnitzler and Kalko 2001), but for these types of information are only effective at very short distances because low intensity, high frequency calls attenuate rapidly in warm, humid environments (e.g., 45 -90 kHz attenuate at 1.4 to 4 dB/m at 25°C and 80% humidity; Jakobsen et al. 2013). Additionally, surrounding foliage can produce acoustic masking effects that may complicate fruit detection (Arlettaz et al. 2001, Korine and Kalko 2005). Therefore, flexibility in olfaction versus echolocation use could be highly beneficial for frugivorous bats given the limitations of each sensory mode within complex forest environments.

Here, we study the primary plant cues from vegetation and fruits and how this relates to the roles of echolocation and olfaction for fruit detection and localization in the chestnut short-tailed fruit bat, *Carollia castanea*, a highly abundant frugivore and ecologically important seed disperser that inhabits forests in Central and South America (Bonaccorso 1979, Fleming 1991) (Figure 1). This research builds upon the seminal work of Thies et al. (1998), which demonstrated the importance of olfaction for fruit detection in *Carollia;* here we investigate the relative contributions of vegetative and fruit scent cues that drive *Carollia castanea's* foraging decisions, and how varying foraging scenarios (i.e. changing of plant scent cues) may affect the relative reliance of these bats on olfaction versus echolocation for fruit detection. *Carollia castanea* feeds primarily on infructescences of Neotropical *Piper* plants (Piperales: Piperaceae), particularly *P. scintillans* (previously *P. sancti-felicis*; Hammel et al. 2014) in our study locality (Lopez and Vaughan 2007). We test if foraging decisions in *C. castanea* are primarily driven by clear signals of food availability (i.e., *P. scintillans* ripe fruit scent). We predict bats will cue in on ripe *P. scintillans* fruit scent and secondarily on its vegetation scent, since vegetation is a salient and fragrant part of the plant that could also aid in fruit localization. Since this prediction relies on the assumption that ripe fruit and vegetation scents differ in their chemical composition, we present analyses contrasting the volatile chemical composition of these plant parts. We also hypothesize that *C. castanea* potentially re-weight their sensory inputs to account for available environmental cues, and predict that foraging bats emit echolocation calls more frequently when scent cues are absent. This is because *Carollia* can use echolocation for the final localization of fruit at close range (Thies et al. 1998; Corlett 2011) and perhaps also when searching for potentially edible fruit patches at a longer range. To test our hypotheses, we conducted a series of experiments to mimic the sensory challenges fruit bats may encounter in nature, and quantified differences in the bats' behavioral responses when exposed to different sensory cues. Our study contributes to the understanding of which chemical cues bats use for fruit selection, which contexts facilitate alternating between sensory modes, and the behavioral and sensory adaptations fruit bats have evolved for foraging.

3.3 MATERIALS AND METHODS

3.3.1 *Study animals*

We used mist nets (avinet, sizes; 4m, 6m, 9m, 12m) to capture *carollia castanea* along forest trails at la selva biological station in sarapiquí, heredia province, costa rica (supplementary table 1) through August – September of 2016. All individuals were experimentally naïve and were used in experiments only on the night of capture. Upon capture, each bat was kept in a clean cotton bag prior to experiments. We conducted experiments on 21 bats (16 adult males and 5 adult non-lactating, non-pregnant females), and if individual had a positive trial, we subsequently collected biometric data and a 2-3 mm wing biopsy (disposable biopsy punches, integra miltex) from the uropatagium of each individual (see below). This was done for future genetic analyses, and helped ensure we did not use recaptured individuals in subsequent experiments. All individuals were released near the site of capture after the behavioral experiments and processing were completed. All procedures were approved by the university of washington institutional animal care and use committee (protocol# 4307-02).

3.3.2 *Experimental set-up*

We conducted two-choice behavioral experiments without reward inside a flight cage (Coleman, 3.048 x 3.048 x 2.1336 m) under natural ambient conditions at La Selva. As shown in Figure 2, we placed an infrared-sensitive handycam (4K HD Video Recording, Sony, Japan) on a tripod (30 cm from the ground), which allowed us to record the bats' foraging behaviors under infrared light conditions (>700nm; beyond the spectral range of vision of phyllostomids; Jones et al. 2013). We recorded the bats' echolocation calls with a condenser microphone (microphone capsule CM16, CMPA preamplifier unit, Avisoft Bioacoustics, Berlin, Germany) mounted at the top, center of the flight cage. During experiments, we visualized real-time calls using an ultrasound acquisition board (UltraSoudGate 116, Avisoft Bioacoustics, Germany; sampling rate 375 kHz, 16-bit resolution). At the back end of the flight cage, we placed a custom-built platform (90 cm long x 125 cm tall) which held two 50 ml falcon tubes, 40 cm apart, onto which we mounted each of the target ("fruit") choices (Figure 2). To control for size and shape of these targets, we used 3D-printed dummy fruits (Form 2 printer with FGLPWH02 resin) of the same shape and size of an average *Piper scintillans* fruit. To mimic a ripe fruit, we smeared a dummy (3D printed) fruit with a standardized amount of ripe *P. scintillans* fruit pulp (approx. 0.62 g, ~1/3 of a total fruit) we used fruit collected on the same day of each experimental night. We harvested vegetation (branches) from the same plants from which we collected ripe fruit. In trials with vegetation present, we placed the vegetation at the base of the dummy fruit, which is the natural configuration within the plant. Between each night of experiments, we cleaned dummy fruits with 95% ethanol to remove scents, rinsed them with water, and let them air dry at least 24 hours before reusing.

To test our hypotheses, we presented each bat with a choice between two of the following targets during each experimental trial: 1) dummy with fruit scent (pulp from *P. scintillans*), 2) dummy with vegetation only, 3) dummy with fruit scent and vegetation, and 4) dummy with no fruit scent or vegetation (Table 1). We ran each trial for a maximum of 20 minutes per bat, and subjected bats to up to four trials, conditional on their performance on the initial trial. If the bat did not perform within 20 minutes, we released the individual. Conversely, if the bat attempted to capture the fruit within the 20-minute duration of the trial, we considered it a positive trial and began a new trial. To begin a new trial, one of us entered the flight cage and switched out choice targets. We randomized both the order we presented each trial and the position (left, right) of the target choice on the platform between consecutive trials to minimize confounding effects due to bat spatial learning (Thiele and Winter 2004). At the end of trials, we used a hand net to recapture the bat inside the flight cage, processed and released it, as described above.

