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

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**Abstract**

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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  $\beta$ -caryophyllene, germacrene D and  $\beta$ -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

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

29

## 30 1.2 INTRODUCTION

31

32 Echolocation is critical to bats and other animals to perform tasks such as spatial orientation,  
33 navigation, social communication, and foraging (Norris et al. 1961, Møhl et al. 2003, Schnitzler  
34 et al. 2003, Thomas and Jalili 2004, Brinkløv et al. 2013, Jung et al. 2014). Because the functions  
35 of echolocation are diverse, various factors have been associated with the diversity of  
36 echolocation call structure across animals, including phylogeny, social communication  
37 (including mate choice, individual recognition, and distress signaling), diet, and habitat (Nyby  
38 and Whitney 1978, Wilkinson and Wenrick Boughman 1998, Lammers et al. 2003, Russ et al.  
39 2005, Voigt-Heucke et al. 2010, Jones and Siemers 2011, Schuchmann et al. 2012, Jung et al.  
40 2014, Puechmaille et al. 2014). Among these factors, foraging ecology (e.g., foraging habitat and  
41 diet) seems to be a strong predictor of call structure in bats (Jones 1999). However, assessments  
42 of call structure differences across guilds are usually based on broadly defined foraging  
43 categories (i.e., aerial-hawking versus gleaning bats; Jones 1999) comparing ecologically distinct  
44 families. Furthermore, the call characteristics that are typically compared, such as the distinction  
45 between constant-frequency (CF) or frequency-modulated (FM) calls, represent coarse  
46 assessments of echolocation calls. Less is known about call structure differences on a fine-

47 resolution scale within families of bats, particularly for families that are ecologically diverse  
48 and/or have calls that are difficult to record (e.g., ‘whispering’ or high-flying bats).

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

64         Broadly, phyllostomid species are narrow-space foragers that primarily hunt in the forest  
65 understory or canopy (Wilson and Reeder 2005), thus their main echolocation task is short-range  
66 detection in a highly cluttered acoustic space (e.g., overcoming acoustic masking echoes from  
67 foliage and other obstacles, Schnitzler and Kalko 2001). Traditionally, they have been  
68 considered ‘whispering’ bats because they typically emit calls at lower intensities than species in  
69 other bat families (Griffin 1958), but research shows that some species may be capable of calling

70 at louder intensities than previously thought (Brinkløv et al. 2009). Even so, knowledge about  
71 phyllostomid echolocation calls is still poor because of limitations associated with recording  
72 these low-intensity, high-frequency calls in the hot, humid and densely forested environments  
73 where most species live (Griffin 1971). Given these constraints, phyllostomid bats are  
74 underrepresented in acoustic comparative studies, and previous, largely qualitative, research has  
75 deemed phyllostomid calls as relatively uniform in structure across species (Brigham, Kalko,  
76 Jones, Parsons, & Limpens, 2002). Quantitative analyses of larger datasets, consistently collected  
77 in terms of recording environments, equipment used, and methods for call extraction, thus have  
78 the potential to reveal that phyllostomid calls are more variable than previously thought.  
79 Interestingly, phyllostomids seem to deviate from the allometric patterns in some call parameters  
80 that are exhibited by other taxa (e.g., bats; Hipposideridae, Rhinolophidae, Emballonuridae,  
81 Vespertilionidae, and Molossidae, Jones 1999; frogs, Ryan, 1985; birds, Martin, Doucet, Knox,  
82 & Mennill, 2011; Ryan & Brenowitz, 1985). This suggests that phylogeny and dietary ecology  
83 may contribute to echolocation call diversity in phyllostomids (Jacobs et al. 2007).

84 Echolocation call parameters have specific functions in shaping an acoustic field of view.  
85 Frequency is particularly important for encoding audible echo reflection (Møhl, 1988; Pye,  
86 1993), range accuracy (Stamper et al. 2009), and for detecting targets against forest clutter (Bates  
87 et al. 2011). For the detection of a specific object, such as a prey item, acoustic theory predicts  
88 that spheres reflect weak echoes if their circumference is smaller than the wavelength of the  
89 impinging sound (Pye, 1993). Ensonification experiments further suggest that insects reflect  
90 sound in a similar way to spheres, and therefore bats must use high frequencies (short  
91 wavelengths) to obtain an audible echo from small insects (Møhl 1988, Safi and Siemers 2009).  
92 Previous work has demonstrated a relationship between emitted call frequency and prey size for

93 some bat species (Vespertilionidae), corroborating the hypothesis that call frequency and prey  
94 size can be functionally linked (Houston et al. 2004). To date, it is unknown if this basic  
95 relationship exists for phyllostomids bats.

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

111

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## 115 1.3 MATERIALS AND METHODS

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### 117 1.3.1 *Acoustics*

118

119 We used mist nets to collect free-ranging bats in Palo Verde National Park, Guanacaste,  
120 Costa Rica and La Selva Biological Station, Sarapiquí, Costa Rica from 2015 to 2018, through  
121 the months of January – March, and July – December. We recorded release calls from 153  
122 individuals spanning 21 genera and 35 species (Table 3) using a microphone condenser  
123 (UltraSoundGate 116) at 375 kHz sampling rate and 16-bit recording. We held each bat in hand,  
124 placed a microphone approximately six inches from its face, and then released the bat away from  
125 environmental clutter while recording the calls emitted as the individual flew away. We  
126 measured call parameters for 2-11 individuals per species, with the exception of species that  
127 were rare or difficult to capture at our study localities (*Chrotopterus auritus*, *Glyphonycteris*  
128 *sylvestris*, *Phyllostomus hastatus*, *Glossophaga leachii*, and *Mesophylla macconnelli*), for which  
129 we only recorded one individual per species (Table 3). All collecting and handling procedures  
130 were approved by the University of Washington’s Institutional Animal Care and Use Committee  
131 (protocol# 4307-01).

132 We analyzed release calls using Avisoft SASLabPro v. 5.2.12 (Avisoft Bioacoustics,  
133 Berlin, Germany). To optimize both frequency and temporal resolution, we set the frequency  
134 resolution parameters for the spectrogram at a fast Fourier transform (FFT; Brigham, 1988)  
135 length of 256, 100% frame size, with a flattop window. We also set the temporal resolution for  
136 the spectrogram with a window overlap of 93.75%. We then set the automatic measurements  
137 algorithm to take measurements of call duration, peak frequency, maximum frequency, minimum

138 frequency, bandwidth, and number of harmonics at appropriate locations for each call within  
139 each file (Figure 1). We manually inspected each call classified by the automatic measurements  
140 algorithm to ensure accuracy in element detection. In the case that ultrasonic background noise  
141 above -20 dB was influencing measurements, we manually erased this noise from the  
142 spectrogram and recalculated measurements. To further reduce the influence of high-intensity,  
143 low-frequency sounds generated by background noise, we filtered all call files with a high-pass  
144 band filter set at 20 kHz, with the exception of the calls of *Phyllostomus hastatus*. This species  
145 had calls with a lower minimum frequency than other phyllostomids, so we set a high-pass band  
146 filter at 10 kHz. To determine valid calls in a recorded file, we set element separation at a hold  
147 time of 2 ms (i.e., within the range of call duration for phyllostomids, Kalko and Condon 1998;  
148 Thies et al. 1998; Brigham et al. 2002; Jennings et al. 2004; Weinbeer and Kalko 2007), with the  
149 exception of *Centurio senex*, for which we set a hold time of 10 ms because of the extended  
150 duration of this species' call.

151 We averaged call sequences per individual (a minimum of 5 calls per file) and calculated  
152 means and standard deviations of each measured parameter. We also report the range of each call  
153 parameter in the form of the maximum and minimum value recorded (Table 3). To estimate the  
154 minimum prey size detectable given an emitted frequency, we used the following equation (Yang  
155 2010):

$$156 \quad \lambda = v/f \quad (1.1)$$

157  
158 Where  $\lambda$  is the wavelength,  $v$  is the wave velocity (speed of sound in air), and  $f$  is the frequency.

159 For speed of sound, we used the speed 347 m/s for 25°C and 68% humidity, which reflects



160 average annual environmental conditions of the sites where we recorded calls (sourced from  
161 worldweatheronline.com).

162

### 163 1.3.2 *Statistical Analyses*

164 First, we tested each call parameter for phylogenetic signal using a pruned version of the  
165 Rojas, Warsi, & Dávalos (2016) phylogeny. For duration, maximum frequency, minimum  
166 frequency, peak frequency, bandwidth, and harmonics, we calculated both Blomberg's K  
167 (Blomberg et al. 2003) and Pagel's lambda (Pagel 1992, Freckleton et al. 2004) in R version  
168 5.3.0 (R Core Team 2018) using the function *phylosig* from the package 'phytools' version 0.6-  
169 60 (Revell 2012).

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

176 To test if specific call parameters are associated with dietary guild, we grouped species  
177 into one of six taxonomic dietary categories (animalivores, insectivores, nectarivores, frugivores,  
178 omnivores, and sanguinivores; Giannini & Kalko, 2004). However, accumulating research  
179 suggests that many species of phyllostomids may not be as restricted to a specific dietary guild.  
180 Instead, many species may be opportunistic feeders with a broader trophic breadth than  
181 traditionally described (Giannini & Kalko, 2004). For this reason, we also tested for differences  
182 in call structure between two "functional" dietary guilds: predators of non-mobile, non-evasive

183 prey (nectarivores, frugivores, and sanguinivores), and predators of mobile, evasive prey  
184 (omnivores, insectivores, and animalivores). To identify call parameters that differentiate  
185 echolocation calls between dietary guilds, we conducted a Linear Discriminant Analysis (LDA)  
186 using the function `lda` from the R package ‘MASS’ version 7.3- 49 (Hulsey et al. 2007). We then  
187 used the model derived from the LDA to test if echolocation call traits are predictors of a  
188 species’ dietary guild. We used the function `sim.char` from the R package ‘geiger’ version 2.0.6.1  
189 (Harmon et al. 2019), to simulate the evolution of call parameters on the phylogeny for each  
190 level of dietary assignment (i.e., taxonomic categories and functional guilds), and we tested if  
191 differences in call parameters are greater than expected under a Brownian motion model of trait  
192 evolution. Finally, to test for differences in minimum detectable prey size among taxonomical  
193 and functional dietary guilds, we performed phylogenetic ANOVAs using the function `aov.phyl`  
194 from the R package ‘geiger’ version 2.0.6.1 (Harmon et al. 2019).

195

## 196 1.4 RESULTS

### 197 1.4.1 *Phylogenetic signal and scaling of call parameters*

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

203 We found a significant relationship between forearm length and maximum echolocation  
204 call frequency (PGLS;  $p = 0.007$ ,  $R^2 = 0.2$ ; Figure 3), but this proxy for body size was not a

205 significant predictor of call duration, minimum frequency, peak frequency, number of  
206 harmonics, or sweep rate.

207

#### 208 1.4.2 *Discrimination of call structure among dietary guilds*

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

226 When functionally-defined guilds are considered, bandwidth has a strong positive loading  
227 on the first discriminant axis (+LD1) and a strong negative relationship with number of

228 harmonics (-LD1, Figure 4B). This axis largely separates species feeding on non-mobile/non-  
229 evasive prey (+LD1) from species feeding on mobile/evasive prey (-LD1, Figure 4B). LDA  
230 predictions correctly assigned 77.1% of species to the correct functionally-defined dietary guild  
231 ( $P = 0.02$ ).

232

### 233 1.4.3 *Minimum detectable prey size*

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

249

## 250 1.5 DISCUSSION

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

264 We found a weak yet significant negative relationship between maximum call frequency  
265 and body size. Given that maximum frequency defines the upper limit of echolocation call  
266 capability, this result can be explained by a known relationship in which an increase in the linear  
267 size of structures associated with sound production results in lower produced call frequencies  
268 (Pye, 1979). However, we did not find any scaling relationship between any other call parameter  
269 and body size. Therefore, our results largely corroborate Jones' (1999) findings in that  
270 phyllostomids diverge from the allometric pattern found in other bat families. Other  
271 morphological features, such as vocal tract geometry (Hartley and Suthers 1988, Neuweiler  
272 2000) or nose leaf morphology (Hartley and Suthers 1987; Vanderelst et al. 2010; Leiser-Miller

273 and Santana, *in revision*) might be better predictors of emitted frequency than body size in  
274 phyllostomids, as the geometry of sound producing structures can also influence the frequency  
275 emitted (Hartley and Suthers 1988, Neuweiler 2000, Jakobsen et al. 2013b). For example,  
276 accessory nasal chambers along the vocal tract in Rhinolophidae absorb specific frequencies,  
277 resulting in a filtered emitted call (Neuweiler 2000). Moreover, given that phyllostomids use  
278 frequency modulated calls, and can exploit a wide range of frequencies, perhaps this relaxes  
279 constraint on call parameters and some of these (e.g., maximum frequency) may be more  
280 constrained by the physical limitations of sound production, while others (e.g., peak frequency)  
281 may be more plastic to match tasks associated with foraging habitat or prey detection (Jacobs et  
282 al. 2007).

283         Consistent with our predictions, both taxonomic and functional dietary guilds differ in  
284 some major parameters that define echolocation call structure. Call parameters were more  
285 effective at predicting functionally-defined dietary guilds than taxonomically-defined guilds;  
286 however, there was some overlap among guilds. This suggests that call structure may be  
287 specialized for diet to some extent, and that call parameters may be more reflective of  
288 specialization associated with specific foraging behaviors necessary to capture the different prey  
289 types. For instance, higher call frequencies reduce detection distance (e.g., species searching  
290 along leaf clutter for insects, *Micronycteris microtis*; Geipel et al. 2013) but allow perception of  
291 smaller prey (e.g., detection of small insects, fruits, or flowers). Conversely, lower frequencies  
292 allow for detection over longer ranges, but provide less resolution, which is only suitable for  
293 detecting larger prey (Neuweiler 2000, Brock Fenton et al. 2016). Based on our findings, these  
294 functional requirements and tradeoffs on echolocation parameters may be more influential than  
295 simple prey taxonomy in call evolution. Even so, some species have diverged in the call structure

296 that would be predicted for their dietary guild. This interesting finding suggests that more  
297 detailed, quantitative studies of foraging behavior and diet are still needed to further elucidate the  
298 relationship between call structure and dietary ecology in phyllostomids.

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

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

316 Acoustic detection of preferred prey size is constrained by wavelength and has only been  
317 studied in a few bat species (Houston et al. 2004). Houston et al. (2004) found that species  
318 emitting the highest frequencies (shortest wavelengths) fed on the smallest insects. However, the

319 species that emitted the lowest frequencies (longest wavelengths) fed on insects that were  
320 smaller than predicted by wavelength alone. We calculated the minimum detectable prey size  
321 across phyllostomid species and found no significant differences among guilds when phylogeny  
322 is considered, but some guilds do exhibit greater variance than others in minimum detectable  
323 prey size (frugivores and insectivores, calculated using emitted peak frequency). Frugivory and  
324 insectivory are the dominant and most speciose dietary guilds within Phyllostomidae (Wilson  
325 and Reeder 2005), therefore a greater variance in detectable prey size may reflect both their  
326 phylogenetic (species) and ecological diversity. The variance in echolocation call design within  
327 guilds can further reflect dietary adaptation and niche partitioning through sensory biases. For  
328 instance, small differences in vespertilionid bats' (insectivores) echolocation call structure has  
329 been shown to contribute to niche differentiation within guilds (Siemers and Schnitzler 2004a,  
330 Siemers and Swift 2005). Further investigation is needed to determine if phyllostomid  
331 echolocation signals actually reflect fine-resolution differences in acoustic niches among species.

332         Phyllostomids have evolved other sensory specializations beyond echolocation, which  
333 they also use for prey detection. For example, *Desmodus rotundus* (sanguivore) uses infrared  
334 sensing pits to sense warm mammals (Jones et al. 2013) and *Trachops cirrhosus* and other  
335 animalivorous species use passive hearing to detect prey (Kalko et al. 1999). Many plant-eating  
336 and omnivorous species use olfaction and vision and rely on multimodal sensing approach for  
337 prey detection (Bell and Fenton 1986, Thies et al. 1998, Kalko and Condon 1998, Neuweiler  
338 2000, Korine and Kalko 2005). Alternative or complementary sensory modalities are expected to  
339 relax selection on echolocation call specialization, but it is still poorly understood how  
340 multimodal sensing plays into unique foraging scenarios in phyllostomids. Even though these  
341 bats are diverse in their sensory abilities, there is growing experimental evidence that



342 phyllostomid species across different dietary guilds use echolocation to find prey (Geipel, Jung,  
343 & Kalko, 2013; Gonzalez-Terrazas et al., 2016; Kalko & Condon, 1998; Thies et al., 1998).  
344 Therefore, the evolution of echolocation in the context of the phyllostomid dietary radiation  
345 likely involves a nuanced and complex interaction with the evolution of other sensory modalities.

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

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

365 echolocation calls of a representative sample of phyllostomids is valuable for future studies  
366 aiming to understand the diversity and functionality of bat echolocation systems.

367

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372 commitment to this project: Alvaro Cerdas Cedeño, Luis Girón, Kathe Ela, Sergio Padilla  
373 Alvarez, and David Villalobos-Chaves.

