# Diet and trophic niche partitioning of three aerial hawking bat species in Venezuela

Yara Azofeifa Romero (1, 2, †), Jafet M Nassar (1), Jesús Mavárez (3, ‡)

(1) Laboratorio de Biología de Organismos, Centro de Ecología, Instituto Venezolano de Investigaciones Científicas, Carretera Panamericana km 11, Caracas 1204, Venezuela.

(2) Laboratorio de Sistemática, Genética y Evolución (LabSGE), Escuela de Ciencias Biológicas, Universidad Nacional. Heredia 40101, Costa Rica.

(3) Laboratoire d'Ecologie Alpine, Université Grenoble Alpes, Université Savoie Mont Blanc, CNRS, F-38000 Grenoble, France.

Correspondence:

(†): LabSGE, Campus Omar Dengo, Calle 9, Avenidas 0 y 1, Costa Rica; yara.azofeifa.romero@una.ac.cr

(‡): LECA, Biologie Bâtiment D, 2233 rue de la Piscine, 38610 Gières, France; jesus.mavarez@univ-grenoble-alpes.fr

## ABSTRACT

Since Neotropical insectivorous bats (IB) often share feeding sites and prey, their coexistence likely depends on processes promoting divergence at trophic niche dimensions. Here we conducted DNA metabarcoding analyses of diets and activity times of three coexisting aerial hawking IB species — *Molossus molossus, Eptesicus furinalis*, and *Myotis nigricans* — from rice fields in the Venezuelan Llanos, aiming to understand their patterns of trophic niche partitioning. Insect prey were identified using DNA sequences obtained from bat fecal samples and then compared against GenBank and a local sequence database. Additionally, we recorded bat vocalizations over rice fields simultaneously with the bat captures. We compared diversity, composition, and breadth of the three IB diets, and employed statistical models to understand: (1) the dietary and activity time overlap among IB species, and (2) the effect of IB species' morphology and hunting strategies over prey's hardness and flight speed. We found high prey richness for *M. molossus* (32 families), *M. nigricans* (19 families), and *E. furinalis* (13 families). Bats exhibited differences in prey richness, proportions, types consumed, and in their activity periods. For instance, as expected from its larger size and fast flight, we found that *M. molossus* prefers larger, fast-flying, and medium-to-high sclerotized preys such as Coleoptera and Lepidoptera, with hunting activities starting and peaking significantly earlier than in the other two bat species. On the other hand, also agreeing with its size and flight type, *M. nigricans* exhibits a significant preference for small, slow-flying, and weekly sclerotized preys such as Diptera, although with relatively high dietary and temporal overlaps with *E. furinalis.* Thus, in concordance with niche theory predictions, the coexistence between these bats, particularly between *M.*

*molossus* and the other two species, seems enhanced by reduced competition driven by divergence at trophic niche dimensions.

**Keywords:** agricultural lands, diets, DNA metabarcoding, insectivorous bats, trophic niche, Venezuela

## INTRODUCTION

Insectivorous bats (IB) are remarkably diverse predators with 20-45 species coexisting in syntopy in tropical forests and crops in the Neotropics, Western Africa, and Southeast Asia (Kingston et al. 2003; Williams-Guillén and Perfecto 2011; Reardon and Schoeman 2017). The high local species richness of IB is likely a result of ecomorphological differences allowing for a wide variety of hunting and echolocation strategies among coexisting species, with which trophic niche differentiation is possible. Indeed, coexisting IB usually differ in relation to wing morphology, body mass, skull shape, and echolocation call design, all of which correlate with habitat use, foraging strategies employed, and types of prey consumed (Marinello and Bernard 2014). Foraging strategies and diets appear thus to be important axes of ecological differentiation, contributing directly to resource partitioning among IB species.

Knowledge about foraging strategies in IB has been accumulating steadily in recent decades, particularly with the advent of ultrasonic recording devices. Studies based on acoustic surveys have indicated that sympatric aerial IB species partition food habitats both horizontally and vertically, even at fine-grain spatial scales, in tropical and temperate environments (Marques et al. 2016; Roemer et al. 2019; Beilke et al. 2021). On the other hand, several studies have highlighted temporal differences in the onset

67 and/or maximum feeding activity periods among Neotropical IB species (Esbérard and
68 Bergallo 2010; Gomes et al. 2020). These spatiotemporal differences in foraging
69 strategies and patterns of activity time most likely led to dietary differences among IB
70 species, particularly in relation to size, hardness, position in the air column, and flight
71 mode of consumed prey (Segura-Trujillo et al. 2016, 2022). Regarding diets,
72 metabarcoding genetic analyses in IB from temperate regions have provided good
73 evidence supporting trophic flexibility, with different degrees of dietary overlap that can
74 only be explained through subtle spatiotemporal partitioning of food resources (Aizpurua
75 et al. 2018; Arrizabalaga-Escudero et al. 2019; Gordon et al. 2019; Andriollo et al.
76 2021). In contrast, molecular-based diet analyses in neotropical IB are still relatively
77 scarce, usually have low taxonomic resolution, and rarely make inter-specific
78 comparisons (but see Emrich et al. 2014, Salina-Ramos et al. 2015, Aguiar et al. 2021,
79 and Segura-Trujillo et al. 2022).