3.3.3 *Analysis of flight behavior during target search and approach*

We watched videos of the behavioral trials at normal playback speed on a computer at the University of Washington, Seattle, WA. From each video, we recorded: the amount of time it took the bat to capture one of the target choices presented, the real time of capture (to synchronize with acoustic calls), and the individual's choice. We defined a capture event as a bat landing on a target choice and attempting to bite it. We noted additional characteristics of the bat's flight behavior (e.g., exploratory flights around the flight cage) and target exploration (i.e., hovering over fruit) for all trial videos.

3.3.4 *Analysis of echolocation behavior during target approach*

We analyzed echolocation calls emitted during target approach using Avisoft SASLabPro v. 4.40 (Avisoft Bioacoustics, Berlin, Germany). We used the time of the capture events from video recordings and matched them with the time stamps of the call files to synchronize acoustics with recorded capture events. These files were used in the subsequent analyses and included the acoustic calls for one minute prior to the capture event, which we defined as the search/approach window. We chose a minute interval prior to capture because we were not only interested in sensory behavior for target localization (typically seen in the approach phase), but also the sensory behavior when 'searching' for food. *Carollia castanea*, similar to other phyllostomids, emits calls well above 20kHz (Thies et al. 1998, Brinkløv et al. 2011), so we used this as the cut-off frequency to avoid including noise from recording at high gain in ambient conditions (Geipel et al. 2013). We filtered each acoustic sequence using a high-pass filter (at 20 kHz) and visualized spectrograms using a Hamming window (512 fast Fourier transform, 98.95% overlap). We extracted the following echolocation call parameters for comparisons across trial types: maximum frequency (kHz), minimum frequency (kHz), peak frequency (i.e. frequency with the highest amplitude, kHz), call duration (ms), call interval (ms), and total bandwidth (kHz) from the spectrograms at the maximum energy of each call. We compiled sequences per individual (approximately 8 – 20) and calculated mean and standard deviation for each call parameter per trial type per individual (Table 2).

3.3.5 *Statistical analyses of behavioral experiments*

We performed all statistical analyses in R v. 3.2.4 (R Core Team, 2018). We used chi-squared tests to assess the differences in bat preference among target types. We tested the normality of the echolocation call data using Shapiro-Wilks tests (Shapiro and Wilk 1965) and subsequently log-transformed these data to improve normality. We compared the differences in call parameters among trial types using analysis of variance (ANOVA).

3.3.6 *Target comparisons*

To determine if *P. scintillans* ripe fruit and vegetation present different olfactory cues to *C. castanea*, we compared the volatile organic compounds (VOCs) that make up the scents of these plant parts. We collected vegetation (7.0 – 7.8 g of fresh weight leaf material; one branch) and ripe fruit (19.9 –20.6 g, fresh weight; 13 – 15 fruits) samples from four *P. scintillans* plants at La Selva. This larger sample of ripe fruit was necessary for VOC capture and detectability by our experimental setup (below). We collected VOCs from these samples via dynamic headspace adsorption using a push–pull system (Raguso and Pellmyr 2006, Riffell et al. 2008). Within two hours of collection, we placed each sample in a 3L teflon bag (Reynolds, Richmond, VA, USA) and connected the bag to a diaphragm pump (400-1901, Barnant Co., Barrington, IL, USA) that pulled the fragrant headspace air through a sorbent cartridge trap (50 mg Porapak Q with silanized glass wool Waters Corp., Milford, MA, USA) and pushed air though a charcoal filter. We collected VOCs in this manner for 20 hours per sample, following previously established protocols and to ensure characterization of the full chemical profile (Byers et al. 2014). We eluted trapped volatiles from each sample's sorbent cartridge with 600 μl of HPLC-grade hexane into a 2mL borosilicate glass vial with a Teflon-lined cap. Subsequently, we stored all of the samples at –20°C to –80°C. We analyzed a 3 μl aliquot of each sample using an Agilent 7890A GC (gas chromatograph) and a 5975C Network Mass Selective Detector (Agilent Technologies, Palo Alto, CA, USA). To separate the VOCs, we used a DB-5MS GC column (J&W Scientific, Folsom, CA, USA; 30 m, 0.25 mm, 0.25 μm) with helium as the carrier gas flowing at a constant rate of 1 cc per min (Byers et al. 2014). The initial oven temperature was 45°C for 4 min, followed by a heating gradient of 10°C min^{-1} to 230°C, which was then held isothermally for 4 min. We initially identified the chromatogram peaks with the aid of NIST 08 mass spectral library (v. 2.0f; ca. 220,460 spectra of 192,108 different chemical compounds) followed by verification using alkane standards and comparing with published Kovats indices. We integrated the peaks for each compound using ChemStation software (Agilent Technologies) and present them in Table 4.

3.4 RESULTS

3.4.1 *Target prefernces*

Most individuals performed exploratory flights prior to showing interest in the presented target choices. These behaviors consisted of circling flights around the cage without approaching the target. In most trials (83%), bats attempted to capture a target by landing and trying to bite the dummy fruits (Supplementary Video 1). Comparisons across treatments revealed that *C. castanea* strongly preferred targets consisting of a dummy fruit with fruit scent (n = 10, χ^2= 6.21, P = 5.69e-05) or a dummy fruit with fruit scent and vegetation (n = 13, χ^2= 22.154, P = 2.52e-06) over targets that had an unscented dummy fruit and vegetation. The presence of vegetation did not affect the bats' preferences for fruit scent (dummy fruit with fruit scent vs. dummy fruit with fruit scent and vegetation: n = 13, χ^2= 0, P = 0.99). Bats never chose unscented dummy fruits, either alone or with vegetation.

3.4.2 *Echolocation behavior*

All bats used in the experiments emitted echolocation calls throughout the trials. We did not find statistically significant differences in the echolocation call parameters among treatment types during the search/approach window (minimum frequency, maximum frequency, peak frequency, bandwidth, duration, pulse interval, all $P > 0.05$; Table 3). However, there were marked trends in call duration and interval in some trial types. Bats emitted echolocation calls more frequently (shorter interval) and of longer duration in treatments where no fruit scent was present (unscented dummy vs. unscented dummy with vegetation; Figure 4).

3.4.3 *Chemical differences between targets*

The scent profiles of *P. scintillans* vegetation and ripe fruit differ slightly in VOC composition, and greatly in the proportion of specific VOCs (Table 4). The vegetation scent of *P. scintillans* is dominated by 3-hexene-1-ol, which is not found in the ripe fruit scent. Conversely, the ripe fruit scent is characterized by a greater abundance of β-caryophyllene, germacrene D and β-elemene. Both vegetation and fruit scents have a low abundance of p-cymene and β-pinene (Table 4).