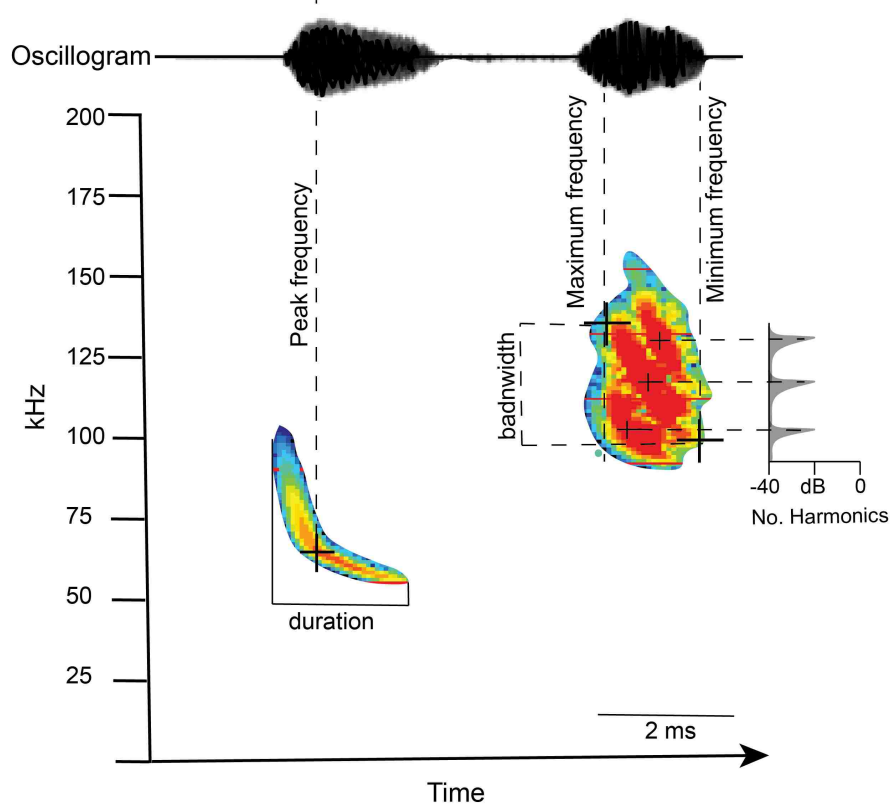
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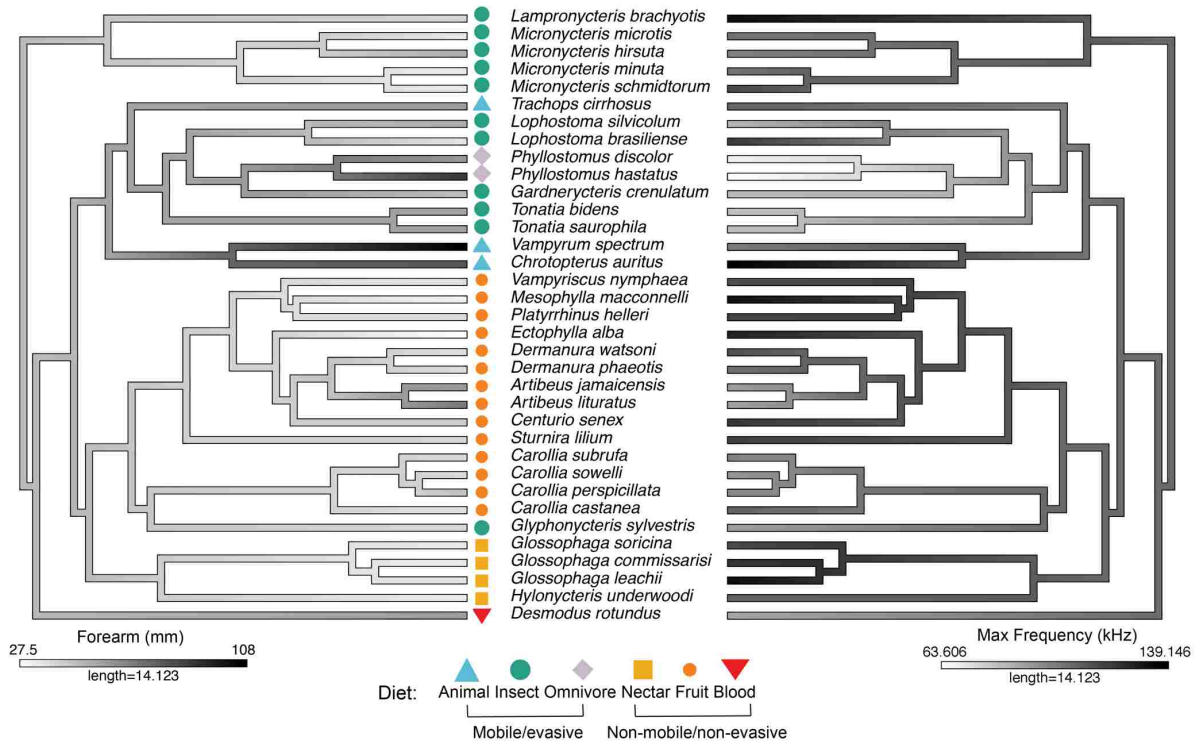
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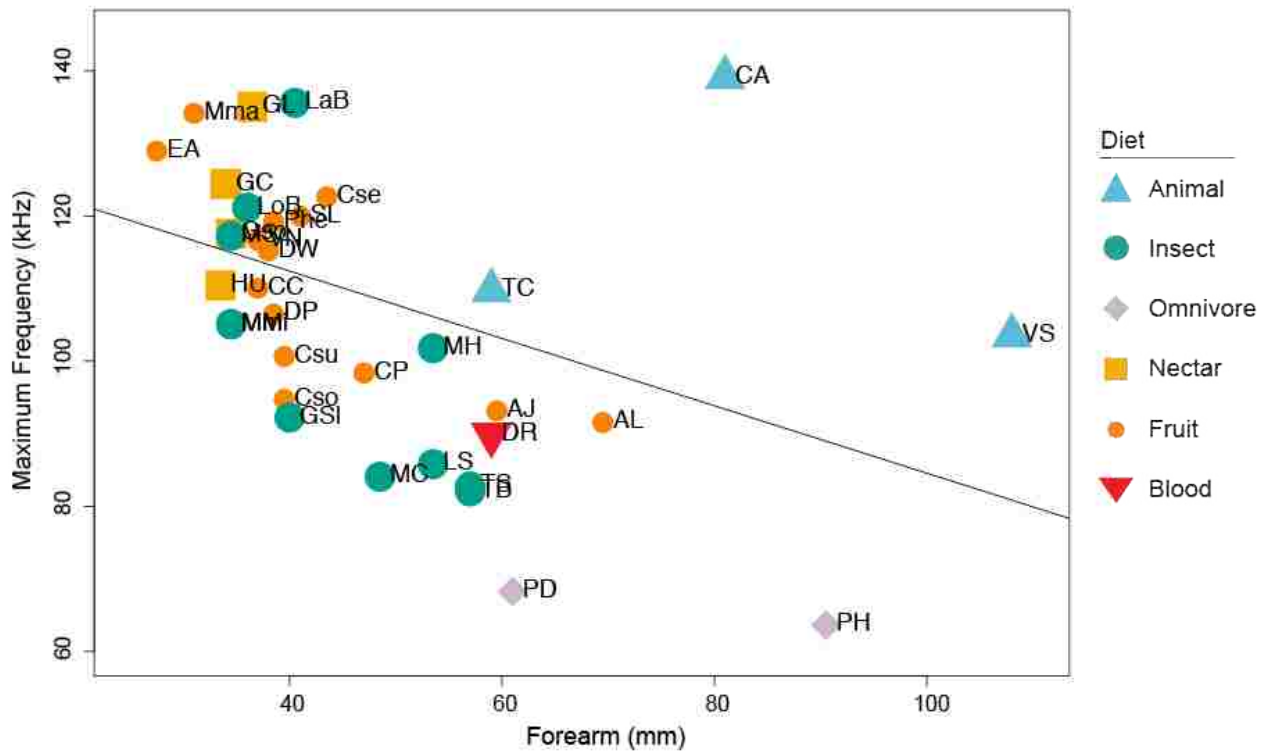


379  
 380 Figure 1.1. Schematic of spectrogram of *Hylonycteris underwoodi* (left) and *Platyrhinus helleri*  
 381 (right) illustrating measurement points of echolocation call parameters used in the analyses.  
 382 Oscillogram (top) represents amplitude of calls. Duration of the call is calculated as the length of  
 383 the call at an amplitude above -20dB relative to the maximum amplitude of the call, maximum  
 384 frequency is taken at maximum amplitude at start of call, minimum frequency is taken at  
 385 maximum amplitude at end of call, peak frequency is the maximum frequency over the entire  
 386 call, bandwidth is calculated as the difference between the maximum and minimum frequency  
 387 over the entire call, and number (No.) of harmonics is taken as the number of peaks with  
 388 amplitude greater than -20dB relative to the maximum amplitude of the individual spectra  
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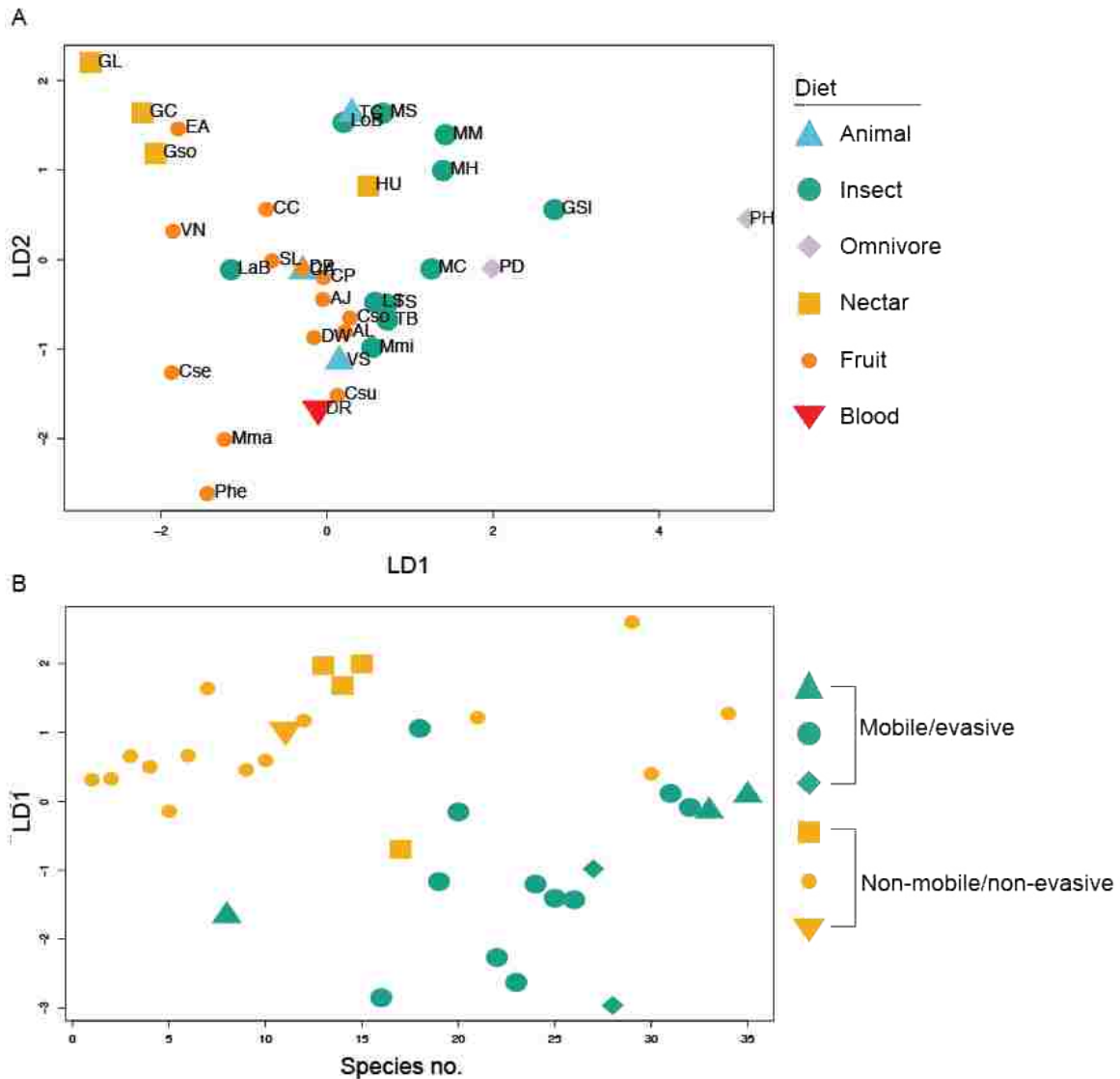


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Figure 1.2. Forearm (left) and maximum frequency (right) reconstructed on the Phyllostomidae phylogeny using function in the ‘phytools’ package (Revell 2012) in R studio version 3.5.0 (R Core Team 2018). Taxonomic and functional dietary guilds denoted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

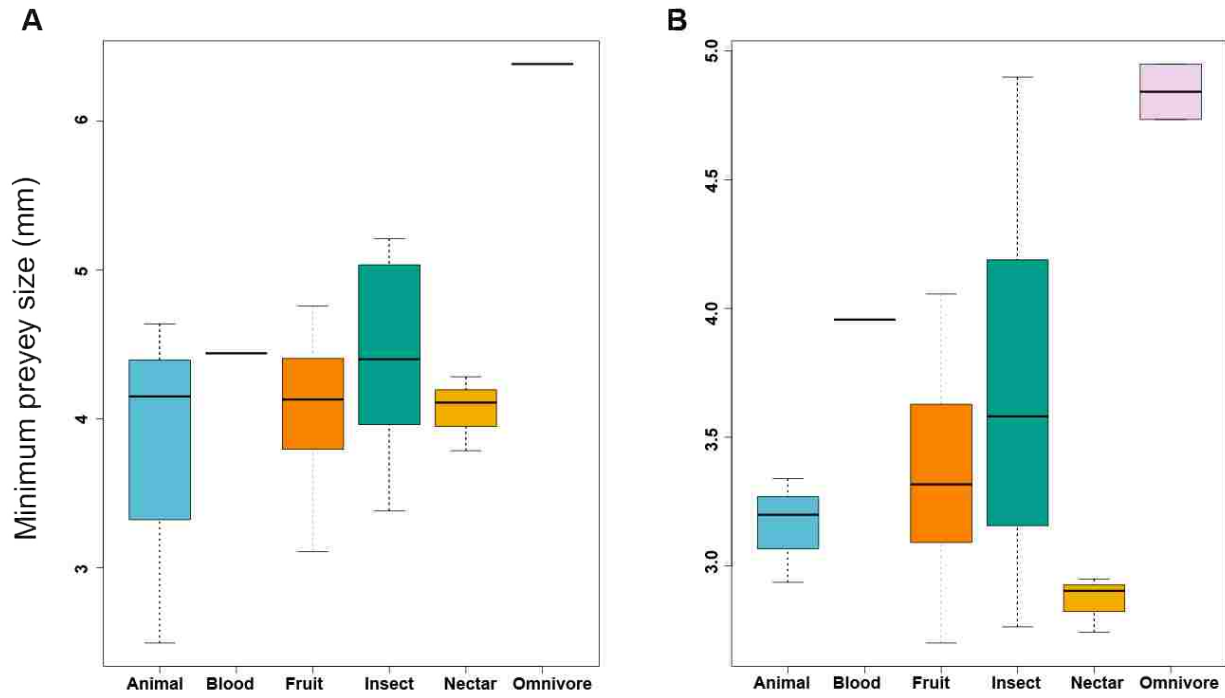


396  
 397 Figure 1.3. Phylogenetic generalized least squares regression of maximum frequency and  
 398 forearm length,  $y = -0.4766 + 129.24x$ ,  $R^2 = 0.2$ . Species codes are listed as AJ-*Artibeus*  
 399 *jamaicensis*, AL -*Artibeus lituratus*, CC- *Carollia castanea*, CP- *Carollia perspicillata*, Cso -  
 400 *Carollia sowelli*, Csu -*Carollia subrufa*, Cse – *Centurio senex*, CA -*Chrotopterus auritus*, DP-  
 401 *Dermanura phaeotis*, DW- *Dermanura watsonii*, DR -*Desmodus rotundus*, EA-*Ectophylla alba*,  
 402 GC-*Glossophaga commissarisi*, GL-*Glossophaga longirostris*, Gso-*Glossophaga soricina*, GSI-  
 403 *Glyphonycteris sylvestris*, HU-*Hylonycteris underwoodi*, LaB-*Lamproncycteris brachyotis*, LoB-  
 404 *Lophostoma Brasiliense*, LS-*Lophostoma silvicolum*, Mma-*Mesophylla macconnelli*, MH-  
 405 *Micronycteris hirsute*, MM-*Micronycteris microtis*, Mmi-*Micronycteris minuta*, MS-  
 406 *Micronycteris schmidtorum*, MC-*Gardnerycteris crenulatum*, PD-*Phyllostomus discolor*, PH-  
 407 *Phyllostomus hastatus*, Phe-*Platyrrhinus helleri*, SL-*Sturnira lilium*, TB-*Tonatia bidens*, TS-  
 408 *Tonatia saurophila*, TC-*Trachops cirrhosis*, VN-*Vampyriscus nymphaea*, VS-*Vampyrum*  
 409 *spectrum*  
 410