80 In this study, we performed a DNA metabarcoding analysis of diets of three aerial
81 hawking IB species from the Northwestern Llanos of Venezuela: *Molossus molossus*
82 (Molossidae), *Myotis nigricans*, and *Eptesicus furinalis* (Vespertilionidae). These bats
83 are frequently sympatric and can even inhabit the same roosts (Wilson and LaVal 1974).
84 They usually feed in the same areas, including relatively open spaces, forest gaps and
85 margins, and mosaics of agriculture and forestland in rural environments (Linares 1998).
86 However, the three species differ in various morphological and behavioral aspects that
87 greatly affect their feeding strategies. For example, *M. molossus* has larger body,
88 forearm, skull, and bite force than *M. nigricans*, while *E. furinalis* presents values that
89 are either similar to *M. nigricans* (body weight and skull length) or to *M. molossus*

(forearm length and bite force) (Linares 1998; Aguirre et al. 2002). Besides, differences in wing shape and aspect ratio imply a fast and high flight in unobstructed spaces in *M. molossus* (Marinello and Bernard 2014; Amador et al. 2020); but a comparatively slower and more maneuverable flight in background-cluttered spaces in *M. nigricans* and *E. furinalis* (Amador et al. 2020).

We analyzed diet composition and activity time of echolocation calls in the three species to understand their patterns of trophic niche partitioning. We predicted that given *M. molossus*' relatively larger size and distinctive flight type, its diet should both be composed by a broader spectrum of prey and show relatively low overlap with the other two species. Under the same argument, the diets of *E. furinalis* and *M. nigricans* should exhibit some overlap given their smaller size and similar flight type. Furthermore, *M. molossus*' relatively strong skull, fast flight, and long-term calls, should allow a diet with important proportions of highly sclerotized and fast-flying insects. In contrast, these types of insects should be relatively infrequent in the diets of *E. furinalis* and *M. nigricans*, and be replaced by less sclerotized and slow-flying insects. Some predictions can also be made in relation to bat activity time. For instance, if the activity time in these bats is maximal during periods of high availability of preferred prey, *E. furinalis* and *M. nigricans* should exhibit a significant degree of activity overlap over time given their probably similar diets. For *M. molossus*, its overlap could be high if the availability of its preferred prey is synchronous with that of the other two bats, and relatively low if it is not.

## MATERIALS AND METHODS

### Study area

This study was conducted in the Northwestern Llanos, Portuguesa state, Venezuela. Two rice farms located in this region were selected: Colonia Agrícola Turén, Turén (hereafter "Turén"; 9.215005267° N, -69.122405063° W, 140 m asl.) and Agropecuaria Durigua, Acarigua (hereafter "Acarigua"; 9.542531825° N, -69.115494594° W, 180 m asl.) (Fig. 1).

## Sampling

**Bats.** Bat captures followed the guidelines recommended by the American Society of Mammalogists (Sikes et al. 2016). The two farms were visited six times between November 2013 and September 2014 and bats were captured using mist nets placed near their roosting sites. In Turén, the three bat species cohabitated the same roosting site, while in Acarigua only *M. molossus* and *M. nigricans* were found in the selected roosting site. However, *E. furinalis* certainly roosted in the forest remnants nearby this farm, as it was recorded foraging in the rice fields. Bats were captured between 20:00 and 21:30 h, when they returned to their roost after their first food intake. Fecal pellets collected were placed under a small cotton ball at the bottom of a sterile 15 mL polypropylene centrifuge tube, and dried by filling the tube with silica gel type III (Sigma-Aldrich H S7625-500 G). In total, fecal samples from 136 bats were collected (*M. molossus*: 71, *M. nigricans*: 44, *E. furinalis*: 21).

**Insects**. Nocturnal insects were captured in the rice fields on the same dates and hours of bat captures. The insects were identified and preserved in ethanol 70%. Of the 110.767 insects captured, we identified 11 orders, 52 families, and 98 morphospecies (Moreno et al. 2020).