3.5 DISCUSSION

An animal's sensory ecology and behavior often reflects the environment it inhabits, as well as its evolutionary history. As such, certain sensory modalities play key roles in mediating ecological interactions. Mammalian frugivores are able to locate and acquire ripe fruit by using and integrating across sensory modalities: they use vision to detect differences in fruit color and luminance (Burkhardt 1989; Osorio and Vorobyev 2008; Melin et al. 2008; Hiramatsu et al.

2008; Valenta et al. 2013), olfaction to detect individual volatile organic compounds (VOCs) or entire odor plumes (Sánchez et al. 2006; Valenta et al. 2013) and, in the case of phyllostomid bats, echolocation to gather information about fruit shape and location (Kalko and Condon 1998, Von Helversen and Von Helversen 1999). Our results indicate that *C. castanea* makes foraging decisions based on ripe fruit scent over all other cues presented, but may rely more heavily on echolocation when olfactory cues are absent. Importantly, in the absence of plant scent cues, a *P. scintillans* fruit shape does not elicit a prey capture response from *C. castanea*. This gives insight to the primary role of olfaction, followed by echolocation, when these bats forage for fruit.

The importance of olfaction for foraging in *C. castanea* is supported by previous research on other *Carollia* species that rely less on *Piper* as a food resource. For example, *C. perspicillata* can recognize minute concentrations of particular chemical components (fruit-typical odor components like ethyl butyrate, n-pentyl acetate, or linalool; Laska 1990), are attracted to fruit scent even when no other cues are present (Hessel and Schmidt 1994), and visit mist nets spiked with the essential oil of *Piper gaudichaudianum* more frequently (Mikich et al. 2003). Here, we link the bouquet of VOCs from a known, preferred food source with the behavioral preferences of *C. castanea*. Our experimental results strongly suggest that *C. castanea* uses ripe fruit scent, as opposed to a combination of ripe fruit and/or vegetation scent, or fruit shape, as the cue to locate food items. Our chemical analyses of ripe fruit and vegetation VOCs provide an explanation for this pattern: the scent profile of the *P. scintillans* ripe fruit and vegetation are somewhat similar in composition, but differ greatly in abundance of some specific VOCs. Additionally, the ripe fruit scent profile contained a few distinct VOCs that were not found in vegetation, and vice versa. Considering that *C. castanea* forages in a complex sensory environment, the forest understory, it may be advantageous for the bats to cue in specific chemicals that unmistakably signal fruit ripeness against the background of unripe fruit and vegetation within a *Piper* bush, as well as adjacent vegetation. Our results motivate future work to examine whether some of these key volatiles or their ratios may signal fruit ripeness amongst the vegetative mélange.

Echolocation call parameters did not differ significantly in frequency between trial types, suggesting that *C. castanea* has a stereotyped call structure regardless of their foraging tasks. While this has not been broadly studied, having a stereotyped echolocation call is common in phyllostomids (Kalko and Condon 1998; Thies et al. 1998; Korine and Kalko 2005; Geipel et al. 2013). Nevertheless, our experiments revealed that *C. castanea* potentially modulates time-linked echolocation traits (i.e., duration of the call and time between calls, interval) when confronted with different prey cues. As in all mammals, phyllostomid bats process olfactory cues by inhaling air through their nose, but also emit echolocation calls out of their nose. Because of the potential conflict between these two functions performed by the nasal cavity, we propose that frugivorous phyllostomids exhibit behavioral modulation in their nasally-linked senses to alternate between and maximize effectiveness of one sensory cue versus another, when appropriate.

Echolocation provides bats with high-resolution information about shape, surface texture, and material of an object at close range (Schnitzler et al. 1983, Ostwald et al. 1988, Kober and Schnitzler 1990), but bats also use echolocation for navigation and detection of plants that signal through morphology for better acoustic detection. We saw a general trend of longer duration of echolocation calls and shorter intervals between calls when bats were offered choices that did not include a ripe fruit scent cue. Decreased time between calls (interval) and longer duration means these bats were calling more frequently in the absence of ripe fruit scent. We hypothesize that, when ripe fruit odor cues are absent, *C. castanea* relies more heavily on echolocation to locate a potential food item, in this case one that may resemble an edible *Piper* fruit. In contrast, when bats were presented with any target that had ripe fruit scent (one or two choices), they emitted shorter echolocation calls at longer intervals, thus echolocating less frequently. We hypothesize that the decrease in echolocation call duration and an increase in interval could be linked to an increase in the bats' use of olfaction as they attempt to locate edible ripe fruits or determine which one is the 'most edible' option.

If ripe fruit scent is the primary cue for fruit location and selection by *C. castanea*, why are there differences in echolocation call duration and interval between treatments with and without ripe fruit scent? Previous studies have demonstrated that phyllostomids bats can use echolocation to determine the position of a fruit (Kalko and Condon 1998), and echolocating bat species, in general, alter call parameters to overcome acoustic masking effects during prey location (Kalko and Schnitzler 1993). This can be accomplished by changing the duration and interval of the call (Siemers and Schnitzler 2000). Bats typically extend the duration of a call when searching for prey and during orientation flights (Kalko and Schnitzler 1993), and decrease the time between calls when approaching a prey item (Kalko and Schnitzler 1993, Siemers and Schnitzler 2000). In our experiments, bats never chose unscented dummy fruits as potential food options, but our behavioral and acoustic recordings demonstrated that they did explore them via echolocation. We propose that *C. castanea* has a series of criteria (e.g., fruit scent, shape, configuration of fruit in relation to vegetation), which may be hierarchical, and are integrated during the search and localization of a potential food item.

The use of echolocation and olfaction for food selection has been documented in other frugivorous and omnivorous phyllostomids. *Artibeus jamaicensis* is a specialized frugivore that detects, localizes and classifies ripe fruits primarily by olfaction (Kalko et al. 2010). In contrast, *Phyllostomus hastatus,* a large omnivorous bat, consistently uses echolocation over olfaction when foraging for *Gurania spinulosa*, a pendulous fruit-bearing vine (Kalko and Condon 1998). These examples illustrate an echolocation-olfaction continuum across phyllostomids that forage for fruit, and suggests that multiple sensory modes are important for fruit foraging in complex environments. They also substantiate that sensing mode could be conditional on which food cues are present or the degree of specialization of each species (e.g., omnivores vs. specialized frugivores). To date, it is still unclear which senses are most important for fruit foraging in most bat frugivores, and what facilitates the use of one sense over another.