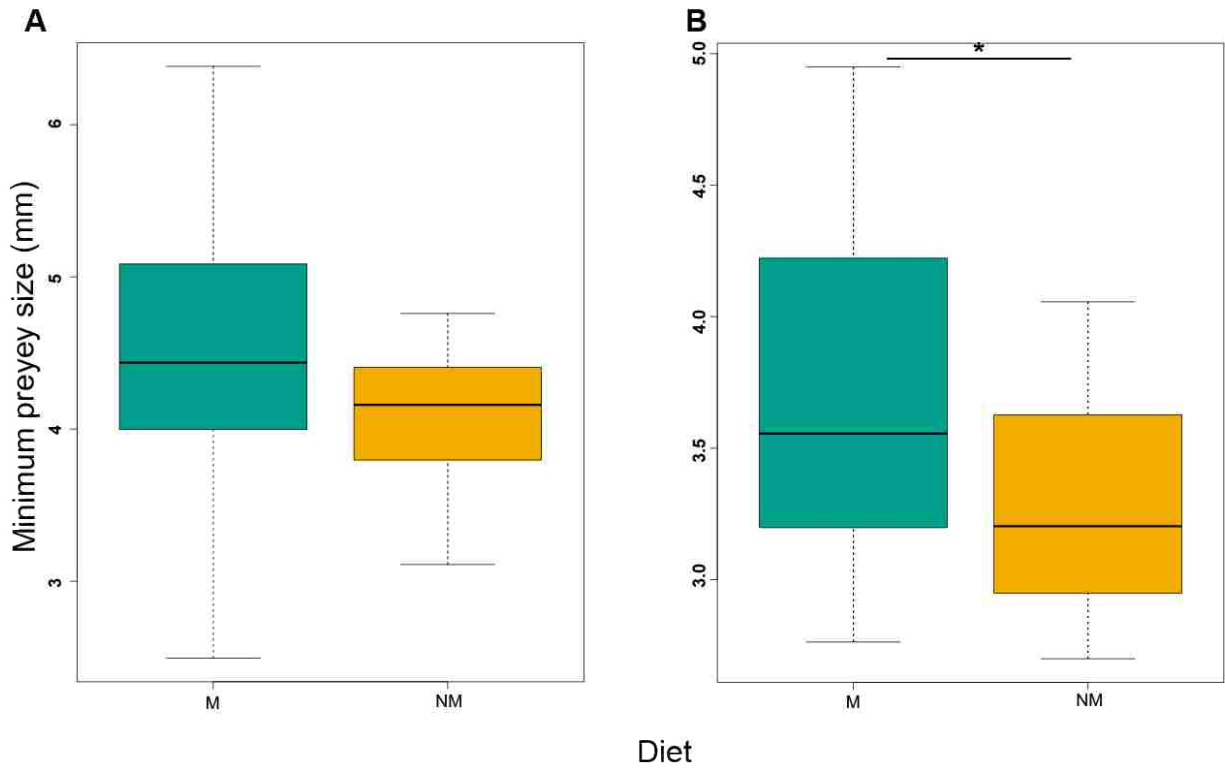


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 412  
 413 Figure 1.4. Linear discriminant analysis (LDA) of A) taxonomically defined dietary guilds and  
 414 B) functionally defined dietary guilds. Species codes are listed as AJ-*Artibeus jamaicensis*, AL -  
 415 *Artibeus lituratus*, CC- *Carollia castanea*, CP- *Carollia perspicillata*, Cso -*Carollia sowellii*, Csu  
 416 -*Carollia subrufa*, Cse – *Centurio senex*, CA -*Chrotopterus auritus*, DP-*Dermanura phaeotis*,  
 417 DW- *Dermanure watsonii*, DR -*Desmodus rotundus*, EA-*Ectophylla alba*, GC-*Glossophaga*  
 418 *commissarisi*, GL-*Glossophaga longirostirs*, Gso-*Glossophaga soricina*, GSI-*Glyphonycteris*  
 419 *sylvestris*, HU-*Hylonycteris underwoodi*, LaB-*Lampronnycteris brachyotis*, LoB-*Lophostoma*  
 420 *Brasiliense*, LS-*Lophostoma silvicolum*, Mma-*Mesophylla macconnelli*, MH-*Micronycteris*  
 421 *hirsute*, MM-*Micronycteris microtis*, Mmi-*Micronycteris minuta*, MS-*Micronycteris*

422 *schmidtorum*, MC-*Gardnerycteris crenulatum*, PD-*Phyllostomus discolor*, PH-*Phyllostoms*  
 423 *hastatus*, Phe-*Platyrrhinus helleri*, SL-*Sturnira lilium*, TB-*Tonatia bidens*, TS-*Tonatia*  
 424 *saurophila*, TC-*Trachops cirrhosis*, VN-*Vampyriscus nympheae*, VS-*Vampyrum spectrum*  
 425  
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427 Diet  
 428 Figure 1.5. Minimum detectable prey size across taxonomically defined dietary guilds for A)  
 429 peak frequency and B) max frequency



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432 Figure 1.6. Minimum detectable prey size across functionally defined dietary guilds (NM- non-

433 mobile/non-evasive prey and M – mobile/evasive prey) for A) peak frequency and B) max

434 frequency

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452 Table 1.1. Definition and functional significance of call parameters. Within each call parameter group (Par. groups) are the specific,  
 453 call parameters (Call specific) measured in this study, along with their function, predictor traits, and associated citations.  
 454

<b>Par. groups</b>	<b>Call specific</b>	<b>Function</b>	<b>Predictor traits</b>	<b>Citation</b>
<b>Harmonics</b>		Distinguish clutter echoes from target echoes.	unknown	Simmons et al. 1975; Bates et al. 2011
<b>Frequency</b>		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	<b>Low values:</b> increase range detection, increase beam width, decrease resolution.	unknown	Neuweiler 2000; Bates et al. 2011; Brock Fenton et al. 2016
	Maximum frequency	<b>High values:</b> 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
<b>Bandwidth</b>		<b>High values:</b> 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	<b>High values:</b> encode information about small frequency changes produced by fluttering insects. <b>Low values:</b> better for detecting larger objects at longer distances.	foraging habitat	Brock Fenton et al. 2016
	Broad-frequency band	<b>High values:</b> 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
<b>Duration</b>		<b>Low values:</b> optimize the resolution of target distance and range accuracy; increases the signal overlap-free window zone (i.e., no echo interference). <b>High values:</b> 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

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458 Table 1.2. Hypotheses and predictions for specific call parameters (Call specific) within each call parameter group (Par. groups), as  
 459 well as predicted dietary guild to exhibit each prediction.  
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<b>Par. groups</b>	<b>Call specific</b>	<b>Prediction</b>	<b>Dietary guild (taxonomic/functional)</b>
<b>Harmonics</b>		Species that forage on cryptic prey in dense clutter will have a higher number of harmonics.	Insectivore/mobile-evasive
<b>Frequency</b>	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.	-
<b>Bandwidth</b>	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
<b>Duration</b>		Species that heavily rely on echolocation to detect and locate prey on leaf background will have shorter duration of calls.	Insectivore/mobile-evasive

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470 Table 1.3. Call parameter means, standard deviations (SD) and range (minimum - maximum values, listed beneath) for duration (dur.),  
 471 maximum frequency (maxF), minimum frequency (minF), peak frequency (pF), bandwidth (bw), and harmonics (harm.) across 35  
 472 species of phyllostomid bats  
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<b>Species</b>	<b>dur.(ms)</b>	<b>maxF (kHz)</b>	<b>minF (kHz)</b>	<b>pF (kHz)</b>	<b>bw (kHz)</b>	<b>harm.</b>
<i>Artibeus jamaicensis</i>	0.78 ±0.42 (0.042-1.5)	93.1 ±3.7 (83.4-96.0)	63.2 ±6.1 (53.1-71.7)	77.1 ±4.4 (69.1-82.5)	39.3 ±12.6 (25.6-58.5)	1.54 ±0.27 (1.10-1.90)
<i>Artibeus lituratus</i>	0.84 ±0.15 (0.61-0.95)	91.5±3.49 (88.3-95.2)	57.7 ±4.23 (52.4 -61.6)	72.9±1.7 (71.8-75.5)	34.8±5.8 (27.3-40.6)	1.33 ±0.33 (1.0-1.80)
<i>Carollia castanea</i>	0.44 ±0.09 (0.31-0.54)	110.0 ±4.29 (115.7-104.4)	56.0 ±5.27 (47.9 -64.1)	80.7 ±4.2 (73.5-86.4)	58.1 ±9.7 (39.7-68.0)	1.35 ±0.345 (1.00-1.80)
<i>Carollia perspicillata</i>	0.65 ±0.30 (0.29-1.11)	98.4 ±11.6 (84.8-124.2)	56.8 ± 11.9 (29.7-74.8)	77.7 ±7.3 (68.1-88.4)	48.8 ±15.2 (29.9-87.7)	1.47 ± 0.23 (1.2-1.86)
<i>Carollia sowelli</i>	0.57 ±0.24 (0.28-1.00)	94.7 ±14.7 (85.0-137.2)	64.5 ±17.1 (41.6-107.1)	78.8 ±15.4 (65.4-121.8)	33.8 ±5.4 (25.1-44.8)	1.41 ±0.13 (1.10-1.60)
<i>Carollia subrufa</i>	0.52 ±0.12 (0.42-0.66)	100.6 ±1.6 (49.3-102.9)	64.6 ±6.8 (55.2-70.6)	85.5 ±2.9 (82.4-89.5)	37.5 ±1.1 (36.4-38.6)	1.29 ±0.14 (1.10-1.40)
<i>Centurio senex</i>	1.04 ±0.22 (0.93-1.37)	122.6 ±12.5 (106.1-141.3)	67.8 ±3.1 (63.7-71.4)	86.7 ±4.1 (83.5-94.0)	34.5 ±7.3 (25.7-42.3)	1.05 ±0.08 (1.00-1.21)
<i>Chrotopterus auritus</i>	0.28	139.1	96.3	118.2	42.1	2.47
<i>Dermanura phaeotis</i>	- 0.53 ±0.08 (0.43-0.70)	- 105.0 ±8.9 (84.9-114.2)	- 59.8 ±5.5 (49.7-68.2)	- 81.3 ±7.9 (72.4-90.1)	- 50.2 ±9.7 (38.6-70.4)	- 1.48 ±0.36 (1.05-2.36)
<i>Dermanura watsoni</i>	0.45 ± 0.10 (0.28-0.57)	115.1 ± 4.8 (109.1-120.4)	63.1 ±10.4 (55.3-80.2)	91.9 ±7.1 (78.5-99.8)	50.7 ±12.1 (30.6-64.8)	1.48 ± 0.17 (1.33-1.73)
<i>Desmodus rotundus</i>	0.74 ±0.18 (0.61-0.95)	89.8 ±7.7 (81.1-96.2)	64.4 ±1.7 (62.4-65.8)	78.1 ±3.7 (73.9-80.9)	30.7 ±1.8 (29.1-32.7)	1.18 ±0.03 (1.13-1.21)
<i>Ectophylla alba</i>	0.63 ±0.11 (0.46-0.74)	128.9 ±10.4 (118.4-144.5)	49.1 ±8.8 (36.6-59.1)	81.1 ±10.7 (69.7-93.7)	71.4 ±15.4 (56.1-95.3)	1.27 ± 0.16 (1.07-1.5)
<i>Glossophaga commissarisi</i>	0.86 ±0.51 (0.33-1.48)	124.4 ±24.1 (91.8-149.9)	49.5 ±16.7 (34.2-71.9)	81.0 ±7.1 (72.3-89.8)	81.3 ±30.7 (43.0-114.3)	1.54 ±0.39 (1.23-1.91)
<i>Glossophaga leachii</i>	0.45	135.0	61.3	91.6	84.1	1.60
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<i>Glossophaga soricina</i>	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)
<i>Glyphonycteris sylvestris</i>	1.49	92.2	51.0	70.8	41.1	2.80
<i>Hylonycteris underwoodii</i>	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)
<i>Lampronnycteris brachyotis</i>	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)
<i>Lophostoma brasiliense</i>	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)
<i>Lophostoma silvicolum</i>	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)
<i>Mesophylla macconnelli</i>	0.63	134.2	84.0	111.6	43.0	1.69
<i>Micronycteris hirsuta</i>	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)
<i>Micronycteris microtis</i>	0.35 ±0.073 (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)
<i>Micronycteris schmidtorum</i>	0.34	117.2	52.2	83.7	65.2	1.96
<i>Mimon crenulatum</i>	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)
<i>Phyllostomus discolor</i>	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)
<i>Phyllostomus hastatus</i>	0.74	63.6	21.3	54.3	56.3	2.3
<i>Platyrrhinus helleri</i>	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)
<i>Sturnira lilium</i>	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)

<i>Tonatia bidens</i>	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)
<i>Tonatia saurophila</i>	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)	(1.17-1.67)
<i>Trachops cirrhosus</i>	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)
<i>Vampyriscus nymphaea</i>	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)
<i>Vampyrum spectrum</i>	0.52 ±0.08	103.5 ±2.8	64.0 ±1.5	83.0 ±0.7	32.8 ±16.0	1.23 ±0.32
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Table 1.4. Phylogenetic signal for call parameters; Pagel's lambda ( $\lambda$ ) and Blomberg's K

<b>Parameter</b>	<b><math>\lambda</math></b>	<b>K</b>
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

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513 Table 1.5. Summary of species, diet and calculated minimum detectable prey size based on peak  
 514 frequency, pF (mm) and maximum frequency emitted, mF (mm)  
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<b>Species</b>	<b>Diet</b>	<b>pF (mm)&lt;</b>	<b>mF (mm)&lt;</b>
<i>Artibeus_jamaicensis</i>	fruit	4.52	3.89
<i>Artibeus_lituratus</i>	fruit	4.76	4.06
<i>Carollia_castanea</i>	fruit	4.30	3.24
<i>Carollia_perspicillata</i>	fruit	4.47	3.54
<i>Carollia_sowellii</i>	fruit	4.41	3.81
<i>Carollia_subrufa</i>	fruit	4.05	3.47
<i>Centurio_senex</i>	fruit	4.00	3.63
<i>Chrotopterus_auritus</i>	animal	2.49	2.94
<i>Artibeus_phaeotis</i>	fruit	4.21	3.40
<i>Artibeus_watsoni</i>	fruit	3.77	3.17
<i>Desmodus_rotundus</i>	blood	4.44	3.96
<i>Ectophylla_alba</i>	fruit	4.28	2.90
<i>Glossophaga_commissarisi</i>	nectar	4.28	2.90
<i>Glossophaga_leachii</i>	nectar	3.79	2.74
<i>Glossophaga_soricina</i>	nectar	4.11	2.95
<i>Glyphonycteris_sylvestris</i>	insect	3.76	4.90
<i>Hylonycteris_underwoodi</i>	nectar	4.44	3.38
<i>Lamproncycteris_brachyotis</i>	insect	3.38	2.76
<i>Lophostoma_brasiliense</i>	insect	3.93	2.90
<i>Lophostoma_silvicolum</i>	insect	4.99	4.02
<i>Mesophylla_macconnelli</i>	fruit	3.11	2.70
<i>Micronycteris_hirsuta</i>	insect	4.52	3.56
<i>Micronycteris_microtis</i>	insect	4.37	3.35
<i>Micronycteris_minuta</i>	insect	4.00	3.61
<i>Micronycteris_schmidtorum</i>	insect	4.14	2.96
<i>Gardnerycteris_crenulatum</i>	insect	5.21	4.33
<i>Phyllostomus_discolor</i>	omn	6.38	4.95
<i>Phyllostomus_hastatus</i>	omn	6.38	4.73
<i>Platyrrhinus_helleri</i>	fruit	3.39	2.95
<i>Sturnira_lilium</i>	fruit	3.89	3.09
<i>Tonatia_bidens</i>	insect	5.08	4.16
<i>Tonatia_saurophila</i>	insect	5.21	4.22
<i>Trachops_cirrhosus</i>	animal	4.64	3.20
<i>Vampyriscus_nymphaea</i>	fruit	3.80	3.17
<i>Vampyrum_spectrum</i>	animal	4.15	3.34

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521 Table 1.6. Group means, standard deviation (SD), and variance for minimum detectable prey size  
 522 for taxonomic and functional dietary guilds, calculated from peak frequency (pF) and maximum  
 523 frequency (mF).  
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<b>Prey size variable</b>	<b>Diet</b>	<b>Mean ± SD</b>	<b>Variance</b>
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

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

549 2.1 ABSTRACT

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

567 and echolocation call parameters. This may be explained by the use of multiple sensory  
568 modes across phyllostomids and plasticity of some echolocation call parameters.

569 6. Species with different diets significantly differed in nose leaf shape, specifically in spear  
570 breadth, presence of a midrib, and cupping and anterior rotation of the horseshoe. This  
571 may relate to different levels of prey type specificity within each diet. Pinnae shape  
572 significantly differed between species that consume non-mobile, non-evasive prey (broad  
573 rounded, cupped pinnae) and mobile, evasive prey (flattened pinnae with a sharp tapering  
574 apex). This may reflect the use of different sound cues to detect prey.

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

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## 578 2.2 INTRODUCTION

579 All animals use sensory cues to perform functions that impact their fitness, and many  
580 possess highly specialized sensory structures that allow them to accomplish challenging tasks  
581 (Catania 1999, 2011, Schmitz and Wainwright 2011). Prey detection is a critical use of the  
582 sensory system that can directly influence organismal fitness and ecology (Persson et al. 1996,  
583 Kalko and Condon 1998, Ferry-Graham et al. 2002, Placyk and Graves 2002, Gracheva et al.  
584 2011), and previous work has provided a solid foundation for understanding how the sensory  
585 structures of many predators have evolved to match the requirements of their dietary niches (e.g.,  
586 Catania, 1999; Safi & Siemers, 2009). However, broad comparative analyses of morphology and  
587 function are still needed to understand sensory adaptations in some of the most diverse  
588 mammalian radiations, and how changes in the sensory system influence organismal ecology and  
589 evolution (Kalko and Condon 1998, Gracheva et al. 2011). Here, we investigate a source of

590 morphological diversity (modularity) in the external sensory organs of the most trophically  
591 diverse family of mammals, the Neotropical leaf-nosed bats (Phyllostomidae), and explore  
592 whether this diversity is linked to echolocation call parameters and dietary ecology.

593         Phyllostomids are an adaptive radiation comprised by over 200 species that vary greatly  
594 in diet, including insectivory, sanguivory, animalivory, nectarivory, omnivory and frugivory  
595 (Rex et al. 2010, Dumont et al. 2012). They are laryngeal echolocators, nasophonators, and  
596 possess a conspicuous leaf-shaped structure surrounding their nares –a nose leaf– that comprises  
597 the major external sensory structure that functions in the emission of echolocation calls  
598 (Bogdanowicz, Csada, & Fenton, 1997; Vanderelst et al., 2010). While the nose leaf acts as an  
599 acoustic reflector that focuses the echolocation sound beam (Wilson & Reeder, 2005; (Hartley  
600 and Suthers 1987, Linnenschmidt and Wiegrebe 2016), the external ears (pinnae) act as acoustic  
601 horns that help detect returning echoes (Fuzessery, 1996; Obrist, Fenton, Eger, & Schlegel,  
602 1993; Jen & Chen, 1988; Vanderelst et al., 2010).