135 **DNA extractions**

136 **Bat fecal samples**. DNA was extracted from ten fecal pellets of each bat using
137 the DNasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). Sterile 1.5 mL
138 microcentrifuge tubes containing fecal pellets and 1 mL lysis buffer solution were placed
139 on a shaker at 30 rpm in an incubator at 55-56°C for two hours. Afterward, samples
140 were centrifuged for 1 min at 1000 rpm, and 100 µL of supernatants were extracted and
141 deposited in new sterile 2 mL microcentrifuge tubes. The remaining protocol steps
142 followed the manufacturer's instructions. To detect cross-contamination, extractions
143 were performed in batches of 24 tubes, including two no-feces negative controls with
144 and without water. Extracted DNA was stored at -18°C until subsequent PCR analysis.

145 **Insects**. DNA was extracted from two legs of insects with body size > 10 mm and
146 from five whole bodies of smaller ones. Extractions were performed using the DNasy
147 Blood & Tissue Kit following the manufacturer's instructions.

148 **PCR amplification and sequencing**

149 Two regions in the mitochondrial 16S rDNA unit were PCR-amplified for both
150 fecal and insect samples: "Inse01" with primers Inse01-F 5'-
151 RGACGAGAAGACCCTATARA-3' and Inse01-R 5'-ACGCTGTTATCCCTAARGTA-3',
152 and "Arth02" with primers Arth02-F 5'-GATAGAAACCAACCTGGYT-3' and LR-N-12945-
153 2 5'-GCGACCTCGATGTTGGATT-3' (Taberlet et al. 2018). All the primers had a
154 common adapter used for Illumina sequencing and an individual 8-bp barcode that
155 allowed the simultaneous sequencing of all samples.

156 DNA extracts were PCR-amplified in triplicate for fecal samples and once for
157 insects. PCR reactions (volume 20 µL) contained: 10 µL of AmpliTaq Gold Master Mix
158 (Applied Biosystems™, Foster City, USA), 0.2 µM of each primer, 5.84 µL of water, 0.04
159 µg of bovine serum albumin (Roche Diagnostic, Basel, Switzerland), and 2 µL of DNA
160 diluted 1:20. The PCR protocols were: 1x: 10 min at 95 °C, 45x: 30 s at 95 °C, 30 s at 52
161 °C (16S-1) or 50 ºC (16S-2), 60 s at 72 °C, 1x: 7 min at 72 °C. The PCR batches
162 included two extraction negatives, a PCR-negative (water), and a blank (empty well).
163 The PCR products were pooled per replicate, resulting in eight pools: six bat diets (three
164 per metabarcode) and two insects (one per metabarcode). Amplicon pools were purified
165 using the MinElute PCR Purification kit (Qiagen), quantified using a Qubit 2.0
166 Fluorometer (Life Technology Corporation), and sent to Fasteris SA (Geneva,
167 Switzerland) for library preparation and sequencing using the MetaFast protocol
168 (www.fasteris.com/metafast; Taberlet et al. 2018).

169 **Bioinformatic analyses**

170 The raw sequences were processed separately for each library using the
171 *OBITools* package (Boyer et al. 2016). Reads were assembled with a minimum quality
172 score of 40 and assigned to samples based on unique tag and primer combinations,
173 allowing for two mismatches with the primer. Sequences more abundant in extraction,
174 PCR and blank negative controls than in samples were considered as contaminants and
175 removed. Sequences with coverage <10 reads, or with lengths outside the expected
176 range (e.g., 76–168 bp for Arth02, Taberlet et al. 2018), or present in only one of the
177 three fecal sample replicates were also discarded. The remaining sequences were
178 clustered into prey MOTUs (*i.e*. Molecular Operational Taxonomic Unit) using the

179 sumaclust algorithm with a sequence similarity of 97% (Mercier et al. 2013). Taxonomic
180 assignment of MOTUs was achieved using the NCBI BLAST algorithm (Altschul et al.
181 1990), first against the 16S sequence database from local insects, and then against
182 GenBank for the sequences that could not be assigned in the previous way. The
183 following *ad hoc* assignment rules were applied in this study: (1) If there was a single
184 best match: species level for similarity ≥ 98%, genus level for 95% ≤ similarity < 98%,
185 family/sub-family level for 92% ≤ similarity < 95%, order level for similarity < 92%; (2) If
186 there were multiple best matches: assigned to the lowest taxonomic level subsuming all
187 best matches (e.g. assigned to the generic level if all best matches correspond to two or
188 more species in the same genus). Sequences that remained unassigned were classified
189 as "unknown". A table of diet items was created by removing rare occurrences (<
190 0.01%) and converting the counts into presence/absence values.

191 **Bat activity time**

192 We used two Echo Meter 3+ detectors placed on top of 2 m tall poles to record
193 bat calls as described by Azofeifa et al. (2019). Bat activity time was recorded
194 continuously for 240 min, beginning at sunset. Each sampling session consisted of two
195 consecutive nights, resulting in four bat recordings per rice farm. This two-night
196 recording scheme was repeated six times (three per climatic season), with a one-month
197 interval between two consecutive sampling sessions, resulting in 24 activity records per
198 farm.