This study provides behavioral links between a frugivore's sensory abilities and plant cues, a relationship that is critical to understanding the ecological dynamics and coevolution between plants and their seed dispersers. There is still much to learn about how vertebrate frugivores perceive and interact with their potential food sources, thus further observational and experimental studies are critical for determining what specific fruit traits (e.g., compounds or combination of compounds in ripe fruit) drive fruit selection by frugivorous species.

All procedures performed in studies involving animals were approved by and in accordance with the ethical standards of the University of Washington Institutional Animal Care and Use Committee (protocol# 4307-02)

3.6 DATA AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Figure 3.1. Study organisms, *Carollia castanea* (left) and *Piper scintillans* (right)

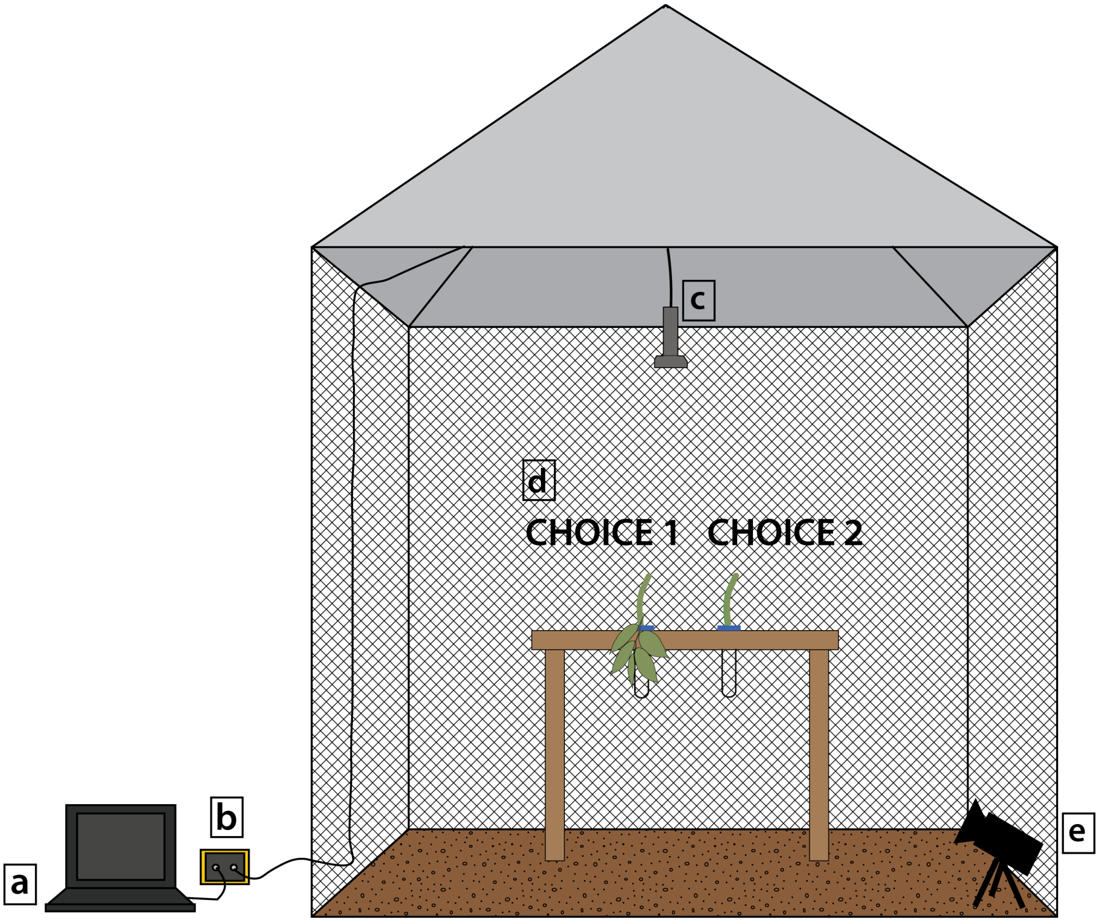

Figure 3.2. Diagram of experimental set-up: echolocation calls were visualized and recorded via a Dell 14 Rugged Extreme laptop (a) connected to a USG 116H recorder (b) that was connected to a CM16 condenser microphone (c). Target choice options were offered on a custom-made platform (d), here showing two example choice options, Choice 1: dummy with fruit scent and vegetation, and Choice 2: dummy with fruit scent only. Bat behaviors were recorded with a Sony infrared-sensitive handycam (e)