603         Computational models and relatively limited experimental evidence has demonstrated  
604 changes in the morphology of the nose leaf and pinnae can drastically alter a bat’s acoustic field  
605 of view (Feng, Gao, Lu, & Mu, 2012; Gao, Balakrishnan, He, Yan, & Müller, 2011; He,  
606 Pedersen, Gupta, Simmons, & Müller, 2015; Müller, 2015; Vanderelst et al., 2010; Zhuang &  
607 Müller, 2007; Zhuang, Wang, Li, Mao, & Wang, 2012). For example, removing particular nose  
608 leaf components can drastically influence the spread of bats’ calls, and curling the pinnae can  
609 change a bat’s ability to localize sound (Zhuang and Müller, 2007; Vanderelst et al., 2010).  
610 Phyllostomids exhibit great morphological diversity in nose leaf and pinnae (Fig. 1) and thus  
611 represent a natural experiment to investigate the ecomorphological diversity of the sensory  
612 system within an evolutionary context. To date, no studies have investigated the evolution of

613 nose leaf morphologies in a broad comparative context, and only a few (Feng, Gao, Lu, &  
614 Müller, 2012; Gao, Balakrishnan, He, Yan, & Müller, 2011; Vanderelst et al., 2010; Zhuang &  
615 Müller, 2007) have explored how specific morphological components are related to sound beam  
616 spreading.

617         Morphological diversity may arise through various mechanisms, including modularity  
618 (Goswami 2006, Goswami and Polly 2010). An anatomical module is defined by sets of highly  
619 correlated traits that have weak correlations with traits outside the module (Goswami and Polly  
620 2010). Modules can either increase or decrease morphological diversity via autonomy among  
621 modules or integration within modules, respectively (Goswami & Polly 2010). Modularity of  
622 morphological structures is a common feature across taxa (e.g., cichlid jaws (Hulsey 2006);  
623 anoles (Sanger et al. 2012); mammal skulls (Goswami 2006), and the evolution of modules  
624 within a structure allows parts of the structure to specialize for different functions. While  
625 modularity has been primarily tested in osteological components involved in feeding, less  
626 attention has been paid to modularity of the components of sensory systems. Here, we investigate  
627 modularity as potential mechanism underlying the morphological diversification of the nose leaf  
628 in phyllostomids. We hypothesize that the nose leaf consists of at least two modules, spear and  
629 horseshoe (Fig. 2), which would allow for specialization of each of these parts on specific  
630 acoustic functions. We expect to find greater covariation among landmarks within each module  
631 than between modules, which would indicate independent variation of the spear and the  
632 horseshoe across species and over evolutionary time. Alternatively, finding a single module  
633 within the nose leaf would indicate that all parts of the nose leaf change together over  
634 evolutionary time.

635         Next, we test if there is a link between morphological variation of the nose leaves and

636 pinnae and echolocation call parameters across phyllostomids. High frequencies, necessary for  
637 small prey detection, need to be of high intensity to produce an audible echo for the bat –high  
638 frequencies attenuate rapidly, especially in the hot, humid habitats where phyllostomids live  
639 (Lawrence and Simmons 1982). High frequency sounds are crucial for insectivorous  
640 phyllostomids because they allow distinguishing cryptic prey from a leaf background and  
641 determining the texture of edible prey (e.g., katydids; Geipel et al., 2013; Simmons et al., 1975).  
642 Therefore, we predict most phyllostomids will have nose leaf morphologies that increase their  
643 ability to focus higher frequencies emitted, and that certain shape components of nose leaf  
644 morphology will be associated with specific echolocation call parameters. The angle in which  
645 sound reflects off a surface can greatly influence either the spread of sound (e.g., wider, less  
646 intense sound beam) or the concentration of sound (e.g., narrower, more intense sound beam;  
647 Herzfeld, 1938). Thus, we predict that an anteriorly rotated, relatively broad spear and an  
648 anteriorly rotated horseshoe will be correlated with higher values of peak frequency, maximum  
649 frequency and bandwidth, as we expect these shape components to be more efficient at  
650 directionally reflecting sound (Herzfeld, 1938; Wahlstrom, 1985; Zhuang & Müller, 2012).  
651 Conversely, a nose leaf that is flattened in the coronal plane and a reduced horseshoe would be  
652 correlated with lower values of peak frequency and minimum frequency. For pinnae, we expect  
653 to find a link between overall shape and the frequency of acoustic cues that may be used for prey  
654 detection. Across several families of bats, absolutely smaller pinnae are not sensitive to detecting  
655 low frequencies (<15 kHz). Typically, smaller pinnae have a more pointed apex and this results  
656 in less surface area compared to rounded, broad morphologies (Obrist, Fenton, Eger, & Schlegel,  
657 1993). Thus, we predict that pointed, narrower pinnae will be correlated with the peak frequency  
658 emitted. Alternatively, broader, rounder pinnae might not be associated with frequencies or

659 bandwidth emitted as these species may be cueing in on low frequency sounds (<15 kHz), such  
660 as prey mating calls (Bernal et al. 2009).

661 Finally, we investigated whether interspecific variation in the morphology of the nose  
662 leaf and pinnae is associated with dietary ecology in phyllostomids. Phyllostomid bats rely on  
663 different sensory modalities depending on the task they are performing, and the type of prey they  
664 pursue. For example, insectivores use echolocation for navigation, and to detect, locate and  
665 classify prey (Geipel et al. 2013). In contrast, frugivores use echolocation for navigation, but rely  
666 heavily on olfaction to initially find ripe fruit (Reiger and Jakob 1988, Laska 1990, Bloss 1999,  
667 Hodgkison et al. 2007). We hypothesize that different dietary ecologies and foraging behaviors  
668 have imposed different selective pressures on the echolocation system of phyllostomids,  
669 including the nose leaf and pinnae, leading to morphological variation in these structures among  
670 dietary categories. More specifically, we predict that species within dietary categories that rely  
671 heavily on precise echolocation information to find and capture more elusive prey (i.e.,  
672 insectivory) will exhibit more specialized nose leaf and pinnae morphologies that enable them to  
673 better focus echolocation calls and receive a wider array of sound frequencies. In contrast, we  
674 predict that frugivores and nectarivores, which rely on other senses such as olfaction, will exhibit  
675 sensory morphologies that are less functionally tied to specific echolocation call parameters.

## 676 2.3 METHODS

### 677 2.3.1 *3D Imaging and shape analyses*

678 We quantified the three-dimensional morphology of nose leaves and pinnae for 47 adult bats  
679 from 31 phyllostomids species that span the diversity in body size, nose leaf and pinnae  
680 morphologies, and dietary ecology within the family. The majority of species used (26) were

681 collected in the field following approved methods (IACUC protocol 4307-01), and the remainder  
682 (5) were fluid-preserved museum specimens (supplemental Table 1). Based on information and  
683 classifications from the literature, we grouped species into six taxonomic dietary categories  
684 (animalivores, insectivores, nectarivores, frugivores, omnivores, and sanguinivores; Giannini &  
685 Kalko, 2004), and two functional dietary categories: predators of non-mobile/non-evasive prey  
686 (nectarivores, frugivores, omnivores and sanguinivores), and predators of mobile, evasive prey  
687 (insectivores, animalivores).

688         Specimen preservation often alters the shape of soft tissue structures (e.g., bent nose  
689 leaves). To mitigate this problem, we captured pinnae and nose leaf morphology by taking casts  
690 from freshly euthanized animals. We used a President Jet dispenser gun to apply President dental  
691 molding epoxy (Epo-tek 301) to the pinnae and nose leaf (Fig. 2A). We allowed casts to dry on  
692 the specimen for a minimum of five minutes before carefully removing them. We used both this  
693 casting method and select fluid-preserved specimens that did not have deformed soft tissues to  
694 compile our dataset. We created 3D digital models of the nose leaf and pinnae by scanning either  
695 specimens or epoxy casts on a Skyscan 1174 micro-CT scanner (Bruker MicroCT, Kontich,  
696 Belgium) at a 17- 30.1  $\mu\text{m}$  resolution, depending on the size of the cast or specimen. We used  
697 NRecon (Microphotronics, Allentown, PA) to convert CT shadow images into image stacks  
698 (“slices”), and imported these into Mimics 17.0 (Materialise NV, Leuven, Belgium, 2014) to  
699 segment nose leaf and pinnae and produce 3D surface (\*.stl) files. We imported raw stl files into  
700 Geomagic Studio 2014.1.0 (3D Systems, SC, USA, 2014) to remove scanning artifacts (e.g.,  
701 debris in molds) from the models.

702         To quantify nose leaf and pinnae shape, we used 3D geometric morphometric analyses  
703 (Bookstein 1997, Zelditch et al. 2004). These were based on single point landmarks and surface

704 patches, all placed on 3D models using Stratovan Checkpoint© (Stratovan Corporation, Davis,  
705 CA). For the nose leaf, we placed: (1) single-point landmarks at the base of each nostril and the  
706 apex of the spear, (2) evenly-spaced semi-landmarks around the nose leaf perimeter, and (3) two  
707 “patches” of semi-landmarks in a grid across the surface of the spear and the surface of the  
708 horseshoe, respectively (Fig. 2D). To analyze shape changes of subcomponents of the nose leaf  
709 separately (i.e., spear and horseshoe), we modified landmark placement to ensure each  
710 subcomponent had a sufficient number of true landmarks. For the spear, we placed a single-point  
711 landmark at the apex of the spear, two landmarks at the point where the spear meets the  
712 horseshoe, and a patch of semi-landmarks over the anterior surface of the spear (Fig. 2D). For  
713 the horseshoe, we placed a single-point landmark on each nostril and one patch over the surface  
714 of horseshoe. For pinnae, we placed two single-point landmarks at the points where the pinna  
715 attaches to the head, and a patch of semi-landmarks across its surface (Fig. 2C). We exported  
716 landmark coordinates for each specimen as .csv files and computed species means for landmark  
717 coordinates in Excel. We then performed Procrustes superimposition analyses to scale, align and  
718 rotate landmark configurations (Rohlf 1990), and obtain a set of variables describing the shape of  
719 the entire nose leaf, spear, horseshoe and pinnae across species. We used the package  
720 “geomorph” (Adams and Otárola-Castillo 2013) in R v 99.903 (R Core Team, 2017) for  
721 geometric morphometric analyses.

722

### 723 2.3.2 *Acoustics*

724 Phyllostomid bats produce low-intensity calls (Griffin 1958, Brinkløv et al. 2009) that are  
725 difficult to capture on passive recording devices. Consequently, call parameter data are sparse for  
726 most phyllostomid species. For this study, we collected 16-bit recordings of release calls using a



727 microphone condenser (UltraSoundGate 116). Our sample included 101 individuals spanning 33  
728 species. We held each bat in hand, placed a microphone approximately six inches from its face,  
729 and then released the bat away from environmental clutter while recording the calls it emitted as  
730 it flew away. Since bats had to be released to document their natural calls, we did not use these  
731 same individuals in morphological analyses. We measured call parameters for 3–7 individuals  
732 per species, with the exception of species that were rare or difficult to capture at our study  
733 localities (*Chrotopterus auritus*, *Glyphonycteris sylvestris*, *Phyllostomus hastatus*, *Sturnira*  
734 *lilium*), for which we were able to record 1 individual per species. We analyzed release calls  
735 using Avisoft SASLabPro v. 5.2.12 (Avisoft Bioacoustics, Berlin, Germany) to extract the  
736 following echolocation call parameters: minimum frequency (kHz), maximum frequency (kHz),  
737 peak frequency (kHz) (i.e. frequency with the highest amplitude), and total bandwidth (kHz)  
738 across the call. We averaged call sequences per individual (a minimum of 5) and calculated  
739 means and standard deviation of each parameter.

740

### 741 2.3.3 *Statistical analyses*

742 To test whether the nose leaf consists of two modules (spear and horseshoe; Fig. 2D), we  
743 computed Covariance Ratio (CR) values for a two-module hypothesis using the nose leaf  
744 landmark data, and estimated the p-value for this relationship via 1,000 random permutations.  
745 The CR ratio indicates the degree of covariation among landmarks within possible modules;  
746 values from 0 to 1 indicate less covariation *between* modules than *within* each module,  
747 supporting the modularity hypothesis, CR values greater than 1 describe greater covariation  
748 between modules than within modules, supporting the null hypothesis of no modules (Adams  
749 2016).

750 To identify major axes of shape variation across sensory structures, we conducted  
751 phylogenetic Principal Component Analyses (pPCA), using the Rojas, Warsi, & Dávalos (2016)  
752 phylogeny, on the Procrustes (shape) coordinates for each structure/substructure using the R  
753 package “phytools” (Revell 2012). We assessed the significance of pPCA axes via Horn’s  
754 parallel analysis from the ‘paran’ function in R (Dinno, 2015). Then, to identify if shapes of  
755 external sensory structures are correlated with call parameters, we ran separate phylogenetic  
756 generalized least squares (PGLS) regressions under Brownian motion of acoustic parameters  
757 across the call (minimum frequency, maximum frequency, peak frequency, and total bandwidth)  
758 against significant pPCs shape scores (see Results; nose leaf pPC 1-5; pinnae pPC 1-4). Finally,  
759 we ran phylogenetic ANOVAs and post-hoc analyses to test for an association between diet  
760 category and nose leaf and pinnae shape, respectively. We used significant pPCs axes as  
761 response variables, and dietary category as the predicting factor.

762

## 763 2.4 RESULTS

### 764 2.4.1 *Modularity in the nose leaf*

765 Our Covariance Ratio analyses indicate that landmarks within the spear and the horseshoe  
766 covary more within each of these subcomponents than between them (CR: 0.882,  $P < 0.001$ ).

767 This strongly suggests that the spear and the horseshoe constitute separate modules within the  
768 nose leaf.

769

### 770 2.4.2 *Morphological diversity of sensory structures*

771 For the complete nose leaf, we identified five significant axes (pPCs) representing 68.3% of the

772 total shape variation. Principal components 3-5 combined explain less than one-third of the total  
773 shape variation, and very subtle morphological differences. The same is true for other structures  
774 examined, thus we focus on describing the shape differences for pPC1-2 for all structures. Nose  
775 leaf pPC1 (32.4% of total shape variation) is positively correlated with shapes that are flattened  
776 in the coronal plane and have a reduced ventral edge of the horseshoe, and negatively correlated  
777 with shapes that have distinct midribs and a horseshoe that is rotated anteriorly (see extremes on  
778 Fig. 3A). Lower values of nose leaf pPC2 (13.2% of total shape variation) describe spear shapes  
779 that are wider, taper sharply towards the apex and have a slight anterior rotation of the outer  
780 edges, and a more pronounced horseshoe that protrudes from the rostrum. In contrast, higher  
781 values along nose leaf pPC2 describe shapes characterized by narrow spears that taper smoothly  
782 towards the apex.

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

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

801

#### 802 2.4.3 *Links among morphology, call structure and diet*

803 *Nose leaf.* PGLS regressions revealed a significant relationship between pPC1 and maximum  
804 frequency and bandwidth (Table 1); species with a flattened nose leaf in the coronal plane and a  
805 reduced ventral edge of the horseshoe have lower maximum frequency and bandwidth in their  
806 echolocation calls. Phylogenetic ANOVAs also revealed significant differences in nose leaf  
807 shape for the whole nose leaf and spear (pPC2 and pPC4, and pPC2, respectively) among dietary  
808 categories (Table 3). *Post-hoc* tests revealed that whole nose leaf shape was significantly  
809 different between frugivores and all other dietary groups, and spear shape between frugivores  
810 and omnivores and all other dietary categories (supplemental Table 2). For the horseshoe, we  
811 found no significant differences across all dietary groups (Table 3). *Post-hoc* test revealed a  
812 difference in pPC3 between omnivores and all other dietary groups and in pPC5 between  
813 frugivores and nectarivores and all other diets ( $p=0.02$ ,  $p=0.04$ , supplemental Table 2).  
814 However, these axes represent less than 13% of the total shape variation.

815

816 *Pinnae.* We found no significant relationships between pinnae shape and echolocation call  
817 parameters (Table 2). We found no significant differences in pinnae shape among all

818 taxonomically-defined dietary categories (Table 3), but *post-hoc* tests reveal sanguivores and  
819 omnivores are different in pinnae shape (pPC1) compared to all other dietary groups ( $p = 0.03$ ,  $p$   
820  $= 0.04$ ; supplemental Table 2). Sanguivores and omnivores both possess highly tapered pinnae,  
821 that can be posteriorly recurved. We also find that pinnae shape pPC1 was significantly different  
822 between bat species that pursue non-mobile, non-evasive prey and species that pursue mobile,  
823 evasive prey ( $p = 0.006$ , supplemental Table 3). These differences are defined by two distinct  
824 morphologies: pinnae that are broad, with a rounded apex (-pPC1), versus those with a sharply  
825 pointed apex (+pPC1) (Fig. 3D).

826

## 827 2.5 DISCUSSION

828 A major goal of functional ecology is to link morphological diversity to ecology (Bickel &  
829 Losos, 2002; Santana, Dumont, & Davis 2009; Santana, Grosse, & Dumont, 2012; Schluter,  
830 1993), but only a few studies have focused on the ecomorphology of sensory systems (e.g.,  
831 Coombs, Janssen, & Webb, 1988; Pankhurst, 1989; van der Meer, Anker, & Barel, 1995). Using  
832 an ecological radiation (Phyllostomidae), we investigated whether and how an evolutionary  
833 mechanism (modularity; Olson & Miller, 1999) may underlie extreme diversity of sensory  
834 structures, explored if morphological differences in external sensory structures are related to  
835 differences in functional outputs, and tested if morphological differences match expectations  
836 related to different ecological demands. Altogether, these analyses explore some of the potential  
837 intrinsic and extrinsic factors shaping the diversity of sensory structures in bats.