199 **Data analyses**

We analyzed the diet composition of the three bat species using only insect presence/absence data. Observed and expected richness of insect taxa in the bat diets were calculated throughout the entire study period using the nonparametric estimator Incidence-Based Bootstrap (specpool function, R package Vegan 2.6-4, Oksanen et al. 2022). Differences in diet composition were analyzed with PerMANOVA tests based on Jaccard distance matrices using PRIMER v7 software (Clarke and Gorley 2015), followed by pairwise comparisons among bat species using non-parametric pseudo-t tests (akin to t-tests).

We conducted a comprehensive literature review to classify the feeding behavior of adult insects into three main categories: phytophagous, saprophagous, and zoophagous. Additionally, whenever possible, we assigned secondary categories (e.g. anthophagous, detritivorous, hematophagous, etc). This review also searched for biological characteristics of prey such as the degree of sclerotization (strong, moderate, or weak), and flight behaviors (fast, intermediate, or slow).

We employed Generalized Linear Models (GLMs) with a Poisson distribution, using the R package *MuMIn* 1.47.5 (Bartoń 2023), to assess the impact of bat factors such as species, functional group, forearm length, skull length, or bite force on characteristics of the prey consumed, using the prey count for each category as the response variable. We determined the relative importance of each variable using Akaike's Information Criterion (AIC) and the weight of evidence (wi). Statistical significance was determined by $p < 0.05$, with confidence intervals excluding zero indicating a significant impact for the predictor.

The trophic niche width, based on the prey proportion in the diet of each bat species, was calculated using Levin's index (Levins 1968) with the niche.width function in the R package *Spaa* 0.2.2 (Zhang 2016). The Levin's index was transformed into the Best index, which standardizes it on a scale of 0 (*i.e.* specialized diet) to 1 (*i.e.* diversified diet) (Krebs 1999). Diet overlap between species was evaluated using Pianka's niche overlap index (Pianka 1973). We used the function niche_null_model in the R package *EcoSimR* 0.1.0, to compare Pianka's index obtained with 100,000 replicates of null models (Gotelli et al. 2015). To illustrate the dietary overlap among the three species, we conducted a Principal Component Analysis (PCA) using the Hill and Smith method with the function dudi.hillsmith from the R package ade 4 1.7-22 (Dray et al. 2023).

We also analyzed the overlap in activity time of the three bat species using a non-parametric approach based on a kernel density estimated with the function density in the R package *Stats* 4.3.2. We obtained estimates of the Weitzman Superposition Coefficient Δ4 and its 95% confidence intervals using the *R* package *Overlapp* 0.3.9 (Meredith *et al*., 2024). We used the Watson and Watson-Wheeler tests available in R package *Circular* 0.5-0 (Agostinelli and Lund 2023) to evaluate the goodness-of-fit of the activity time data for individual species, and to compare the activity time data distributions across pairs of bat species. Finally, we plotted the kernel density-time curves with default smoothing parameters of the density function with the R package *ggplot2* (Wickham 2016).

## RESULTS

### Richness and composition of the bat diets

245 A total of 65 insect taxa were identified in the feces of the three bats species, with
246 identifications ranging from order and family to, in some instances, the species level
247 (Table 1, Supplementary Data SD1). Diets included 50 MOTUs from 71 *Molossus*
248 *molossus*, 31 MOTUs from 44 *Myotis nigricans*, and 22 MOTUs from 21 *Eptesicus*
249 *furinalis*. Expected richness of insect taxa were 60.02 (SD = 2.89) for *M. molossus*,
250 36.17 (SD = 2.05) for *M. nigricans*, and 25.90 (SD = 1.89) for *E. furinalis*. Estimated
251 completeness of the diets were 83% for *M. molossus*, 86% for *M. nigricans*, and 85% for
252 *E. furinalis*. Statistical exploration of these MOTUs suggested differences in the dietary
253 composition of the bat species (Pseudo-$F_{2, 133}$ = 3.64, *P*-perm = 0.001). Specifically, the
254 pairwise comparisons *M. molossus* vs. *M. nigricans* (pseudo-*t* = 2.39, *P*-perm = 0.001)
255 and *M. molossus* vs. *E. furinalis* (pseudo-*t* = 1.70, *P*-perm = 0.003) were significantly
256 different.