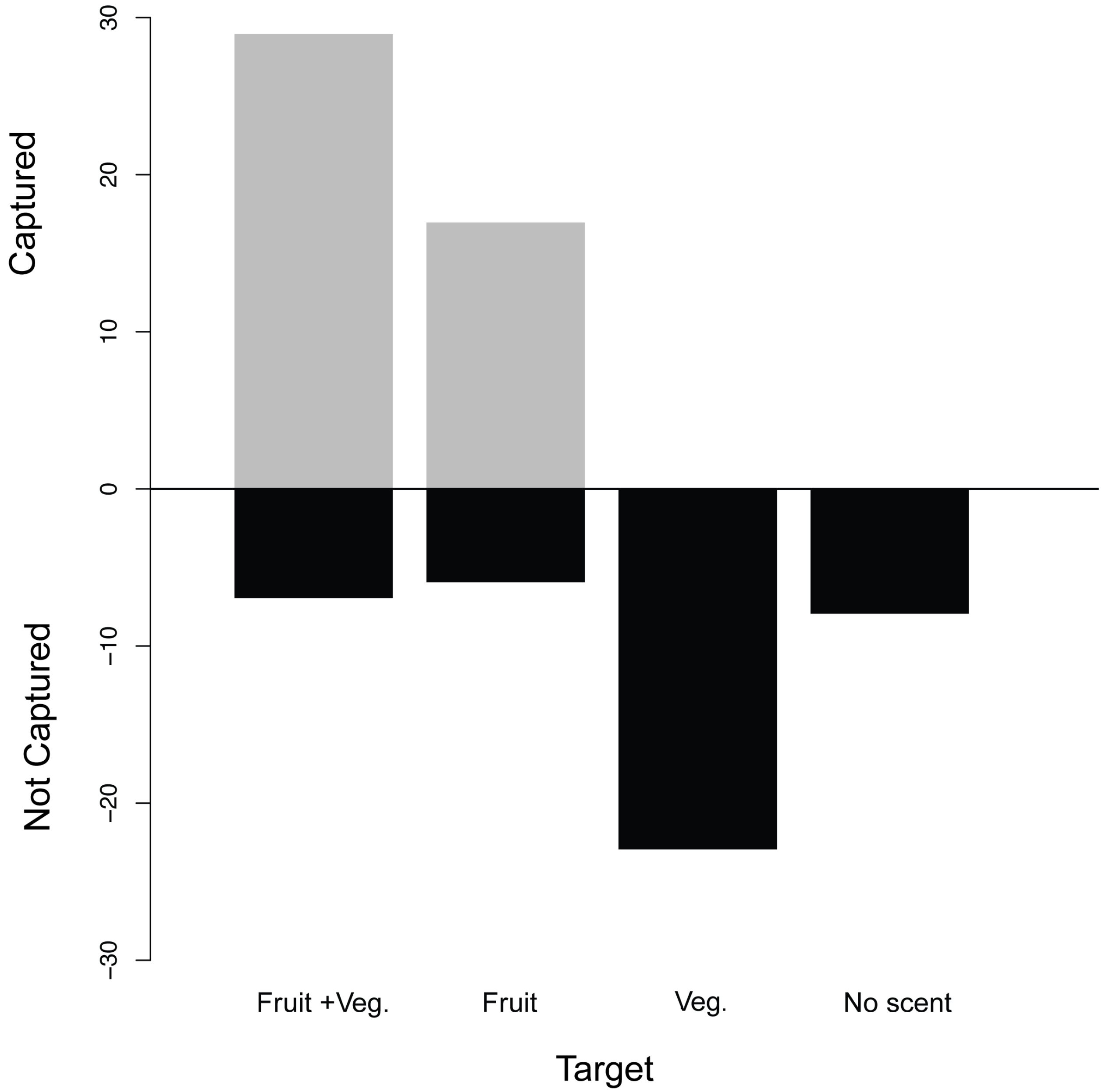

Figure 3.3. Summary of successful target captures by *Carollia castanea* for each of the four target types across behavioral experiments: dummy with fruit scent and vegetation (Fruit + Veg.), dummy with fruit scent only (Fruit), dummy unscented with vegetation only (Veg.), and dummy unscented (No scent)

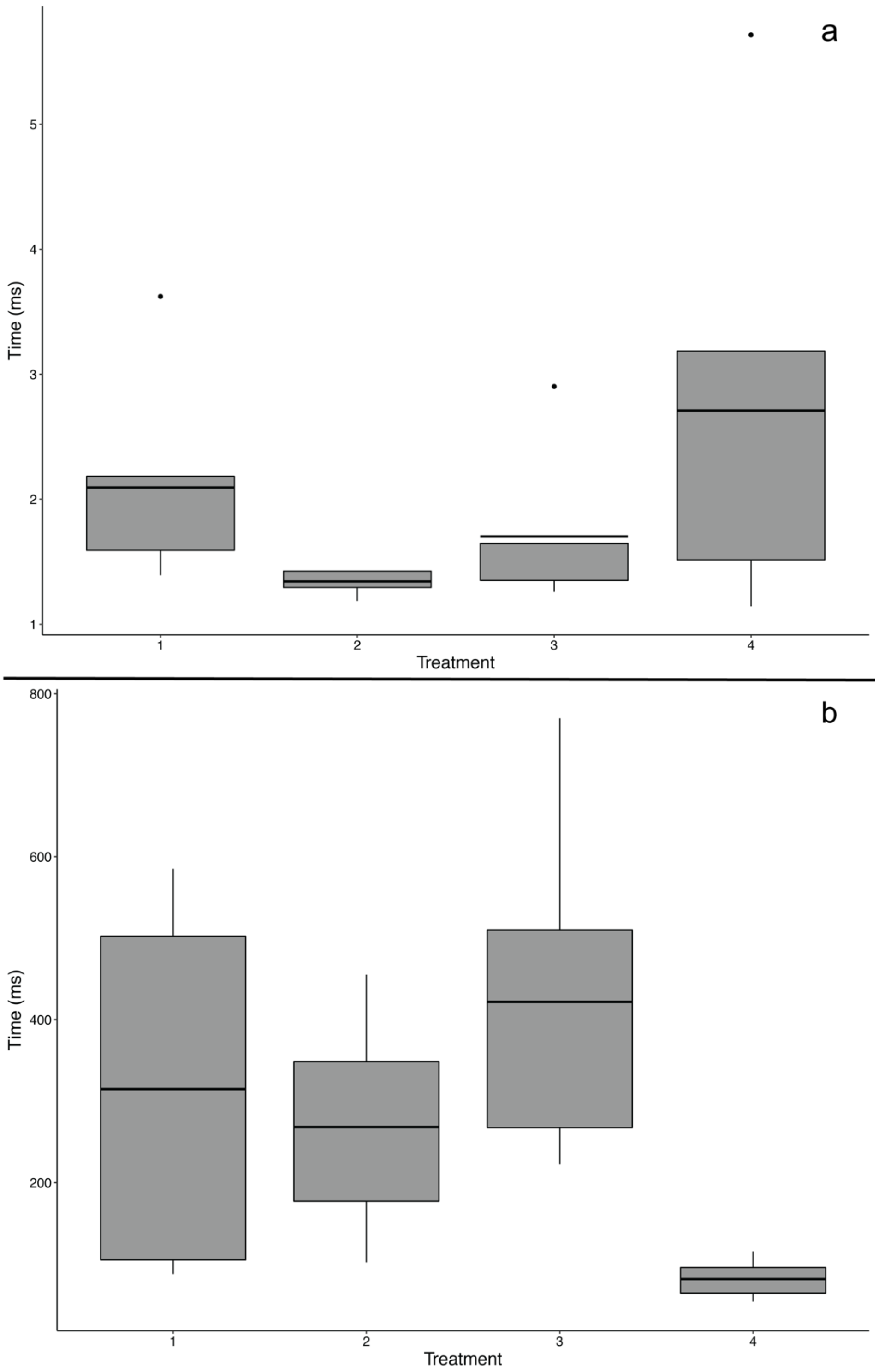

Figure 3.4. Summary of the duration of *Carollia castanea's* echolocation calls across treatments (a), and summary of the interval of *Carollia castanea's* echolocation calls across treatments (b). Treatments are described in Table 1

Table 3.10. Description of the two target choices offered within each experimental treatment, and the response being tested during behavioral experiments on *Carollia castanea*. Number of trials differed among treatments due to differences in the number of positive responses of experimental bats (see Methods)

Treatment	***Choice 1***	***Choice 2***	***Test***	***Number of Trials***
1	Dummy unscented + vegetation	Dummy with fruit scent + vegetation	Preference for fruit scent in the presence of vegetation scent	36
2	Dummy unscented + vegetation	Dummy with fruit scent	Preference between vegetation and fruit scents	23
3	Dummy with fruit scent + vegetation	Dummy with fruit scent	Preference for vegetation scent in the presence of fruit scent	23
4	Dummy unscented	Dummy unscented + vegetation	Preference for vegetation scent	8