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

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

857         We proposed that the detection of particular prey items influenced the morphological  
858 evolution of the nose leaf and pinnae across phyllostomids, via selection on the function of these  
859 structures. A few studies have investigated the three-dimensional morphology of the bat sensory  
860 system and its relationship with functional outputs (Feng, Gao, Lu, & Müller, 2012; Gao,  
861 Balakrishnan, He, Yan, & Müller, 2011; He, Pedersen, Gupta, Simmons & Müller, 2015;  
862 Vanderelst et al., 2010). These have found a significant link between nose leaf and pinnae  
863 morphology and echolocation call frequency in other groups of nasophonating bats

864 (Rhinolophidae and Hipposideridae; Robinson 1996; Huihua, Shiyu, Mingxue, & Jiang 2003).  
865 Here, we found a relationship between one axis of nose leaf shape and two echolocation call  
866 parameters across the species, maximum frequency and bandwidth, but not between any other  
867 shape axes or with pinnae shape. This raises the question, why is there a weak relationship  
868 between nose leaf shape and echolocation call parameters in phyllostomids?

869         In comparison to other nasophonators, many phyllostomids (e.g., frugivores and  
870 nectarivores) rely on multiple sensory modalities (e.g., olfaction and echolocation) for prey  
871 detection (Thies et al. 1998, Kalko and Condon 1998, Korine and Kalko 2005, Sánchez et al.  
872 2006). It is highly likely that sensory mode specialization and plasticity varies across species,  
873 complicating the relationship between external sensory morphology and echolocation  
874 parameters. Modeling studies have found that nose leaf and pinnae function differs across  
875 morphologically-similar species with different sensory specializations (Obrist, Fenton, Eger, &  
876 Schlegel, 1993; Vanderelst et al., 2010). For example, *Phyllostomus discolor* and *Micronycteris*  
877 *microtis* share similar gross morphologies, but the nose leaf shape of *M. microtis*, which relies  
878 heavily on echolocation for prey detection, focuses the energy to a different extent than *P.*  
879 *discolor*, which relies on vision and olfaction for the same task. Phyllostomids also use diverse  
880 foraging behaviors (Bloss 1999, Korine and Kalko 2005, Weinbeer and Meyer 2006, Kunz et al.  
881 2011), which can also alter the bat's perceptual field and reconcile physical differences of the  
882 sensory system. For example, *M. microtis* flies closely along the vegetation inspecting small  
883 areas to detect motionless prey and relies on high-resolution acoustic information about texture  
884 differences between vegetation and prey items (Geipel et al. 2013). *Phyllostomus* often forages  
885 in groups and detects patches of fruits or flowers that can be detected at longer ranges  
886 (Kwiecinski 2006).

887 Additional factors in the sound production system of phyllostomids likely influence the  
888 relationship between external sensory morphology and call parameters, including the  
889 morphology of the vocal tract, nasal cavity, and the size of sensory structures. Accessory folds,  
890 along the vocal tract, can influence the frequencies emitted (Neuweiler 2000). Phyllostomids also  
891 vary greatly in the size of their nose leaves, both relative to body size and relative to the size of  
892 the pinnae. For example, some insectivorous and nectarivorous species share a similar nose leaf  
893 shape but differ greatly in its size (10mm vs. 5mm in height). The size of the structure may be an  
894 important factor linking phyllostomid echolocation and ecology, as the size of a surface can  
895 influences how sound is reflected, and the size of a reflector must be greater than the size of the  
896 wavelength being reflected. Therefore, the size of the nose leaf could evolve in tandem with the  
897 minimum echolocation call frequency (longest wavelength in call).

898 Supporting our hypothesis, we found differences in sensory morphologies across dietary  
899 categories. Presumably, these differences in nose leaf shape among dietary categories could be  
900 driven by pressures on echolocation performance, specifically how sound is reflected and  
901 directed. Insectivores and frugivores are the most divergent in whole nose leaf shape, whereas  
902 frugivores and nectarivores differ the most when spear shape alone is compared. However, not  
903 all axes of nose leaf shape variation are significantly different across dietary categories,  
904 suggesting that some shape changes may be more functionally important for some foraging  
905 strategies than others. Frugivorous and some animalivorous species have a wider spear with  
906 more anteriorly rotated outer edges, and a more pronounced horseshoe that protrudes from the  
907 rostrum (lower pPC1 in Fig. 3), a morphology that provides a more curved reflecting surface.  
908 Curved surfaces (e.g., parabolic surfaces) reflect sound in a very concentrated area, increasing its  
909 intensity (Wahlstrom 1985) and directionality (e.g., reducing spread). Curved morphologies



910 specialized for sound concentration are found in diverse taxa and in structures that aid in both  
911 sound emission and reception, such as the parabolic skulls of odontocetes that help focus emitted  
912 sound (Norris and Harvey 1900, Huggenberger et al. 2014), the antlers of the bull moose that  
913 help focus received sound toward their pinnae (Bubenik and Bubenik 2008), and the spherical  
914 facial disks of owls aid in sound localization (Coles and Guppy 1988). In contrast, nectarivorous  
915 and some insectivorous species have narrower and more flattened spears that taper smoothly  
916 towards the apex (higher pPC2 in Fig. 3B). Insectivores also have a slightly more pronounced  
917 horseshoe, which is almost non-existent in nectarivores. Planar (more flattened) surfaces reflect  
918 sound off-axis and at the same angle of incidence (Herzfeld 1938), possibly increasing the call  
919 beam width. Overall, differences in nose leaf shape likely influence the bat's perceptual field,  
920 and the relationship between nose leaf morphology and foraging ecology.

921         Pinnae shape did not differ significantly among species grouped into taxonomic dietary  
922 categories (e.g., insectivore, frugivore, nectarivore), but it did when species were grouped into  
923 functional diet categories that represented challenges of prey capture (mobile, evasive prey  
924 versus non-mobile, non-evasive prey). Specifically, we found that species that use passive  
925 acoustic cues for prey detection (e.g., *Chrotopterus auritus*) have broader and large pinnae. This  
926 is similar to the pattern observed in other mammal taxa with sensitive hearing (e.g., Serval cats;  
927 Smithers, 1978). Broader and large pinnae presumably perform better at detecting the low  
928 frequency, low intensity sounds produced by potential prey rustling in the understory or in water  
929 (Arlettaz, Jones, & Racey, 2001; Halfwerk, Jones, Taylor, Ryan, & Page, 2014), and low  
930 frequency mating calls produced by potential prey, such as the túngara frogs (calling at 6-11  
931 kHz; Bernal, Page, Ryan, Argo, & Wilson, 2009).

932         Finally, it is important to note that external sensory structures are dynamically controlled,

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

943

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960

961 2.8 AUTHOR CONTRIBUTIONS

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

965

966 2.9 DATA ACCESSIBILITY

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

969

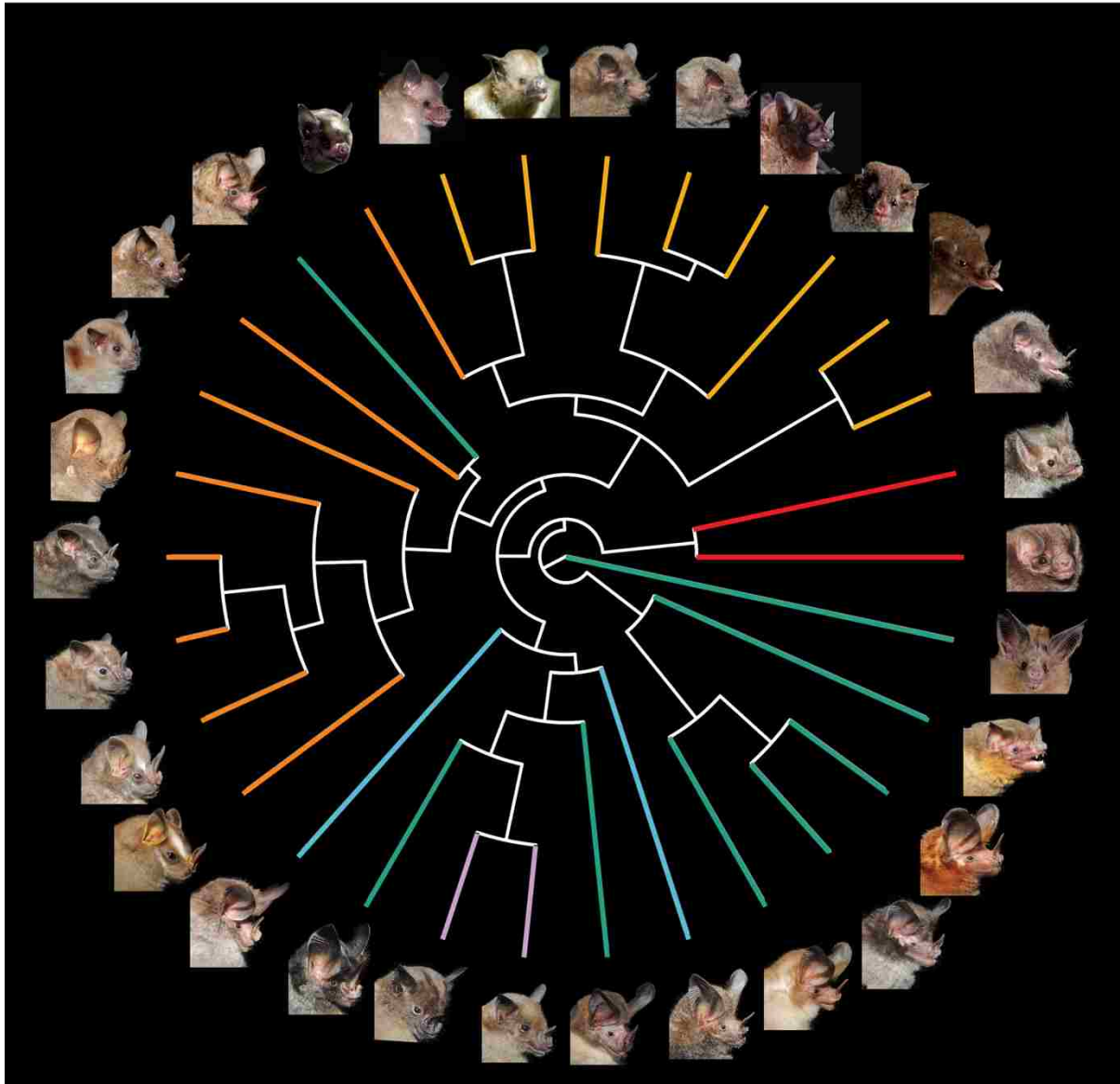
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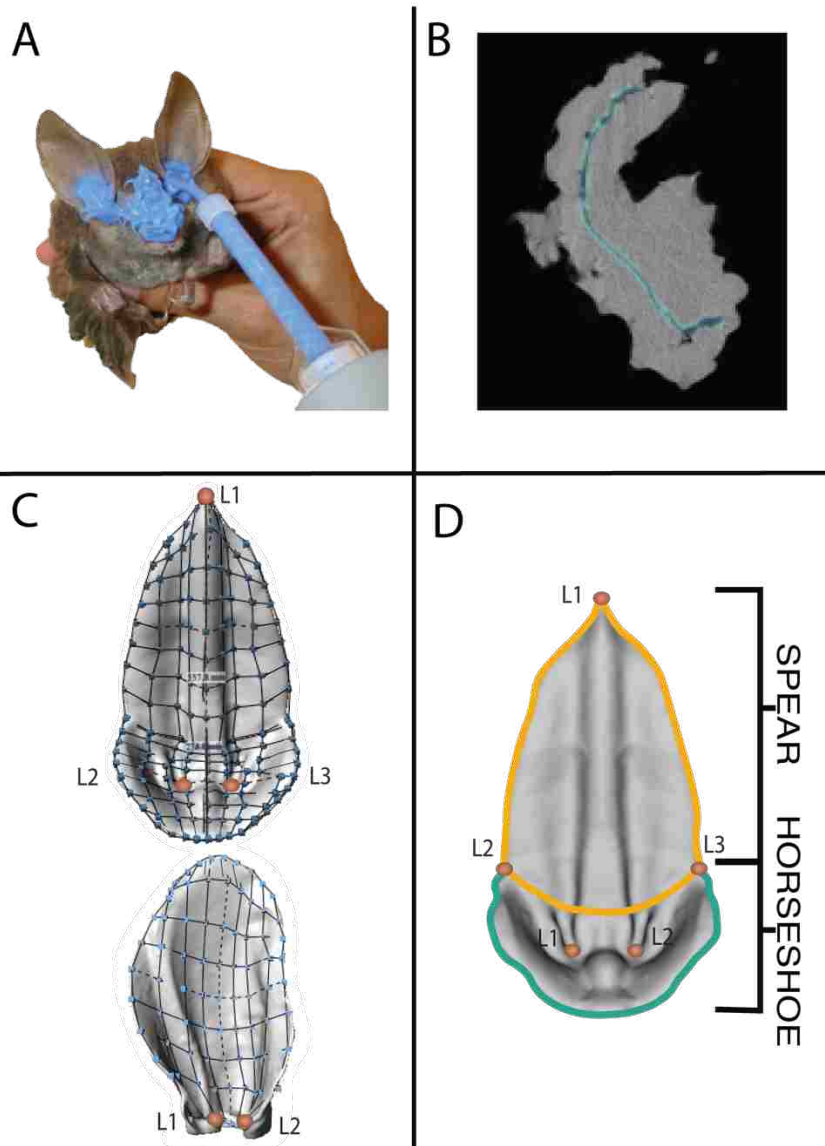
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975  
976

977 Figure 2.1. Phylogeny of phyllostomid species in our dataset. Branches do not contain  
 978 information about scale and are colored based on diet. Species starting at the root and moving  
 979 clock-wise: *Macrotus waterhousii*, *Lamproncyteris brachyotis*, *Micronycteris microtis*,  
 980 *Micronycteris hirsuta*, *Micronycteris minuta*, *Trachops cirrhosus*, *Lophostoma silvicolum*,  
 981 *Phyllostomus discolor*, *Phyllostomus hastatus*, *Gardnerycteris crenulatum*, *Chrotopterus auritus*,  
 982 *Platyrrhinus helleri*, *Artibeus phaeotis*, *Artibeus jamaicensis*, *Artibeus lituratus*, *Phyllops*  
 983 *falcatus*, *Sturnira lilium*, *Carollia perspicillata*, *Glyphonycteris sylvestris*, *Brachyphylla nana*,  
 984 *Phyllonycteris poeyi*, *Erophylla bombifrons*, *Glossophaga soricina*, *Glossophaga commissarisi*,  
 985 *Glossophaga longirostris*, *Monophyllus redmani*, *Lichonycteris obscura*, *Hylonycteris*

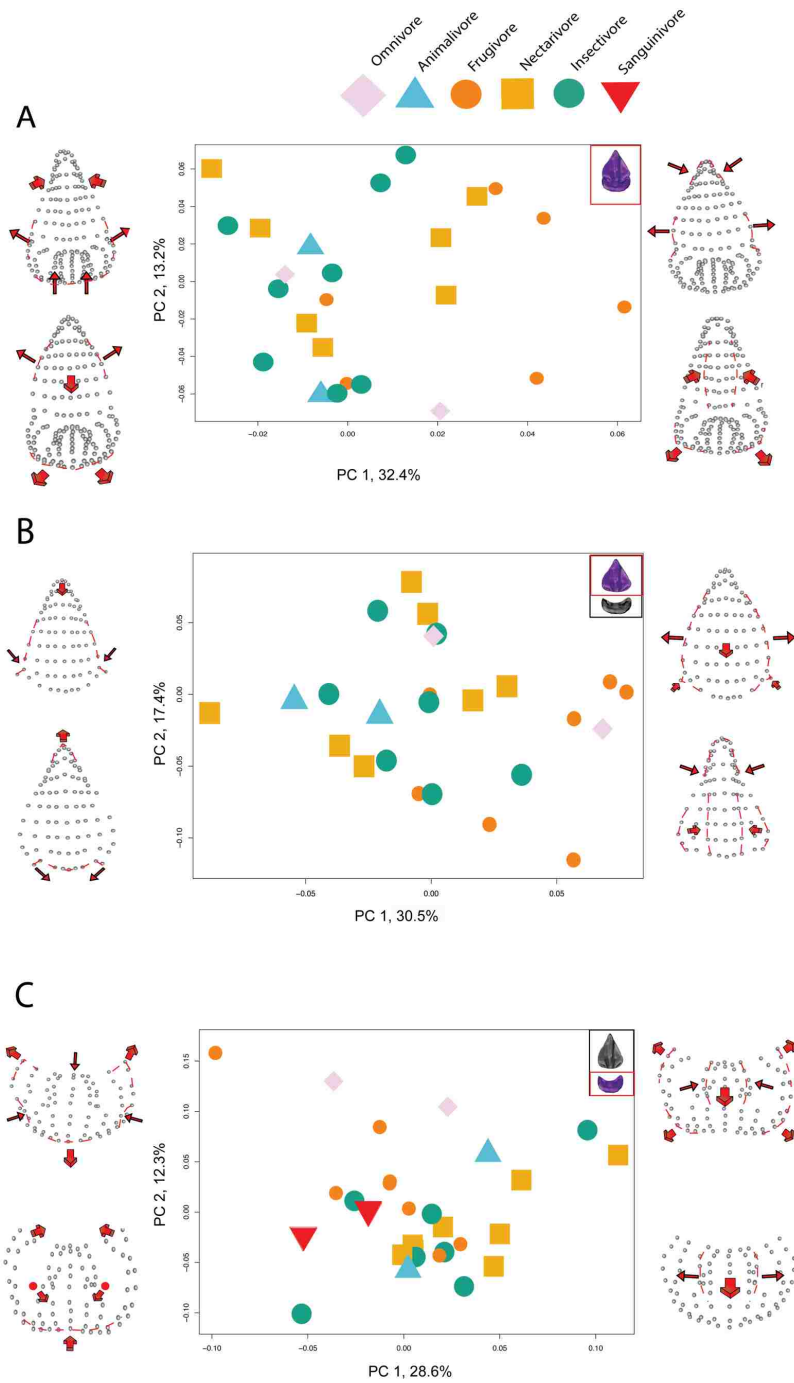
986 *underwoodii*, *Desmodus rotundus*, and *Diphylla ecaudata*. Photo credit: David Villalobos-  
987 Chaves, Brock Fenton, José G. Martínez-Fonseca, Sharlene Santana, Joaquín Ugarte, and Laurel  
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1000 Figure 2.2. Epoxy molding technique on freshly collected specimen (A), microCT scan slice with  
 1001 molding space highlighted in blue for 3D reconstruction (B), reconstructed 3D model of nose  
 1002 leaf and pinna with landmarks (L, orange) and semilandmarks (blue) labeled (C), and modularity  
 1003 hypothesis tested (D), where the spear (yellow) and horseshoe (green) constitute separate  
 1004 modules, with landmarks (L) labeled (D)