257 Diet composition of the three bat species differed significantly at the order level
258 (Pseudo-$F_{2, 133}$ = 4.83, *P*-perm = 0.001). Ten insect orders were identified in the fecal
259 samples, with nine in *M. molossus*, seven in *M. nigricans*, and five *E. furinalis*. Highly
260 significant differences in composition of insect orders occurred between *M. molossus* vs.
261 *M. nigricans* (Pseudo-*t* = 2.52, *P*-perm = 0.001) and *M. molossus* vs. *E. furinalis*
262 (Pseudo-*t* = 2.31, *P*-perm = 0.003). For *M. molossus*, the preferred prey sequence was
263 Coleoptera, Lepidoptera, and Diptera. In contrast, *M. nigricans* primarily favored Diptera,
264 followed by Coleoptera and Hemiptera; whereas *E. furinalis* favored Coleoptera,
265 followed by Diptera and Hemiptera (Fig. 2A).

266 At the family level, differences were also notable among bat species (Pseudo-$F_{2,}$
267 $_{133}$ = 3.92, *P*-perm = 0.001). Forty insect families were identified in the fecal samples,

268 with 32 in *M. molossus*, 19 in *M. nigricans*, and 13 in *E. furinalis*. As above, the most
269 notable differences in composition of insect families were also observed between *M.*
270 *molossus* vs. *M. nigricans* (Pseudo-*t* = 2.39, *P*-perm = 0.001) and *M. molossus* vs. *E.*
271 *furinalis* (Pseudo-*t* = 1.85, *P*-perm = 0.002). For *M. molossus*, favored insect families,
272 from most to least preferred, were Tipulidae, Elateridae, Crambidae, Curculionidae,
273 Noctuidae, and Scirtidae. *Myotis nigricans*, on the other hand, primarily favored
274 Tipulidae, followed by Cicadelliadae, Heteroceridae, Crambidae, Ptilodactylinae, and
275 Culicidae, whereas *E. furinalis* preferred Tipulidae, followed by Cicadellidae, Carabidae,
276 Ptilodactylinae, Curculionidae, and Culicidae (Fig. 2B). Some prey were assigned to
277 genus or species level in orders Coleoptera (*Aeolus* sp., *Lissorhoptrus* sp.), Lepidoptera
278 (*Spodoptera frugiperda*, *Leucania* sp., *Condica sutor*, *Rupela albinella*, *Diatraea*
279 *saccharalis*, *Eoreuma loftini*), and Hemiptera (*Graminella* sp., *Tagosodes orizicolus*,
280 *Oebalus ypsilon-griseus*), most of which were ingested by *M. molossus* (Table 1).

281 No notable differences among bat species were detected as a function of prey
282 trophic groups. The three bat species primarily feed on phytophagous insects (39.9% of
283 diet in *M. molossus*, 31.1% in *E. furinalis*, 29.2% in *M. nigricans*, Fig. 2C).
284 Saprophagous prey represented 10.8% in *M. nigricans*, 8.2% in *E. furinalis*, and 5.2% in
285 *M. molossus*, while zoophagous prey accounted for just 8.2% in *E. furinalis*, 6.2% in *M.*
286 *nigricans*, and only 2.4% in *M. molossus*. Regarding the secondary functional
287 classification of the diet, *M. molossus* and *E. furinalis* exhibited four categories of
288 phytophagous insects (anthophagous, seed-feeding, phyllophagous, and xylophagous),
289 while *M. nigricans* displayed three categories (anthophagous, seed-feeding, and
290 phyllophagous). Additionally, four secondary categories within saprophagous insects

291 (detritivores and fungivores) and zoophagous insects (predators and hematophagous)
292 were detected in the diets of all three bat species.

293 **Prey types**

294 **Sclerotization**. The primary components in the diet of the two species with the
295 strongest bites, *M. molossus* and *E. furinalis*, were prey with a high degree of
296 sclerotization (Fig. 3). The results from the GLMs suggested that the lower bite force of
297 *M. nigricans* was negatively associated with highly sclerotized prey (wi = 0.63, *P* =
298 0.0114, CI = -0.92 to -0.12). Nonetheless, a notable similarity among the three species
299 was observed regarding the consumption of prey with intermediate sclerotization (Fig.
300 3). On the other hand, GLMs suggested that the lower bite force of *M. nigricans* was
301 positively associated with weakly sclerotized prey (wi = 0.29, *P* =0.001, CI = 0.27 to
302 0.98), although this type of prey was also present in the diets of the other species with
303 stronger bite force (see Fig. 3). Confidence intervals of these GLMs do not include zero,
304 indicating that bite force influences prey consumption with varying degrees of
305 sclerotization (Supplementary Data SD2).