Table 3.11. Means (± standard deviation) of echolocation call parameters for *Carollia castanea* during each experimental treatment (from Table 1). Means for the call parameters were calculated by averaging the calls for each individual bat across its entire calls sequence (the approach call for one individual, for one treatment), and averaging each of these values across individuals within each treatment

Treatment	**Duration (ms)**	**Interval (ms)**	**Peak Frequency (kHz)**	**Min. Frequency (kHz)**	**Max. Frequency (kHz)**	**Bandwidth (kHz)**
1	2.09 ± 1.02	314.7 ± 252.8	83.9 ± 3.44	62.5 ± 10.0	108.3 ± 9.131	45.8 ± 15.0
2	1.34 ± 0.0103	268.1 ± 151.5	85.9 ± 6.89	68.0 ± 9.79	111.7 ± 12.54	43.7 ± 19.1
3	1.70 ± 0.0589	421.8 ± 20.63	83.1 ± 5.69	60.7 ± 6.16	111.3 ± 4.531	50.6 ± 4.92
4	2.71 ± 0.206	81.6 ± 26.8	87.2 ± 10.65	64.7 ± 10.7	108.9 ± 11.51	44.2 ± 11.6

Table 3.12. Summary of ANOVA results comparing each call parameter trait across the four experimental treatments (from Table 1).

Variable	**Df**	**Sum. Sq.**	**Mean Sq.**	**F-Value**	**P(>F)**
Duration	1	0.852	0.852	0.728	0.405
Interval	1	1.37	1.37	1.209	0.287
Peak Freq.	1	0.36	0.361	0.194	0.665
Min. Freq.	1	0.00	0.0001	0.00	0.995
Max. Freq.	1	0.00	0.002	0.001	0.975
Bandwidth	1	0.00	0.0004	0.000	0.989

Table 3.13. Volatile organic compounds found in the scent of *Piper scintillans* ripe fruit and vegetation. Values are the mean proportion across all samples (n = 2 fruit; n = 2 vegetation). KRI: Kovats retention indices; IUPAC: International Union of Pure and Applied Chemistry nomenclature

Chemical Name	**IUPAC**	**KRI**	**Class**
3-Hexene-1-ol	(Z)-hex-3-en-1-ol	858	aliphatic alcohol
β-Pinene	6,6-dimethyl-4-methylidenebicyclo[3.1.1]heptane	981	monoterpene
α-Phellandrene	2-methyl-5-propan-2-ylcyclohexa-1,3-diene	1008	monoterpene
3-Carene	4,7,7-trimethylbicyclo[4.1.0]hept-3-ene	1010	monoterpene
α-Terpinene	1-methyl-4-propan-2-ylcyclohexa-1,3-diene	1020	monoterpene
p-Cymene	1-methyl-4-propan-2-ylbenzene	1028	aromatic
β-Ocimene	3,7-dimethylocta-1,3,6-triene	1050	monoterpene
γ-Terpinene	1-methyl-4-propan-2-ylcyclohexa-1,4-diene	1064	monoterpene
Terpinolene	1-methyl-4-propan-2-ylidenecyclohexene	1091	monoterpene
Ethyl benzoate	ethyl benzoate	1175	aromatic
α-Cubebene	4,10-dimethyl-7-(propan-2-yl)tricyclo[4.4.0.$0^{1,5}$]dec-3-ene	1354	sesquiterpene
β-Elemene	2,4-Diisopropenyl-1-methyl-1-vinylcyclohexan	1394	sesquiterpene
α-Cedrene	Cedr-8-ene	1419	sesquiterpene
β-Caryophyllene	4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	1433	sesquiterpene
α-Bergamotene	4,6-dimethyl-6-(4-methylpent-3-enyl)bicyclo[3.1.1]hept-3-ene	1440	sesquiterpene
α-Caryophyllene	2,6,6,9-tetramethylcycloundeca-1,4,8-triene	1470	sesquiterpene
γ-Muurolene	7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene	1485	sesquiterpene
Germacrene D	1-methyl-5-methylidene-8-propan-2-ylcyclodeca-1,6-diene	1495	sesquiterpene
Alloaromadendrene	1,1,7-trimethyl-4-methylidene-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulene	1504	sesquiterpene

Caryophyllene oxide	4,12,12-Trimethyl-9-methylene-5-oxatricyclo[8.2.0.$0^{4,6}$]dodecane	1581	sesquiterpene

SUPPLEMENTARY TABLES & FIGURES

Table S.14.1. Detailed data for specimens included in this study. Locality and specimen numbers are field ids or Slater Natural History Museum numbers. Locality abbreviations are Palo Verde, Costa Rica (PV), Dominican Republic (DR), La Selva, Costa Rica (LS), and Grenada (GR).

Subfamily	**Genus**	**Species**	**Spec. #**	**Locality**
Stenodermatinae	*Artibeus*	*jamaicensis*	022614-25	PV
Stenodermatinae	*Artibeus*	*lituratus*	022614-29	PV
Stenodermatinae	*Artibeus*	*lituratus*	20302	PV
Stenodermatinae	*Brachyphylla*	*nana*	dr856fha	DR
Carollinae	*Carollia*	*perspicillata*	02614-27	PV
Carollinae	*Carollia*	*perspicillata*	022614-34	PV
Phyllostominae	*Chrotopterus*	*auritus*	022614-5	PV
Phyllostominae	*Chrotopterus*	*auritus*	20502	PV
Stenodermatinae	*Dermanura*	*phaeotis*	022614-24	PV
Stenodermatinae	*Dermanura*	*phaeotis*	022714-5	PV
Desmodontinae	*Desmodus*	*rotundus*	82017-01	LS
Desmodontinae	*Desmodus*	*rotundus*	022714-6	PV
Desmodontinae	*Diphylla*	*ecaudata*	022714-4	PV
Phyllonycterinae	*Erophylla*	*bombifrons*	dr1134fha	DR
Glossophaginae	*Glossophaga*	*commisarisi*	82017-02	LS
Glossophaginae	*Glossophaga*	*commisarisi*	030114-5	PV
Glossophaginae	*Glossophaga*	*longirostris*	gr51-2015	GR
Glossophaginae	*Glossophaga*	*soricina*	022814_10	PV
Glossophaginae	*Glossophaga*	*soricina*	30902	PV
Phyllostominae	*Glyphynycteris*	*sylvestris*	022714-1	PV
Glossophaginae	*Hylonycteris*	*underwoodi*	82017-03	LS
Micronycterinae	*Lampronycteris*	*brachyotis*	022614-6	PV
Micronycterinae	*Lampronycteris*	*brachyotis*	0116-2	PV
Micronycterinae	*Lampronycteris*	*brachyotis*	21103	PV
Glossophaginae	*Lichonycteris*	*obscura*	82017-04	LS
Phyllostominae	*Lophostoma*	*silvicolum*	022814-3	PV
Phyllostominae	*Lophostoma*	*silvicolum*	82017-05	LS
Phyllostominae	*Lophostoma*	*silvicolum*	022814_3	PV