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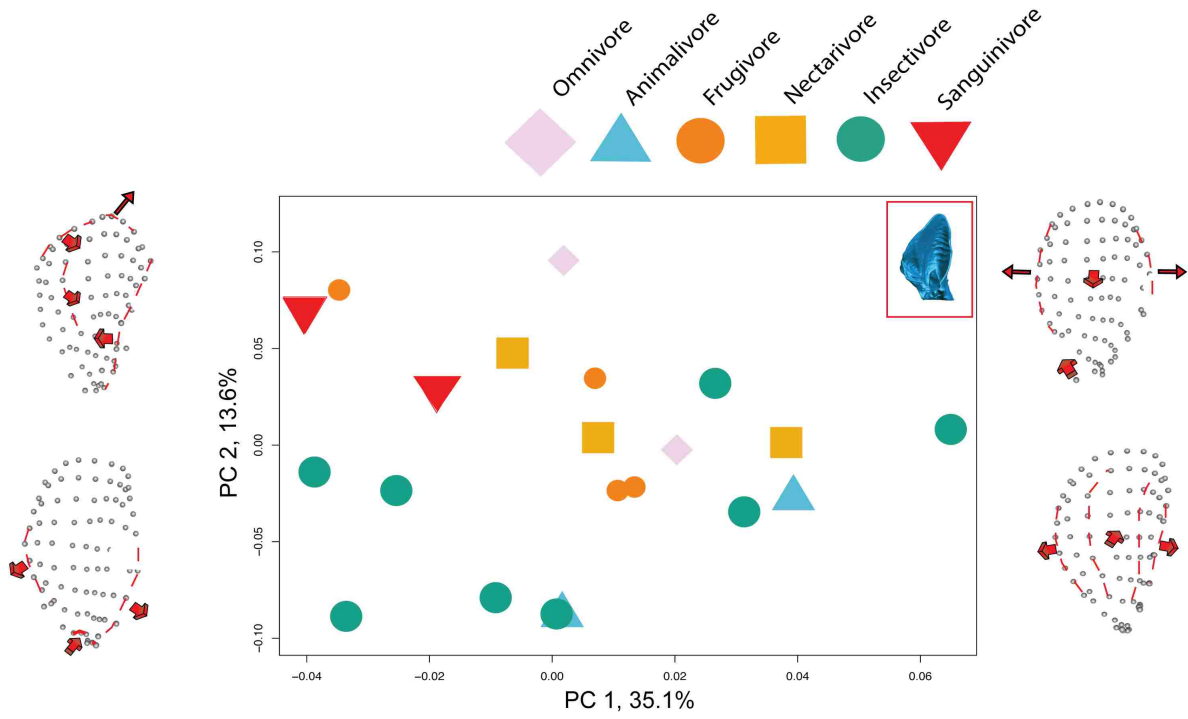
1006

1007 Figure 2.3. Morphospace (pPC) plots for nose leaf (A), spear (B), horseshoe (C), and pinna (D).

1008 Landmark clouds with arrows show shape changes along pPC1 (right) and pPC2 (left). Areas of

1009 change are outlined in red dashed lines

1010



1011 Figure 2.4. Morphospace (pPC) plots for pinnae. Landmark clouds with arrows show shape  
 1012 changes along pPC1 (right) and pPC2 (left). Areas of change are outlined in red dashed lines  
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1034 Table 2.7. Phylogenetic Generalized Least Squares regression of nose leaf shape variables (PCs)  
 1035 on acoustic call parameters.  
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	<b>Nose leaf shape PC</b>	<b>Call parameter</b>	<b>B</b>	<b>SE</b>	<b>t</b>	<b>P</b>
1		Peak frequency	-0.524	0.708	-0.74	0.47
		Min. frequency	-0.117	0.776	-1.51	0.15
		<b>Max. frequency</b>	<b>-0.255</b>	<b>0.100</b>	<b>2.54</b>	<b>0.02*</b>
		<b>Bandwidth</b>	<b>-0.138</b>	<b>0.589</b>	<b>-2.35</b>	<b>0.03*</b>
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

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1039 Table 2.8. Phylogenetic Generalized Least Squares regression of pinna shape variables (PCs) on  
 1040 acoustic call parameters.  
 1041

<b>Pinna shape PC</b>	<b>Call parameter</b>	<b>B</b>	<b>SE</b>	<b>t</b>	<b>P</b>
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

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1046 Table 2.9. Phylogenetic ANOVA results comparing nose leaf, spear, horseshoe, and pinna shape  
 1047 across dietary categories: animalivore, insectivore, frugivore, nectarivore, sanguinivore, and  
 1048 omnivore.  
 1049

<b>Structure</b>	<b>Shape PC</b>	<b>df</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Nose leaf	1	4	0.0070	0.0018	0.96	0.45
	2	4	0.0048	0.0012	2.8	<b>0.052*</b>
	3	4	0.0018	0.00045	1.02	0.42
	4	4	0.0057	0.0014	3.4	<b>0.027*</b>
	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	<b>0.026*</b>
	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

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1064 Chapter 3. A FRUITFUL ENDEAVOR: SCENT CUES AND  
1065 ECHOLOLOCATION BEHAVIOR USED BY *CAROLLIA CASTANEA* TO  
1066 FIND FRUIT

1067

1068 3.1 ABSTRACT

1069 Frugivores have evolved sensory and behavioral adaptations that allow them to find ripe fruit  
1070 effectively, but the relative importance of different senses in varying foraging scenarios is poorly  
1071 known. Within Neotropical ecosystems, short-tailed fruit bats (*Carollia*: Phyllostomidae) are  
1072 abundant nocturnal frugivores, relying primarily on plants of the genus *Piper* as a food resource.  
1073 Previous research has demonstrated *Carollia* employ olfaction and echolocation to locate *Piper*  
1074 fruit, but it is unknown how their sensory use and foraging decisions are influenced by the  
1075 complex diversity of chemical cues that fruiting plants produce. Using wild *C. castanea* and their  
1076 preferred food, *Piper scintillans*, we conducted behavioral experiments to test two main  
1077 hypotheses: (1) foraging decisions in *C. castanea* are primarily driven by ripe fruit scent and  
1078 secondarily by vegetation scent, and (2) *C. castanea* re-weight their sensory inputs to account for  
1079 available environmental cues, such that bats rely more heavily on echolocation in the absence of  
1080 adequate scent cues. Our results suggest that *C. castanea* requires olfactory information and  
1081 relies almost exclusively on ripe fruit scent to make foraging attempts. Ripe fruit scent is  
1082 chemically distinct from vegetation scent in *P. scintillans*, with a greater abundance of  $\beta$ -  
1083 caryophyllene, germacrene D and  $\beta$ -elemene, and a few unique compounds. Although variation  
1084 in echolocation call parameters was independent of scent cue presence, bats emitted longer and  
1085 more frequent echolocation calls in trials where no fruit scent was present. Altogether, these

1086 results highlight the adaptations, plasticity, and potential constraints in the sensory system of  
1087 neotropical fruit bats.

### 1088 3.2 INTRODUCTION

1089 Animals rely on multiple sensory modalities to perform even the simplest ecological tasks  
1090 (Burkhardt 1989, Siemers and Schnitzler 2000, Holland et al. 2005, Knaden and Graham 2016).  
1091 One of the main goals of behavioral and sensory ecology lies in understanding the ability of  
1092 different species to employ and modulate their sensory modes in the context of different  
1093 environmental cues, and how the resulting behavioral decisions ultimately affect their ecology  
1094 and evolution. Frugivores use various cues, including components of fruit color (Burkhardt 1989;  
1095 Osorio & Vorobyev 2008, Melin et al. 2008, Hiramatsu et al. 2008; Valenta et al. 2013), shape  
1096 (Helvesen & Helvesen 1998; Kalko & Condon 1998), and scent (Valenta et al. 2013, Sánchez  
1097 et al. 2006) to find and select ripe fruit, and exhibit corresponding sensory specializations in their  
1098 visual, auditory and/or olfactory systems to target those cues (Catania 1999, Muller et al. 2007,  
1099 Vanderelst et al. 2010). Little is known, however, about what scenarios facilitate or constrain  
1100 sensory system use and modulation during fruit location, selection, and acquisition in vertebrate  
1101 frugivores. Insight into these processes would provide mechanistic understanding of the  
1102 behaviors underlying their foraging ecology.

1103 Frugivorous and omnivorous Neotropical leaf-nosed bats (Phyllostomidae) use three  
1104 major senses, echolocation, olfaction and vision, for navigation and foraging (e.g., Bloss 1999;  
1105 Laska 1990; Reiger et al. 1988; Schnitzler et al. 2003; Winter et al. 2003), which makes these  
1106 organisms an exceptional system for investigating the relative role of different sensory modes  
1107 during ecologically important tasks. Although there has been recent work on phyllostomid  
1108 vision, including comparisons of their short- and long-wave opsins (Muller et al. 2007, Müller et

1109 al. 2009), the importance of vision in ripe fruit location and selection has not been  
1110 experimentally tested in phyllostomids. By contrast, experimental evidence strongly suggests  
1111 that omnivorous phyllostomids rely primarily on echolocation to locate fruits (Kalko and Condon  
1112 1998), and specialized frugivores employ either olfaction or a combination of olfaction and  
1113 echolocation to locate ripe fruit (Kalko and Schnitzler 1993, Korine and Kalko 2005, Hodgkison  
1114 et al. 2007, 2013).

1115         The relative importance of different sensory modes for fruit detection may depend on  
1116 which plant cues can be readily perceived within a specific environmental context. To date, it is  
1117 unknown whether and how frugivorous phyllostomids integrate their primary sensory modes  
1118 (echolocation and olfaction) conditional on which plant cues are present. While plant scents can  
1119 travel over short or long distances (Riffell et al. 2014), they are rarely directional and may be  
1120 difficult to detect in saturated scent environments such as rainforests. Conversely, echolocation  
1121 allows for highly precise prey detection (Schnitzler and Kalko 2001; Brinkløv et al. 2009;  
1122 Jakobsen et al. 2013), but phyllostomids emit short, high frequency and low intensity calls (Thies  
1123 et al. 1998; Korine and Kalko 2005). The echoes from these calls provide information about size,  
1124 shape, texture, range and position of an object in space relative to the bat (Simmons et al. 1975,  
1125 1983, Simmons and Stein 1980, Neuweiler 1989, Schmidt et al. 2000, Schnitzler and Kalko  
1126 2001), but for these types of information are only effective at very short distances because low  
1127 intensity, high frequency calls attenuate rapidly in warm, humid environments (e.g., 45 -90 kHz  
1128 attenuate at 1.4 to 4 dB/m at 25°C and 80% humidity; Jakobsen et al. 2013). Additionally,  
1129 surrounding foliage can produce acoustic masking effects that may complicate fruit detection  
1130 (Arlettaz et al. 2001, Korine and Kalko 2005). Therefore, flexibility in olfaction versus

1131 echolocation use could be highly beneficial for frugivorous bats given the limitations of each  
1132 sensory mode within complex forest environments.

1133         Here, we study the primary plant cues from vegetation and fruits and how this relates to  
1134 the roles of echolocation and olfaction for fruit detection and localization in the chestnut short-  
1135 tailed fruit bat, *Carollia castanea*, a highly abundant frugivore and ecologically important seed  
1136 disperser that inhabits forests in Central and South America (Bonaccorso 1979, Fleming 1991)  
1137 (Figure 1). This research builds upon the seminal work of Thies et al. (1998), which  
1138 demonstrated the importance of olfaction for fruit detection in *Carollia*; here we investigate the  
1139 relative contributions of vegetative and fruit scent cues that drive *Carollia castanea*'s foraging  
1140 decisions, and how varying foraging scenarios (i.e. changing of plant scent cues) may affect the  
1141 relative reliance of these bats on olfaction versus echolocation for fruit detection. *Carollia*  
1142 *castanea* feeds primarily on infructescences of Neotropical *Piper* plants (Piperales: Piperaceae),  
1143 particularly *P. scintillans* (previously *P. sancti-felicis*; Hammel et al. 2014) in our study locality  
1144 (Lopez and Vaughan 2007). We test if foraging decisions in *C. castanea* are primarily driven by  
1145 clear signals of food availability (i.e., *P. scintillans* ripe fruit scent). We predict bats will cue in  
1146 on ripe *P. scintillans* fruit scent and secondarily on its vegetation scent, since vegetation is a  
1147 salient and fragrant part of the plant that could also aid in fruit localization. Since this prediction  
1148 relies on the assumption that ripe fruit and vegetation scents differ in their chemical composition,  
1149 we present analyses contrasting the volatile chemical composition of these plant parts. We also  
1150 hypothesize that *C. castanea* potentially re-weight their sensory inputs to account for available  
1151 environmental cues, and predict that foraging bats emit echolocation calls more frequently when  
1152 scent cues are absent. This is because *Carollia* can use echolocation for the final localization of  
1153 fruit at close range (Thies et al. 1998; Corlett 2011) and perhaps also when searching for

1154 potentially edible fruit patches at a longer range. To test our hypotheses, we conducted a series of  
1155 experiments to mimic the sensory challenges fruit bats may encounter in nature, and quantified  
1156 differences in the bats' behavioral responses when exposed to different sensory cues. Our study  
1157 contributes to the understanding of which chemical cues bats use for fruit selection, which  
1158 contexts facilitate alternating between sensory modes, and the behavioral and sensory  
1159 adaptations fruit bats have evolved for foraging.

1160

### 1161 3.3 MATERIALS AND METHODS

#### 1162 3.3.1 *Study animals*

1163 We used mist nets (avinet, sizes; 4m, 6m, 9m, 12m) to capture *carollia castanea* along forest  
1164 trails at la selva biological station in sarapiquí, heredia province, costa rica (supplementary table  
1165 1) through August – September of 2016. All individuals were experimentally naïve and were  
1166 used in experiments only on the night of capture. Upon capture, each bat was kept in a clean  
1167 cotton bag prior to experiments. We conducted experiments on 21 bats (16 adult males and 5  
1168 adult non-lactating, non-pregnant females), and if individual had a positive trial, we subsequently  
1169 collected biometric data and a 2-3 mm wing biopsy (disposable biopsy punches, integra miltex)  
1170 from the uropatagium of each individual (see below). This was done for future genetic analyses,  
1171 and helped ensure we did not use recaptured individuals in subsequent experiments. All  
1172 individuals were released near the site of capture after the behavioral experiments and processing  
1173 were completed. All procedures were approved by the university of washington institutional  
1174 animal care and use committee (protocol# 4307-02).

1175



1176

1177 3.3.2 *Experimental set-up*

1178 We conducted two-choice behavioral experiments without reward inside a flight cage (Coleman,  
1179 3.048 x 3.048 x 2.1336 m) under natural ambient conditions at La Selva. As shown in Figure 2,  
1180 we placed an infrared-sensitive handycam (4K HD Video Recording, Sony, Japan) on a tripod  
1181 (30 cm from the ground), which allowed us to record the bats' foraging behaviors under infrared  
1182 light conditions (>700nm; beyond the spectral range of vision of phyllostomids; Jones et al.  
1183 2013). We recorded the bats' echolocation calls with a condenser microphone (microphone  
1184 capsule CM16, CMPA preamplifier unit, Avisoft Bioacoustics, Berlin, Germany) mounted at the  
1185 top, center of the flight cage. During experiments, we visualized real-time calls using an  
1186 ultrasound acquisition board (UltraSoudGate 116, Avisoft Bioacoustics, Germany; sampling rate  
1187 375 kHz, 16-bit resolution). At the back end of the flight cage, we placed a custom-built platform  
1188 (90 cm long x 125 cm tall) which held two 50 ml falcon tubes, 40 cm apart, onto which we  
1189 mounted each of the target ("fruit") choices (Figure 2). To control for size and shape of these  
1190 targets, we used 3D-printed dummy fruits (Form 2 printer with FGLPWH02 resin) of the same  
1191 shape and size of an average *Piper scintillans* fruit. To mimic a ripe fruit, we smeared a dummy  
1192 (3D printed) fruit with a standardized amount of ripe *P. scintillans* fruit pulp (approx. 0.62 g,  
1193 ~1/3 of a total fruit) we used fruit collected on the same day of each experimental night. We  
1194 harvested vegetation (branches) from the same plants from which we collected ripe fruit. In trials  
1195 with vegetation present, we placed the vegetation at the base of the dummy fruit, which is the  
1196 natural configuration within the plant. Between each night of experiments, we cleaned dummy  
1197 fruits with 95% ethanol to remove scents, rinsed them with water, and let them air dry at least 24  
1198 hours before reusing.