306 **Flight speed**. Flight speed of prey appeared to be closely linked to bat hunting
307 ability in open areas. Specifically, fast-flying prey contribute largely to the diet of *M.*
308 *molossus*, moderately so to *M. nigricans*, and very little to *E. furinalis* (Fig. 4). The GLMs
309 indicated that the diets of *M. nigricans* and *E. furinalis* are negatively influenced by fast-
310 flying prey (wi = 0.38, p-value = 0.002, CI = -1.26 to -0.30). Bats with larger forearm, *M.*
311 *Molossus* and *E. furinalis*, showed a similar consumption of prey with intermediate flight
312 speeds. In fact, the GLMs indicated that the diet of the species with the smallest
313 forearm, *M. nigricans*, was negatively influenced by intermediate-flying prey (wi = 0.66,

314 *p*-value = 0.001, CI = -2.17 to -0.59). Slow-flying prey appeared to be more associated
315 with the edge-space foragers *E. furinalis* and *M. nigricans* (wi = 0.49, *p*-value = 0.00001,
316 CI = 0.34 to 1.02). However, slow-flying prey were also present in the diet of *M.*
317 *molossus*. Confidence intervals for these GLMs excluded zero, suggesting an impact of
318 bat forearm length and foraging strategy on the consumption of prey with diverse flight
319 behaviors (Supplementary Data SD2).

320 **Bat niche breadth**

321 The highest *Levin*'s index was found in *E. furinalis* ($B_{est}$ = 0.48), followed by *M.*
322 *nigricans* ($B_{est}$ = 0.29), and *M. molossus* ($B_{est}$ = 0.26). *Molossus molossus* and *M.*
323 *nigricans* had many low-proportion food items (75th percentile: values ≤ 1.54%), while *E.*
324 *furinalis* had a slightly more balanced diet although still with many low-proportion items
325 (75th percentile: values ≤ 1.64%).

326 **Bat trophic niche overlap**

327 The highest trophic niche overlap was observed in comparisons between *E.*
328 *furinalis* vs. *M. nigricans* (Pianka's index = 0.91), followed by *E. furinalis* vs. *M. molossus*
329 (Pianka's index = 0.76) and *M. molossus* vs. *M. nigricans* (Pianka's index = 0.72). For all
330 the pairwise comparisons, the probability of observing by chance a Pianka index equal
331 or higher than the one recorded was extremely low, indicating that the partial dietary
332 overlaps observed among species were higher than expected by chance alone ($P$ <
333 0.001, n = 10,000 simulations). This result can be visualized in the Hill and Smith PCA,
334 which shows a clear lack of full overlap between the diet of *M. molossus* and the other

335 two species (Fig. 5). Furthermore, these analyses indicated the presence of 24 MOTUs
336 detected in single bat individuals, most of which were found only in *M. molossus*.

337 **Bat activity time**

338 *Molossus molossus* and *M. nigricans* represented 55.20% and 41.97%,
339 respectively, of the sound files obtained in both farms (n = 6007). In contrast, *Eptesicus*
340 *furinalis* had the lowest number of sound files during the entire study period (2.83%).
341 The distribution of activity over time is not uniform for any of the species (Watson test =
342 11.1 to 241.8, all $P < 0.01$). The activity time of *M. molossus* did not overlap much with
343 either *M. nigricans* (Δ4=0.36, CI bootstrap =0.34 and 0.38) or *E. furinalis* (Δ4=0.39, CI
344 bootstrap=0.34 and 0.44) (Fig. 6 A-B). A medium to high overlap in activity time between
345 *E. furinalis* and *M. nigricans* was observed throughout the study (Δ4=0.93, CI
346 bootstrap=0.88 and 0.97) (Fig. 6 C). Additional tests comparing the distributions of
347 activity over time in terms of orientation showed differences between *M. molossus* and
348 both *M. nigricans* and *E. furinalis* (Watson and Watson-Wheeler test = 248.1 to 2312.4,
349 all $P$ = 2.2e-16). In contrast, the result of that same test in the comparison *M. nigricans*
350 vs. *E. furinalis* was not significant (Watson and Watson-Wheeler test = 3.1, $P$ = 0.2)
351 (Supplementary Data SD3).

352 **DISCUSSION**

353 We investigated the diet and activity times of three bat species—*Molossus*
354 *molossus*, *Eptesicus furinalis*, and *Myotis nigricans*—in an agricultural setting,
355 employing metabarcoding of fecal samples and bioacoustic methods. These species
356 show some remarkable differences in the richness, taxonomic affinity, proportions, and

357 functional grouping of prey consumed, as well as in their activity times. As consequence,
358 these bats differ significantly in the breadth and degree of overlapping of their tropic
359 niche, all of which certainly resulting from morphological and behavioral attributes
360 related to bite force, size, and hunting abilities of these predators. Indeed, the diet and
361 activity differences observed suggest a division of food resources among these bats that
362 contribute to trophic niche partitioning, enhancing thereby their coexistence in close
363 sympatry.