Phyllostominae	*Lophostoma*	*brasiliensis*	21604	PV
Phyllostominae	*Lophostoma*	*brasiliensis*	022614-1	PV
Macrotinae	*Macrotus*	*waterhousii*	dr135fha	-
Micronycterinae	*Micronycteris*	*hirsuta*	030214-2	PV
Micronycterinae	*Micronycteris*	*microtis*	022814-1	PV
Micronycterinae	*Micronycteris*	*microtis*	82017-06	LS
Micronycterinae	*Micronycteris*	*microtis*	277689	PV
Micronycterinae	*Micronycteris*	*minuta*	030314-2	PV
Micronycterinae	*Micronycteris*	*minuta*	022814-4	PV
Phyllostominae	*Gardnerycteris*	*crenulatum*	082017-01	LS
Glossophaginae	*Monophyllus*	*redmani*	1086fha	DR
Phyllostominae	*Phyllostomus*	*discolor*	82017-07	LS
Phyllostominae	*Phyllostomus*	*hastatus*	082017-08	LS
Stenodermatinae	*Phyllops*	*falcatus*	1117fha	DR
Phyllonycterinae	*Phyllonycteris*	*poeyi*	dr750fha	DR
Stenodermatinae	*Platyrrhinus*	*helleri*	082017-09	LS
Stenodermatinae	*Sturnira*	*lilium*	278299	PV
Phyllostominae	*Trachops*	*cirrhosus*	022614-17	PV

Table S.15.1. Tukey post-hoc comparisons of results comparing nose leaf, spear, horseshoe, and pinna shape across dietary categories: animalivore, Sanguinivore, frugivore, insectivore, nectarivore, and omnivore.

Structure	**Shape PC**	**Diet**	**Estimate**	**SE**	**T**	**P**
Nose leaf	1	Animalivore	-0.0323	0.0247	-1.31	0.205
		Frugivore	0.0246	0.0302	0.815	0.425
		Insectivore	0.0392	0.0295	1.33	0.199
		Nectarivore	0.0455	0.0295	1.54	0.138
		Omnivore	0.000351	0.0390	-0.009	0.993
	2	Animalivore	-0.00375	0.0119	-0.315	0.756
		Frugivore	0.0329	0.0146	2.26	**0.035***
		Insectivore	-0.00289	0.0142	-0.204	0.841
		Nectarivore	0.00474	0.0142	0.334	0.742
		Omnivore	0.00704	0.0188	0.375	0.712
	3	Animalivore	0.00220	0.0122	0.181	0.859
		Frugivore	0.00746	0.0149	-0.499	0.623
		Insectivore	0.00861	0.0146	0.590	0.562
		Nectarivore	0.00589	0.0146	-0.404	0.690
		Omnivore	0.0215	0.0193	-1.113	0.279
	4	Animalivore	0.00179	0.0118	0.152	0.881
		Frugivore	0.00861	0.0144	0.596	0.558
		Insectivore	-0.0120	0.0141	-0.853	0.404
		Nectarivore	0.00942	0.0141	0.668	0.512
		Omnivore	0.0462	0.0187	2.48	**0.022***
	5	Animalivore	-0.00176	0.0128	-0.137	0.892
		Frugivore	0.0100	0.0157	0.638	0.530
		Insectivore	0.00373	0.0153	0.243	0.810
		Nectarivore	-0.00477	0.0153	-0.312	0.758
		Omnivore	-0.00353	0.0203	-0.174	0.864
Spear	1	Animalivore	-0.00936	0.0335	-0.279	0.783
		Frugivore	-0.0309	0.0380	-0.814	0.425
		Insectivore	-0.00155	0.0380	-0.041	0.968
		Nectarivore	0.0147	0.0380	0.385	0.704

		Omnivore	0.0176	0.0474	0.370	0.715
	2	Animalivore	-0.0375	0.0237	-1.584	0.129
		Frugivore	0.0775	0.0269	2.87	**0.009****
		Insectivore	0.0315	0.0269	1.17	0.255
		Nectarivore	0.0213	0.0269	0.791	0.438
		Omnivore	0.0721	0.0335	2.15	**0.0438***
	3	Animalivore	-0.0217	0.0209	-1.037	0.312
		Frugivore	0.0268	0.0237	1.13	0.271
		Insectivore	0.0209	0.0237	0.882	0.388
		Nectarivore	0.0281	0.0237	1.18	0.251
		Omnivore	0.00447	0.0296	0.151	0.882
	4	Animalivore	0.0173	0.0205	0.846	0.408
		Frugivore	-0.0329	0.0232	-1.42	0.171
		Insectivore	-0.0237	0.0232	-1.02	0.321
		Nectarivore	-0.0199	0.0232	-0.855	0.403
		Omnivore	-0.0228	0.0289	-0.785	0.442
	5	Animalivore	0.000568	0.0161	0.035	0.972
		Frugivore	-0.0236	0.0183	-1.29	0.212
		Insectivore	0.00221	0.0183	0.121	0.905
		Nectarivore	-0.00265	0.0183	-0.145	0.886
		Omnivore	-0.0385	0.0228	-1.69	0.107
Horseshoe	1	Animalivore	0.0575	0.0319	1.80	0.0841
		Sanguinivore	-0.0815	0.0504	-1.62	0.119
		Frugivore	-0.0319	0.0374	-0.855	0.401
		Insectivore	-0.0734	0.0381	-1.93	0.0662
		Nectarivore	-0.0580	0.0374	-1.55	0.134
		Omnivore	0.0509	0.0504	1.01	0.323
	2	Animalivore	-0.0198	0.0206	-0.966	0.343
		Sanguinivore	-0.0129	0.0325	-0.396	0.696
		Frugivore	0.0121	0.0241	0.505	0.618
		Insectivore	0.0322	0.0246	1.31	0.202
		Nectarivore	0.0424	0.0241	1.76	0.0913
		Omnivore	0.00570	0.0325	0.175	0.862
	3	Animalivore	-0.00583	0.0171	-0.340	0.736