1199           To test our hypotheses, we presented each bat with a choice between two of the following  
1200 targets during each experimental trial: 1) dummy with fruit scent (pulp from *P. scintillans*), 2)  
1201 dummy with vegetation only, 3) dummy with fruit scent and vegetation, and 4) dummy with no  
1202 fruit scent or vegetation (Table 1). We ran each trial for a maximum of 20 minutes per bat, and  
1203 subjected bats to up to four trials, conditional on their performance on the initial trial. If the bat  
1204 did not perform within 20 minutes, we released the individual. Conversely, if the bat attempted  
1205 to capture the fruit within the 20-minute duration of the trial, we considered it a positive trial and  
1206 began a new trial. To begin a new trial, one of us entered the flight cage and switched out choice  
1207 targets. We randomized both the order we presented each trial and the position (left, right) of the  
1208 target choice on the platform between consecutive trials to minimize confounding effects due to  
1209 bat spatial learning (Thiele and Winter 2004). At the end of trials, we used a hand net to  
1210 recapture the bat inside the flight cage, processed and released it, as described above.

1211

### 1212 3.3.3 *Analysis of flight behavior during target search and approach*

1213 We watched videos of the behavioral trials at normal playback speed on a computer at the  
1214 University of Washington, Seattle, WA. From each video, we recorded: the amount of time it  
1215 took the bat to capture one of the target choices presented, the real time of capture (to  
1216 synchronize with acoustic calls), and the individual's choice. We defined a capture event as a bat  
1217 landing on a target choice and attempting to bite it. We noted additional characteristics of the  
1218 bat's flight behavior (e.g., exploratory flights around the flight cage) and target exploration (i.e.,  
1219 hovering over fruit) for all trial videos.

1220 3.3.4 *Analysis of echolocation behavior during target approach*

1221 We analyzed echolocation calls emitted during target approach using Avisoft SASLabPro v. 4.40  
1222 (Avisoft Bioacoustics, Berlin, Germany). We used the time of the capture events from video  
1223 recordings and matched them with the time stamps of the call files to synchronize acoustics with  
1224 recorded capture events. These files were used in the subsequent analyses and included the  
1225 acoustic calls for one minute prior to the capture event, which we defined as the search/approach  
1226 window. We chose a minute interval prior to capture because we were not only interested in  
1227 sensory behavior for target localization (typically seen in the approach phase), but also the  
1228 sensory behavior when ‘searching’ for food. *Carollia castanea*, similar to other phyllostomids,  
1229 emits calls well above 20kHz (Thies et al. 1998, Brinkløv et al. 2011), so we used this as the cut-  
1230 off frequency to avoid including noise from recording at high gain in ambient conditions (Geipel  
1231 et al. 2013). We filtered each acoustic sequence using a high-pass filter (at 20 kHz) and  
1232 visualized spectrograms using a Hamming window (512 fast Fourier transform, 98.95% overlap).  
1233 We extracted the following echolocation call parameters for comparisons across trial types:  
1234 maximum frequency (kHz), minimum frequency (kHz), peak frequency (i.e. frequency with the  
1235 highest amplitude, kHz), call duration (ms), call interval (ms), and total bandwidth (kHz) from  
1236 the spectrograms at the maximum energy of each call. We compiled sequences per individual  
1237 (approximately 8 – 20) and calculated mean and standard deviation for each call parameter per  
1238 trial type per individual (Table 2).

1239

1240 3.3.5 *Statistical analyses of behavioral experiments*

1241 We performed all statistical analyses in R v. 3.2.4 (R Core Team, 2018). We used chi-squared  
1242 tests to assess the differences in bat preference among target types. We tested the normality of

1243 the echolocation call data using Shapiro-Wilks tests (Shapiro and Wilk 1965) and subsequently  
1244 log-transformed these data to improve normality. We compared the differences in call  
1245 parameters among trial types using analysis of variance (ANOVA).

1246

### 1247 3.3.6 *Target comparisons*

1248 To determine if *P. scintillans* ripe fruit and vegetation present different olfactory cues to *C.*  
1249 *castanea*, we compared the volatile organic compounds (VOCs) that make up the scents of these  
1250 plant parts. We collected vegetation (7.0 – 7.8 g of fresh weight leaf material; one branch) and  
1251 ripe fruit (19.9 –20.6 g, fresh weight; 13 – 15 fruits) samples from four *P. scintillans* plants at La  
1252 Selva. This larger sample of ripe fruit was necessary for VOC capture and detectability by our  
1253 experimental setup (below). We collected VOCs from these samples via dynamic headspace  
1254 adsorption using a push–pull system (Raguso and Pellmyr 2006, Riffell et al. 2008). Within two  
1255 hours of collection, we placed each sample in a 3L teflon bag (Reynolds, Richmond, VA, USA)  
1256 and connected the bag to a diaphragm pump (400-1901, Barnant Co., Barrington, IL, USA) that  
1257 pulled the fragrant headspace air through a sorbent cartridge trap (50 mg Porapak Q with  
1258 silanized glass wool Waters Corp., Milford, MA, USA) and pushed air through a charcoal filter.  
1259 We collected VOCs in this manner for 20 hours per sample, following previously established  
1260 protocols and to ensure characterization of the full chemical profile (Byers et al. 2014). We  
1261 eluted trapped volatiles from each sample’s sorbent cartridge with 600 µl of HPLC-grade hexane  
1262 into a 2mL borosilicate glass vial with a Teflon-lined cap. Subsequently, we stored all of the  
1263 samples at –20°C to –80°C. We analyzed a 3 µl aliquot of each sample using an Agilent 7890A  
1264 GC (gas chromatograph) and a 5975C Network Mass Selective Detector (Agilent Technologies,  
1265 Palo Alto, CA, USA). To separate the VOCs, we used a DB-5MS GC column (J&W Scientific,

1266 Folsom, CA, USA; 30 m, 0.25 mm, 0.25  $\mu\text{m}$ ) with helium as the carrier gas flowing at a constant  
1267 rate of 1 cc per min (Byers et al. 2014). The initial oven temperature was 45°C for 4 min,  
1268 followed by a heating gradient of 10°C min<sup>-1</sup> to 230°C, which was then held isothermally for 4  
1269 min. We initially identified the chromatogram peaks with the aid of NIST 08 mass spectral  
1270 library (v. 2.0f; ca. 220,460 spectra of 192,108 different chemical compounds) followed by  
1271 verification using alkane standards and comparing with published Kovats indices. We integrated  
1272 the peaks for each compound using ChemStation software (Agilent Technologies) and present  
1273 them in Table 4.

1274

## 1275 3.4 RESULTS

### 1276 3.4.1 *Target preferences*

1277 Most individuals performed exploratory flights prior to showing interest in the presented target  
1278 choices. These behaviors consisted of circling flights around the cage without approaching the  
1279 target. In most trials (83%), bats attempted to capture a target by landing and trying to bite the  
1280 dummy fruits (Supplementary Video 1). Comparisons across treatments revealed that *C.*  
1281 *castanea* strongly preferred targets consisting of a dummy fruit with fruit scent ( $n = 10$ ,  $\chi^2 = 6.21$ ,  
1282  $P = 5.69\text{e-}05$ ) or a dummy fruit with fruit scent and vegetation ( $n = 13$ ,  $\chi^2 = 22.154$ ,  $P = 2.52\text{e-}06$ )  
1283 over targets that had an unscented dummy fruit and vegetation. The presence of vegetation did  
1284 not affect the bats' preferences for fruit scent (dummy fruit with fruit scent vs. dummy fruit with  
1285 fruit scent and vegetation:  $n = 13$ ,  $\chi^2 = 0$ ,  $P = 0.99$ ). Bats never chose unscented dummy fruits,  
1286 either alone or with vegetation.

1287

1288 3.4.2 *Echolocation behavior*

1289 All bats used in the experiments emitted echolocation calls throughout the trials. We did not find  
1290 statistically significant differences in the echolocation call parameters among treatment types  
1291 during the search/approach window (minimum frequency, maximum frequency, peak frequency,  
1292 bandwidth, duration, pulse interval, all  $P > 0.05$ ; Table 3). However, there were marked trends  
1293 in call duration and interval in some trial types. Bats emitted echolocation calls more frequently  
1294 (shorter interval) and of longer duration in treatments where no fruit scent was present  
1295 (unscented dummy vs. unscented dummy with vegetation; Figure 4).

1296

1297 3.4.3 *Chemical differences between targets*

1298 The scent profiles of *P. scintillans* vegetation and ripe fruit differ slightly in VOC composition,  
1299 and greatly in the proportion of specific VOCs (Table 4). The vegetation scent of *P. scintillans* is  
1300 dominated by 3-hexene-1-ol, which is not found in the ripe fruit scent. Conversely, the ripe fruit  
1301 scent is characterized by a greater abundance of  $\beta$ -caryophyllene, germacrene D and  $\beta$ -elemene.  
1302 Both vegetation and fruit scents have a low abundance of p-cymene and  $\beta$ -pinene (Table 4).

1303

1304 3.5 DISCUSSION

1305 An animal's sensory ecology and behavior often reflects the environment it inhabits, as well as  
1306 its evolutionary history. As such, certain sensory modalities play key roles in mediating  
1307 ecological interactions. Mammalian frugivores are able to locate and acquire ripe fruit by using  
1308 and integrating across sensory modalities: they use vision to detect differences in fruit color and  
1309 luminance (Burkhardt 1989; Osorio and Vorobyev 2008; Melin et al. 2008; Hiramatsu et al.

1310 2008; Valenta et al. 2013), olfaction to detect individual volatile organic compounds (VOCs) or  
1311 entire odor plumes (Sánchez et al. 2006; Valenta et al. 2013) and, in the case of phyllostomid  
1312 bats, echolocation to gather information about fruit shape and location (Kalko and Condon 1998,  
1313 Von Helversen and Von Helversen 1999). Our results indicate that *C. castanea* makes foraging  
1314 decisions based on ripe fruit scent over all other cues presented, but may rely more heavily on  
1315 echolocation when olfactory cues are absent. Importantly, in the absence of plant scent cues, a *P.*  
1316 *scintillans* fruit shape does not elicit a prey capture response from *C. castanea*. This gives insight  
1317 to the primary role of olfaction, followed by echolocation, when these bats forage for fruit.

1318         The importance of olfaction for foraging in *C. castanea* is supported by previous research  
1319 on other *Carollia* species that rely less on *Piper* as a food resource. For example, *C. perspicillata*  
1320 can recognize minute concentrations of particular chemical components (fruit-typical odor  
1321 components like ethyl butyrate, n-pentyl acetate, or linalool; Laska 1990), are attracted to fruit  
1322 scent even when no other cues are present (Hessel and Schmidt 1994), and visit mist nets spiked  
1323 with the essential oil of *Piper gaudichaudianum* more frequently (Mikich et al. 2003). Here, we  
1324 link the bouquet of VOCs from a known, preferred food source with the behavioral preferences  
1325 of *C. castanea*. Our experimental results strongly suggest that *C. castanea* uses ripe fruit scent,  
1326 as opposed to a combination of ripe fruit and/or vegetation scent, or fruit shape, as the cue to  
1327 locate food items. Our chemical analyses of ripe fruit and vegetation VOCs provide an  
1328 explanation for this pattern: the scent profile of the *P. scintillans* ripe fruit and vegetation are  
1329 somewhat similar in composition, but differ greatly in abundance of some specific VOCs.  
1330 Additionally, the ripe fruit scent profile contained a few distinct VOCs that were not found in  
1331 vegetation, and vice versa. Considering that *C. castanea* forages in a complex sensory  
1332 environment, the forest understory, it may be advantageous for the bats to cue in specific

1333 chemicals that unmistakably signal fruit ripeness against the background of unripe fruit and  
1334 vegetation within a *Piper* bush, as well as adjacent vegetation. Our results motivate future work  
1335 to examine whether some of these key volatiles or their ratios may signal fruit ripeness amongst  
1336 the vegetative mélange.

1337         Echolocation call parameters did not differ significantly in frequency between trial types,  
1338 suggesting that *C. castanea* has a stereotyped call structure regardless of their foraging tasks.  
1339 While this has not been broadly studied, having a stereotyped echolocation call is common in  
1340 phyllostomids (Kalko and Condon 1998; Thies et al. 1998; Korine and Kalko 2005; Geipel et al.  
1341 2013). Nevertheless, our experiments revealed that *C. castanea* potentially modulates time-  
1342 linked echolocation traits (i.e., duration of the call and time between calls, interval) when  
1343 confronted with different prey cues. As in all mammals, phyllostomid bats process olfactory cues  
1344 by inhaling air through their nose, but also emit echolocation calls out of their nose. Because of  
1345 the potential conflict between these two functions performed by the nasal cavity, we propose that  
1346 frugivorous phyllostomids exhibit behavioral modulation in their nasally-linked senses to  
1347 alternate between and maximize effectiveness of one sensory cue versus another, when  
1348 appropriate.

1349         Echolocation provides bats with high-resolution information about shape, surface texture,  
1350 and material of an object at close range (Schnitzler et al. 1983, Ostwald et al. 1988, Kober and  
1351 Schnitzler 1990), but bats also use echolocation for navigation and detection of plants that signal  
1352 through morphology for better acoustic detection. We saw a general trend of longer duration of  
1353 echolocation calls and shorter intervals between calls when bats were offered choices that did not  
1354 include a ripe fruit scent cue. Decreased time between calls (interval) and longer duration means  
1355 these bats were calling more frequently in the absence of ripe fruit scent. We hypothesize that,



1356 when ripe fruit odor cues are absent, *C. castanea* relies more heavily on echolocation to locate a  
1357 potential food item, in this case one that may resemble an edible *Piper* fruit. In contrast, when  
1358 bats were presented with any target that had ripe fruit scent (one or two choices), they emitted  
1359 shorter echolocation calls at longer intervals, thus echolocating less frequently. We hypothesize  
1360 that the decrease in echolocation call duration and an increase in interval could be linked to an  
1361 increase in the bats' use of olfaction as they attempt to locate edible ripe fruits or determine  
1362 which one is the 'most edible' option.

1363         If ripe fruit scent is the primary cue for fruit location and selection by *C. castanea*, why  
1364 are there differences in echolocation call duration and interval between treatments with and  
1365 without ripe fruit scent? Previous studies have demonstrated that phyllostomids bats can use  
1366 echolocation to determine the position of a fruit (Kalko and Condon 1998), and echolocating bat  
1367 species, in general, alter call parameters to overcome acoustic masking effects during prey  
1368 location (Kalko and Schnitzler 1993). This can be accomplished by changing the duration and  
1369 interval of the call (Siemers and Schnitzler 2000). Bats typically extend the duration of a call  
1370 when searching for prey and during orientation flights (Kalko and Schnitzler 1993), and decrease  
1371 the time between calls when approaching a prey item (Kalko and Schnitzler 1993, Siemers and  
1372 Schnitzler 2000). In our experiments, bats never chose unscented dummy fruits as potential food  
1373 options, but our behavioral and acoustic recordings demonstrated that they did explore them via  
1374 echolocation. We propose that *C. castanea* has a series of criteria (e.g., fruit scent, shape,  
1375 configuration of fruit in relation to vegetation), which may be hierarchical, and are integrated  
1376 during the search and localization of a potential food item.

1377         The use of echolocation and olfaction for food selection has been documented in other  
1378 frugivorous and omnivorous phyllostomids. *Artibeus jamaicensis* is a specialized frugivore that

1379 detects, localizes and classifies ripe fruits primarily by olfaction (Kalko et al. 2010). In contrast,  
1380 *Phyllostomus hastatus*, a large omnivorous bat, consistently uses echolocation over olfaction  
1381 when foraging for *Gurania spinulosa*, a pendulous fruit-bearing vine (Kalko and Condon 1998).  
1382 These examples illustrate an echolocation-olfaction continuum across phyllostomids that forage  
1383 for fruit, and suggests that multiple sensory modes are important for fruit foraging in complex  
1384 environments. They also substantiate that sensing mode could be conditional on which food cues  
1385 are present or the degree of specialization of each species (e.g., omnivores vs. specialized  
1386 frugivores). To date, it is still unclear which senses are most important for fruit foraging in most  
1387 bat frugivores, and what facilitates the use of one sense over another.

1388         This study provides behavioral links between a frugivore's sensory abilities and plant  
1389 cues, a relationship that is critical to understanding the ecological dynamics and coevolution  
1390 between plants and their seed dispersers. There is still much to learn about how vertebrate  
1391 frugivores perceive and interact with their potential food sources, thus further observational and  
1392 experimental studies are critical for determining what specific fruit traits (e.g., compounds or  
1393 combination of compounds in ripe fruit) drive fruit selection by frugivorous species.