364 **Dietary richness and composition**

365 The dietary taxonomic richness of insects in the studied species—*M. molossus*
366 (nine orders, 32 families), *M. nigricans* (seven orders, 19 families), and *E. furinalis* (five
367 orders, 13 families)— often surpassed richness values reported in other molecular diet
368 analyses of molossids and vespertilionids in agricultural areas. Notably, in our study, *M.*
369 *molossus* showed twice and 1.7 times the number of orders and families, respectively,
370 reported by Aguiar et al. (2021) for the same species in urban-agricultural environments
371 in Goiás, Brazil. Furthermore, molecular analyses of diets of other tropical molossid
372 species report family richness nearly three times lower than the value reported for *M.*
373 *molossus* in this study (Bohmann et al. 2011; Aguiar et al. 2021). However, Kolkert et al.
374 (2020) found 7.4 to 10.4 times more MOTUs richness in vespertilionid diets in
375 subtropical agricultural regions in New South Wales compared to our study. These
376 contrasting results suggest that bat dietary diversity is more variable than previously
377 understood, likely influenced by species-specific adaptations to climate, and agricultural
378 ecosystem characteristics (Hedrick and Dumont 2018).

379 In this study, differences in preference of insect orders among bat species were
380 conspicuous. For instance, in *M. molossus*, the three main types of prey were
381 Coleoptera (36.2%), Lepidoptera (22.5%) and Diptera (21.1%); whereas in *M. nigricans*
382 the ranking was Diptera (44.6%), Coleoptera (23.8%) and Hemiptera (13.1%), and in *E.*
383 *furinalis* was Coleoptera (39.3%), Diptera (32.8%) and Hemiptera (16.4%). The most
384 abundant insect orders captured in the study area were Coleoptera (61%), Hemiptera
385 (23%) and Diptera (15%) (Moreno et al. 2020). This suggests that these bats, especially
386 *M. molossus* and *E. furinalis*, are opportunistic predators whose diet compositions reflect
387 to a great extent the availability of potential insect prey. On the other hand, *M. nigricans*
388 exhibited a slightly more specialized behavior, with a marked preference for prey
389 (Diptera) that are not the most abundant in the study area. Thus, as observed in
390 generalist insectivorous birds, the structure of insect communities in agricultural crops
391 likely conditions the diets of aerial hawking bats (Durst et al. 2008; Aizpurua et al. 2018;
392 McClenaghan et al. 2019). However, the device and type of light used by Moreno et al.
393 (2020) to collect insects in our study area are probably biased towards capturing certain
394 taxa, so there is some uncertainty regarding the extent to which the bat diets reflect the
395 actual insect community structure.

396 Important sources of proteins and fats for bats include several phytophagous taxa
397 within Coleoptera, Lepidoptera, and Hemiptera. Notable among them are the elaterid
398 *Aeolus* sp. in the three bats species (Pi = 3.1% - 13.3%); the curculionid *Lissorhoptrus*
399 sp. in *M. molossus* (Pi = 5.6%) and *E. furinalis* (Pi = 4.9%); various moths in Crambidae
400 (*Rupela albinella* and *Diatraea saccharalis*) and Noctuidae (*Spodoptera frugiperda*,
401 *Condica sutor*, and *Leucania* sp.) in *M. molossus* (∑Pi = 8.9%); and the cicadellid

402 *Graminella* sp. in *M. nigricans* (*Pi* = 10.8%) and *E. furinalis* (Pi = 8.2%). Notably, the
403 predominant prey type in the diets of these bats consists of phytophagous insects. This
404 indicates the high abundance of these insects, their nutritional value, and the efficiency
405 of converting them into bat body mass (García et al. 2012; Razeng and Watson 2015).

406 We found that Diptera represent an important fraction of the three bats diets (21.1
407 – 44.6%). For instance, the giant craneflies of the subfamily Limoniinae (Tipulidae)
408 accounted alone for 18.3% – 26.2% of the diets, more than any other food item
409 analyzed at the family/sub-family level. The identification of Diptera and other fragile
410 insects through morphological analysis of feces is not straightforward, which certainly
411 made difficult to understand their importance as food items, and to better ascertain the
412 drivers of food resource selection in non-molecular analyses of bat diets (Muñoz and
413 Molinari 2000; Whitaker and Philips 2012). Our research indicates that although Diptera
414 have lower nutritional value compared to Coleoptera or Lepidoptera, they remain
415 important food sources for bats due to their abundance and ease of capture in altered
416 environments (Liu et al. 2023), a result also observed in birds (Razeng and Watson
417 2015; Bellavance et al. 2018; McClenaghan et al. 2019). These vertebrates may use
418 Diptera either as substitutes of more nutritious but less available prey or as a
419 micronutrient supplement, which could be especially important in artificial wetlands.