		Sanguinivore	-0.00615	0.0271	-0.277	0.822
		Frugivore	0.0243	0.0201	1.21	0.239
		Insectivore	0.00984	0.0205	0.481	0.635
		Nectarivore	0.00479	0.0201	0.239	0.813
		Omnivore	0.0732	0.0271	2.70	**0.0124***
	4	Animalivore	-0.00104	0.0112	-0.094	0.926
		Sanguinivore	-0.00747	0.0177	-0.423	0.676
		Frugivore	0.0125	0.0121	0.952	0.350
		Insectivore	-0.00734	0.0134	-0.549	0.588
		Nectarivore	0.00106	0.0131	0.081	0.936
		Omnivore	0.0312	0.0177	1.77	0.090
	5	Animalivore	0.0217	0.0122	1.78	0.0882
		Sanguinivore	-0.0203	0.0193	-1.05	0.303
		Frugivore	-0.0344	0.0143	-2.40	**0.0243***
		Insectivore	-0.0219	0.0146	-1.50	0.146
		Nectarivore	-0.0315	0.0143	-2.20	**0.0379***
		Omnivore	-0.0391	0.0193	-2.03	0.0541
Pinna	1	Animalivore	-0.0577	0.0320	-1.80	0.0916
		Sanguinivore	0.108	0.0453	2.38	**0.0313***
		Frugivore	0.0751	0.0392	1.92	0.0747
		Insectivore	0.0219	0.0358	0.610	0.551
		Nectarivore	0.0753	0.0413	1.82	0.0885
		Omnivore	0.104	0.0453	2.30	**0.0360***
	2	Animalivore	0.0205	0.0208	0.986	0.340
		Sanguinivore	-0.0500	0.0294	-1.70	0.109
		Frugivore	-0.0214	0.0254	-0.841	0.413
		Insectivore	-0.0184	0.0232	-0.793	0.440
		Nectarivore	-0.00742	0.0268	-0.277	0.786
		Omnivore	-0.00938	0.0294	-0.320	0.754
	3	Animalivore	-0.0256	0.0196	-1.31	0.210
		Sanguinivore	0.0405	0.0277	1.46	0.164
		Frugivore	0.00637	0.0240	0.266	0.794
		Insectivore	0.0279	0.0219	1.27	0.222
		Nectarivore	0.0443	0.0253	1.75	0.0998

		Omnivore	-0.00361	0.0277	-0.130	0.898
	4	Animalivore	0.00111	0.0251	0.044	0.965
		Sanguinivore	-0.0144	0.0355	-0.406	0.691
		Frugivore	0.0130	0.0307	0.422	0.679
		Insectivore	-0.0108	0.0281	-0.383	0.707
		Nectarivore	0.0199	0.0324	0.616	0.547
		Omnivore	0.0441	0.0355	1.24	0.233

Table S.16.1. Phylogenetic ANOVA results comparing nose leaf, spear, horseshoe, and pinna shape across dietary categories: non-mobile, non-evasive prey and mobile, evasive prey.

Structure	**Shape PC**	**df**	**SS**	**MS.**	**F**	**P**
Nose leaf	1	2	0.00236	0.00118	0.63	0.542
	2	2	0.00222	0.00111	2.21	0.134
	3	2	0.00167	0.00083	2.01	0.157
	4	2	0.00541	0.00270	6.87	**0.005****
	5	2	0.00006	0.00003	0.067	0.936
Spear	1	2	0.00236	0.00118	0.63	0.542
	2	2	0.00027	0.00013	0.15	0.856
	3	2	0.00144	0.000723	0.82	0.454
	4	2	0.00643	0.003216	3.012	0.074
	5	2	0.000064	0.000032	0.067	0.936
Horseshoe	1	1	0.00101	0.001009	0.262	0.162
	2	1	0.00001	0.0000141	0.01	0.921
	3	1	0.000871	0.0008714	0.806	0.377
	4	1	0.001112	0.0011124	2.783	0.106
	5	1	0.00191	0.0019104	4.399	**0.0451***
Pinna	1	2	0.02565	0.012827	6.963	**0.0057 ****
	2	2	0.000489	0.0002444	0.281	0.758
	3	2	0.001503	0.0007517	0.861	0.439
	4	2	0.005054	0.002527	2.208	0.139

Table S.17.1. List of individual bats used in experiments and associated biometric data: M(male), F(female), A (adult), NP-NL (Not pregnant, not lactating), NS (non-scrotal)

No.	Species	ID	Sex	Age	Rep. Cond.	Mass (g)	FA (mm)
1	Carollia castanea	083016_1	F	A	NP-NL	14	37.3
2	Carollia castanea	083016_2	M	A	NS	12	35.8
3	Carollia castanea	083016_3	M	A	NS	11	37.4
4	Carollia castanea	083116_1	M	A	NS	14	37.9
5	Carollia castanea	083116_2	M	A	NS	12	36.1
6	Carollia castanea	083116_3	F	A	NP-NL	14	37.1
7	Carollia castanea	090116_2	M	A	NS	13	36.8
8	Carollia castanea	090116_3	M	A	NS	14	37.6
9	Carollia castanea	090816_2c	M	A	NS	14	36.7
10	Carollia castanea	090816_3c	M	A	NS	13	36.3
11	Carollia castanea	090816_4c	F	A	NP-NL	14	36.5
12	Carollia castanea	090816_5c	M	A	NS	11	36
13	Carollia castanea	090816_6c	M	A	NS	11	36.2
14	Carollia castanea	090916_1	F	A	NP-NL	14	38.7
15	Carollia castanea	090916_2	M	A	NS	14	37.1
16	Carollia castanea	090916_3	M	A	NS	11	37.2
17	Carollia castanea	090916_5	M	A	NS	12	36.2
18	Carollia castanea	091016_1	F	A	NP-NL	12	36.2
19	Carollia castanea	091016_2	M	A	NS	13	37.2
20	Carollia castanea	091016_4	M	A	NS	14	37.1
21	Carollia castanea	091216_3	M	A	NS	10	36.15

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VITA

EDUCATION

University of Washington — Seattle, WA
Ph.D. Candidate, Department of Biology — 2013-Present
Advisor: Dr. Sharlene Santana

University of California — Santa Cruz, CA
B.S. Marine Biology — July, 2012

PUBLICATIONS

Leiser-Miller. L. B., Kaliszewska, Z.A., Villalobos-Chaves, D., and Santana, S.E. Cheating the mutualistic contract?. Frontiers EcoPics.

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