1394

1395 All procedures performed in studies involving animals were approved by and in accordance with  
1396 the ethical standards of the University of Washington Institutional Animal Care and Use  
1397 Committee (protocol# 4307-02)

1398

### 1399     3.6    DATA AVAILABILITY

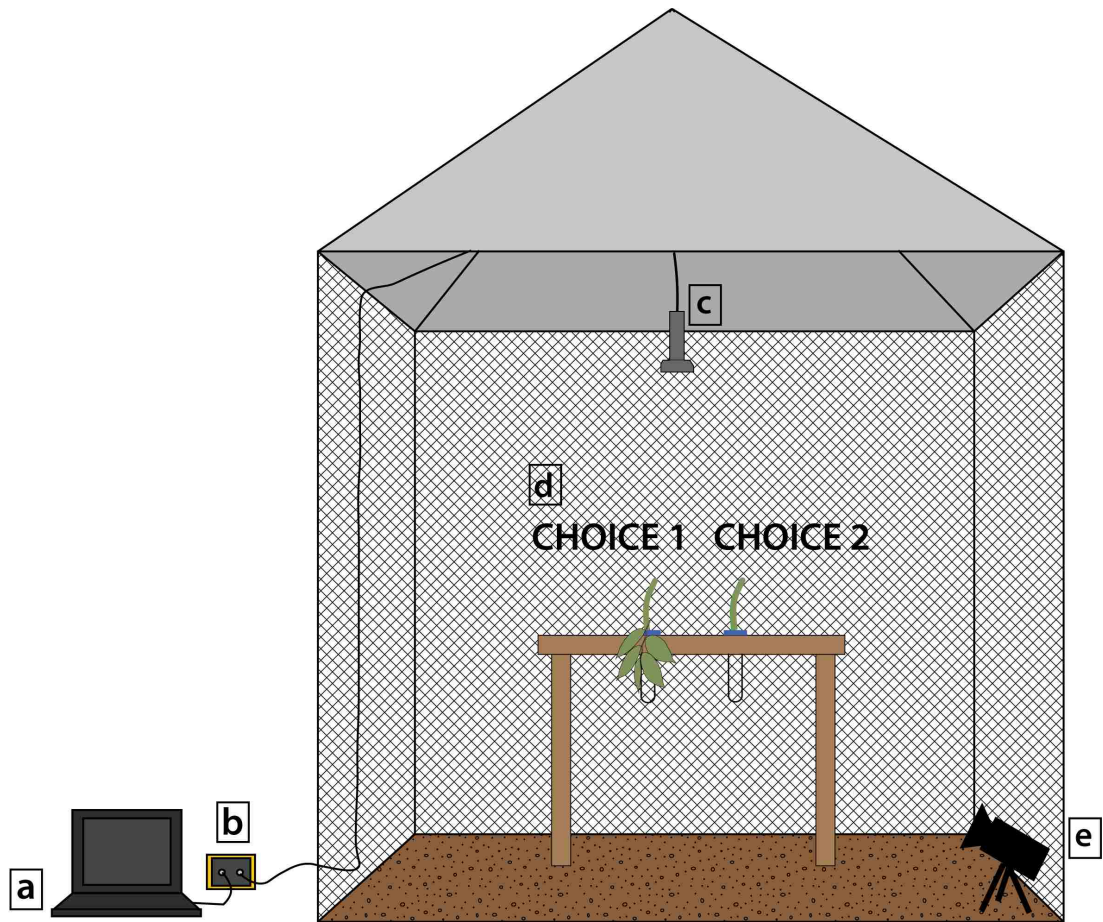
1400 The datasets generated and analyzed during the current study are available from the  
1401 corresponding author on reasonable request.



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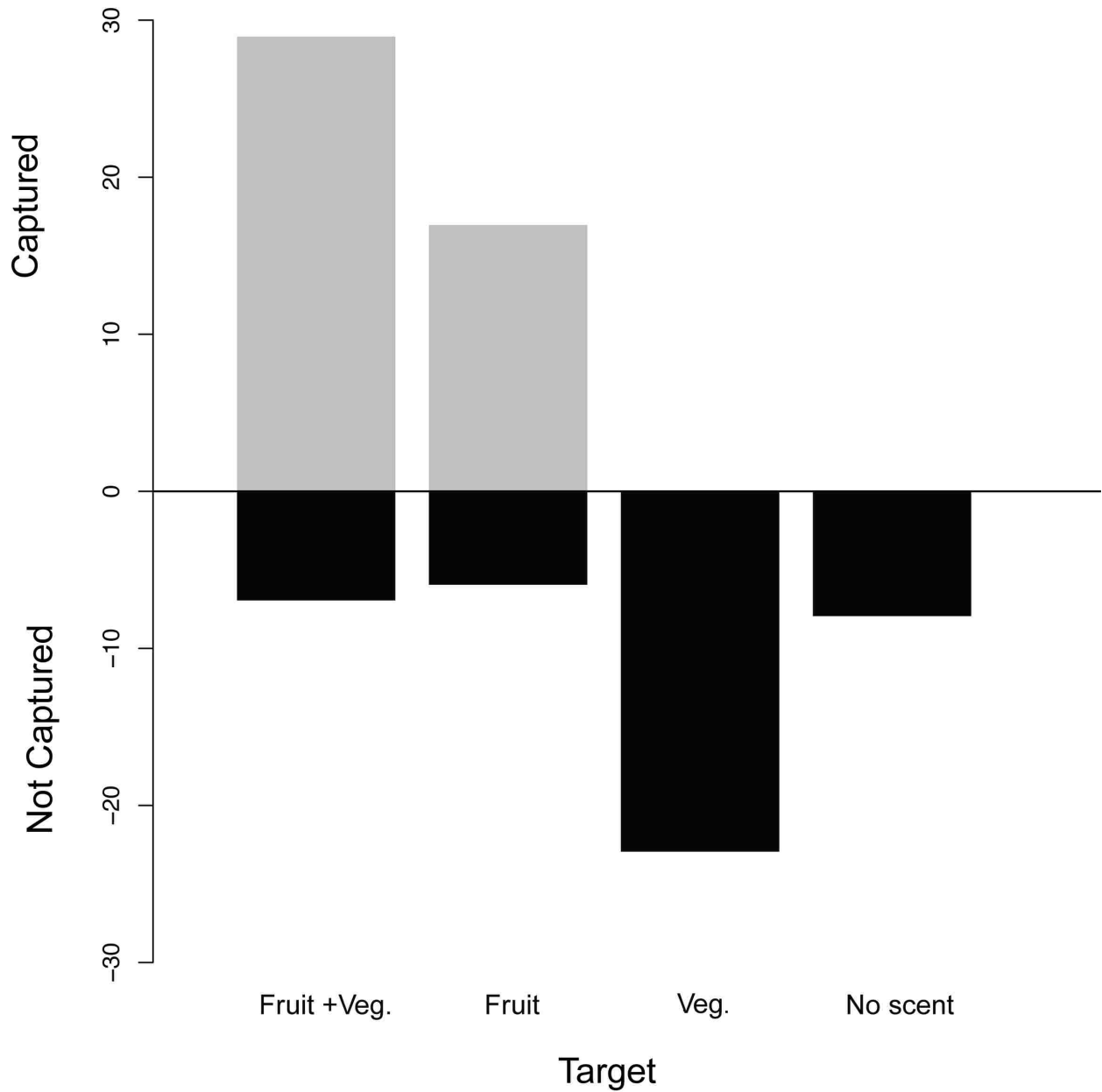
1403 Figure 3.1. Study organisms, *Carollia castanea* (left) and *Piper scintillans* (right)

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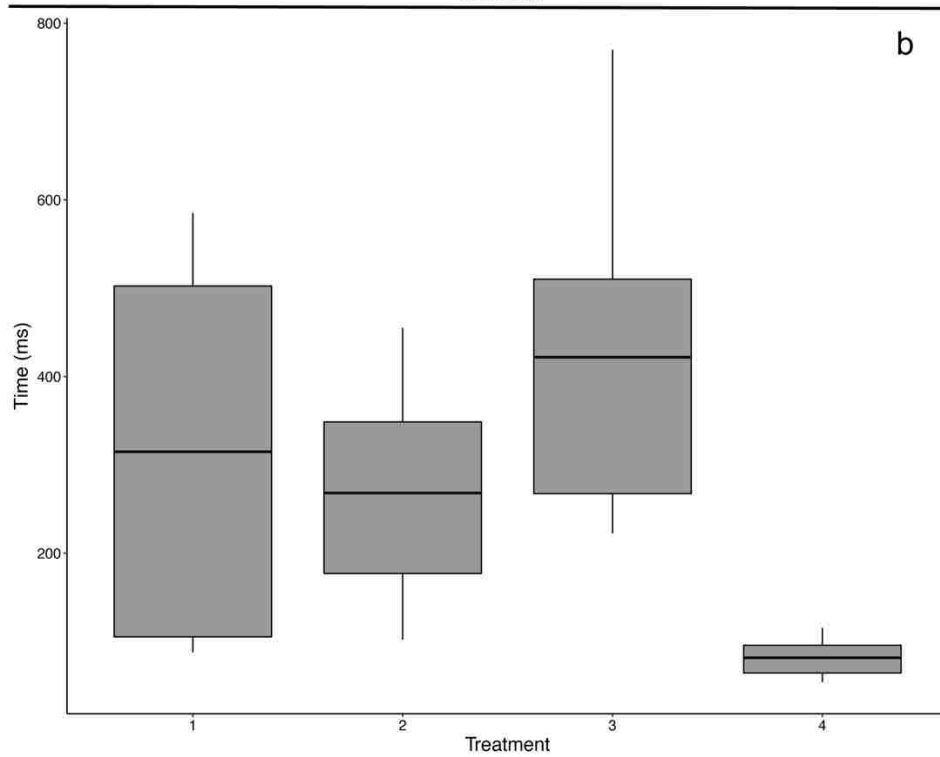
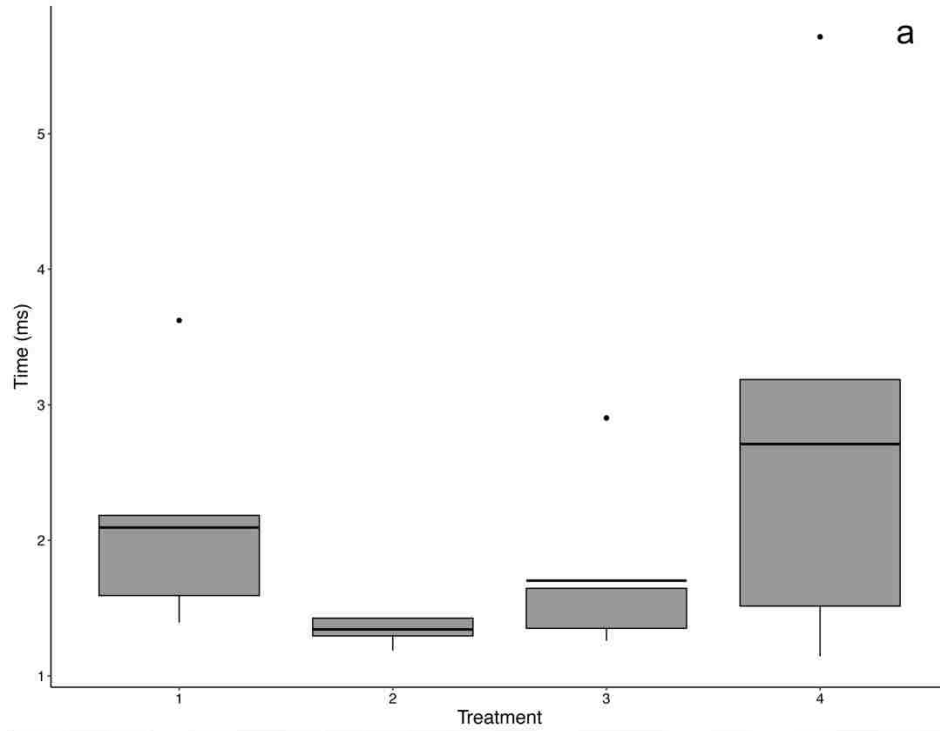
Figure 3.2. Diagram of experimental set-up: echolocation calls were visualized and recorded via a Dell 14 Rugged Extreme laptop (a) connected to a USG 116H recorder (b) that was connected to a CM16 condenser microphone (c). Target choice options were offered on a custom-made platform (d), here showing 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)



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1417 Figure 3.3. Summary of successful target captures by *Carollia castanea* for each of the four  
1418 target types across behavioral experiments: dummy with fruit scent and vegetation (Fruit +  
1419 Veg.), dummy with fruit scent only (Fruit), dummy unscented with vegetation only (Veg.), and  
1420 dummy unscented (No scent)

1421



1422

1423 Figure 3.4. Summary of the duration of *Carollia castanea*'s echolocation calls across treatments

1424 (a), and summary of the interval of *Carollia castanea*'s echolocation calls across treatments (b).

1425 Treatments are described in Table 1

1426

1427

1428 Table 3.10. Description of the two target choices offered within each experimental treatment, and the response being tested during  
 1429 behavioral experiments on *Carollia castanea*. Number of trials differed among treatments due to differences in the number of positive  
 1430 responses of experimental bats (see Methods)

1431

<b><i>Treatment</i></b>	<b><i>Choice 1</i></b>	<b><i>Choice 2</i></b>	<b><i>Test</i></b>	<b><i>Number of Trials</i></b>
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

1432 Table 3.11. Means ( $\pm$  standard deviation) of echolocation call parameters for *Carollia castanea* during each experimental treatment  
1433 (from Table 1). Means for the call parameters were calculated by averaging the calls for each individual bat across its entire calls  
1434 sequence (the approach call for one individual, for one treatment), and averaging each of these values across individuals within each  
1435 treatment

1436

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

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1438



1439 Table 3.12. Summary of ANOVA results comparing each call parameter trait across the four  
1440 experimental treatments (from Table 1).

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1442	<b>Variable</b>	<b>Df</b>	<b>Sum. Sq.</b>	<b>Mean Sq.</b>	<b>F-Value</b>	<b>P(&gt;F)</b>
1443	Duration	1	0.852	0.852	0.728	0.405
1444	Interval	1	1.37	1.37	1.209	0.287
1445	Peak Freq.	1	0.36	0.361	0.194	0.665
1446	Min. Freq.	1	0.00	0.0001	0.00	0.995
1447	Max. Freq.	1	0.00	0.002	0.001	0.975
1448	Bandwidth	1	0.00	0.0004	0.000	0.989

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1462 Table 3.13. Volatile organic compounds found in the scent of *Piper scintillans* ripe fruit and  
1463 vegetation. Values are the mean proportion across all samples (n = 2 fruit; n = 2 vegetation).  
1464 KRI: Kovats retention indices; IUPAC: International Union of Pure and Applied Chemistry  
1465 nomenclature

Chemical Name	IUPAC	KRI	Class
3-Hexene-1-ol	(Z)-hex-3-en-1-ol	858	aliphatic alcohol
$\beta$ -Pinene	6,6-dimethyl-4-methylidenebicyclo[3.1.1]heptane	981	monoterpene
$\alpha$ -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
$\alpha$ -Terpinene	1-methyl-4-propan-2-ylcyclohexa-1,3-diene	1020	monoterpene
p-Cymene	1-methyl-4-propan-2-ylbenzene	1028	aromatic
$\beta$ -Ocimene	3,7-dimethylocta-1,3,6-triene	1050	monoterpene
$\gamma$ -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
$\alpha$ -Cubebene	4,10-dimethyl-7-(propan-2-yl)tricyclo[4.4.0.0 <sup>1,5</sup> ]dec-3-ene	1354	sesquiterpene
$\beta$ -Elemene	2,4-Diisopropenyl-1-methyl-1-vinylcyclohexan	1394	sesquiterpene
$\alpha$ -Cedrene	Cedr-8-ene	1419	sesquiterpene
$\beta$ -Caryophyllene	4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	1433	sesquiterpene
$\alpha$ -Bergamotene	4,6-dimethyl-6-(4-methylpent-3-enyl)bicyclo[3.1.1]hept-3-ene	1440	sesquiterpene
$\alpha$ -Caryophyllene	2,6,6,9-tetramethylcycloundeca-1,4,8-triene	1470	sesquiterpene
$\gamma$ -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-e octahydro-1aH-cyclopropa[e]azulene	1504	sesquiterpene

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Caryophyllene	4,12,12-Trimethyl-9-methylene-5-	1581	sesquiterpene
oxide	oxatricyclo[8.2.0.0 <sup>4,6</sup> ]dodecane		

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## 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).

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

Phyllostominae	<i>Lophostoma</i>	<i>brasiliensis</i>	21604	PV
Phyllostominae	<i>Lophostoma</i>	<i>brasiliensis</i>	022614-1	PV
Macrotinae	<i>Macrotus</i>	<i>waterhousii</i>	dr135fha	-
Micronycterinae	<i>Micronycteris</i>	<i>hirsuta</i>	030214-2	PV
Micronycterinae	<i>Micronycteris</i>	<i>microtis</i>	022814-1	PV
Micronycterinae	<i>Micronycteris</i>	<i>microtis</i>	82017-06	LS
Micronycterinae	<i>Micronycteris</i>	<i>microtis</i>	277689	PV
Micronycterinae	<i>Micronycteris</i>	<i>minuta</i>	030314-2	PV
Micronycterinae	<i>Micronycteris</i>	<i>minuta</i>	022814-4	PV
Phyllostominae	<i>Gardnerycteris</i>	<i>crenulatum</i>	082017-01	LS
Glossophaginae	<i>Monophyllus</i>	<i>redmani</i>	1086fha	DR
Phyllostominae	<i>Phyllostomus</i>	<i>discolor</i>	82017-07	LS
Phyllostominae	<i>Phyllostomus</i>	<i>hastatus</i>	082017-08	LS
Stenodermatinae	<i>Phyllops</i>	<i>falcatus</i>	1117fha	DR
Phyllonycterinae	<i>Phyllonycteris</i>	<i>poeyi</i>	dr750fha	DR
Stenodermatinae	<i>Platyrrhinus</i>	<i>helleri</i>	082017-09	LS
Stenodermatinae	<i>Sturnira</i>	<i>lilium</i>	278299	PV
Phyllostominae	<i>Trachops</i>	<i>cirrhosus</i>	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	<b>0.035*</b>
		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	<b>0.022*</b>
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	<b>0.009**</b>
		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	<b>0.0438*</b>
	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	<b>0.0124*</b>
	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	<b>0.0243*</b>
		Insectivore	-0.0219	0.0146	-1.50	0.146
		Nectarivore	-0.0315	0.0143	-2.20	<b>0.0379*</b>
		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	<b>0.0313*</b>
		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	<b>0.0360*</b>
	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

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4	Omnivore	-0.00361	0.0277	-0.130	0.898
	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

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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	<b>0.005**</b>
	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	<b>0.0451*</b>
Pinna	1	2	0.02565	0.012827	6.963	<b>0.0057**</b>
	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)

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

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Stone, D., Lyons, A.C. , Huang, YJ. S. ,Vanlandingham, D.E, Higgs S. , Blitvich, B.J., Adesiyun, A.A., Santana S.E., **Leiser-Miller, L. B.** , Cheetham, S. (2018). Serological evidence of widespread exposure of Grenada fruit bats to chikungunya virus. *Zoonoses and Public Health*. 505 - 511.

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