420 **Types of prey captured**

421 We predicted that *M. molossus* should consume highly sclerotized prey, while for
422 *E. furinalis* and *M. nigricans* we suggested a higher consumption of weakly ones.
423 However, our findings partially align with these predictions, mainly due to *E. furinalis*.
424 Indeed, the best GLMs suggest that *M. molossus* and *E. furinalis* possess bite forces

425 suitable for consuming highly sclerotized prey, while *M. nigricans*' diet is driven by
426 weakly sclerotized prey due to its lower bite force. Previous studies have suggested that
427 bite force increases with skull length, which in turn correlates positively with the degree
428 of sclerotization of consumed prey (Senawi et al. 2015; Ramírez-Francel et al. 2021).
429 However, *E. furinalis* deviates from this relationship by having a skull length similar to *M.*
430 *nigricans,* yet with a bite force almost six times stronger (Aguirre et al. 2002). Although
431 the bite force of *E. furinalis* is slightly lower than that of *M. molossus*, it is sufficient to
432 overcome the mechanical limitations of processing highly sclerotized items, even with a
433 small skull. This aligns with Hedrick and Dumont (2018) finding that the dental rather
434 than the skull attributes are the primary focus of selective pressures related to food
435 processing, although such pattern seems more evident in phyllostomid than in non-
436 phyllostomid bat families.

437 The best GLM confirmed our prediction that the diets of *M. nigricans* and *E.*
438 *furinalis* should be negatively correlated with fast-flying prey, highlighting *M. molossus*'s
439 ability to hunt such prey through accelerated flight and long-range echolocation calls.
440 Similar to swallows and swifts, molossid bats have long, narrow wings, enabling agile
441 maneuvers during fast flights in open spaces at a lower cost compared to *M. nigricans*
442 and *E. furinalis*, which have short, wide wings (Norberg 1986; Voigt and Holderied 2012;
443 Matyjasiak et al. 2023). This same argument justifies the selection of the functional
444 group edge-space foragers by the GLM to explain the higher proportion of slow-flying
445 prey in *E. furinalis* and *M. nigricans.* The best GLM also indicated that forearm length
446 explains the significant similarities in intermediate-flying prey consumption between *M.*
447 *molossus* and *E. furinalis*. Forearm length, serving as a proxy for wing design (Thiagavel

et al. 2017), suggests that *E. furinalis*'s behavior of capturing slow-flying insects may somewhat deviate from the typical edge-space foragers guild. In fact, *E. furinalis* has a wing loading 1.2 times greater than *M. nigricans* (Amador et al. 2020), which could help increase its flight speed, making it as efficient as *M. molossus* in capturing intermediate-flying insects. However, all these arguments are part of a complex interplay of elements that is barely understood, so they must be approached with caution.

**Trophic niche breadth**

As predicted, the diet of *M. molossus* had the highest taxonomic richness, followed by *M. nigricans* and *E. furinalis*. However, contrary to expectations, the niche breadth, as measured by the Best index, was nearly twice as big in *E. furinalis* compared to the other bats. Thus, although *M. molossus* consumes a taxonomically larger array of prey than *E. furinalis*, it is also less even in its consumption. These results suggest that *M. Molossus* is perhaps better seen as a generalist species with great trophic flexibility, a conclusion supported by our finding that 24% (17/71) *M. molossus* individuals consumed prey detected only once, compared to 11% (5/44) and 10% (2/21) in *M. nigricans* and *E. furinalis*, respectively. On a broader perspective, two types of generalist species have been suggested to exist: somehow like *E. furinalis*, mostly composed by individuals that consume a large array of prey equitably, and like *M. Molossus*. and probably *M. nigricans*, composed by individuals that may consume different kinds of prey (Bolnick et al. 2003). Although the latter category has been little studied in bats, there is now some evidence supporting such pattern (Arrizabalaga-Escudero et al. 2019; Magalhães de Oliveira et al. 2020).

**Trophic niche overlap**

As predicted, Pianka's index confirmed a significantly low dietary overlap between *M. molossus* and the two vespertilionids, which, despite some differences in prey contribution, are in turn highly overlapping. This observation aligns with findings by Aguiar and Antonini (2008), who noted a high dietary overlap through morphological analyses of fecal samples in *M. nigricans* and *E. furinalis* from the Brazilian Cerrado. Other molecular analyses of bat diets have also found values of Pianka's index (0.60-0.83) similar or slightly lower to those we obtained (0.72-0.91). These values have not only resulted from comparisons between cryptic species (Razgour et al. 2011), but also when inter-guild and even intra-guild comparisons are made (Salinas-Ramos et al. 2015; Gordon et al. 2019). In contrast, Segura-Trujillo et al. (2022) reported lower dietary overlap among species from the same locality (Pianka's index ≤ 0.5) compared to the same species from different localities (Pianka's index = ?), suggesting a coexistence favored by reduced inter-specific competition via partitioning of trophic niches. We believe that a similar process may enhance coexistence in the bats of this study, although with higher overlapping of trophic niches, probably as a consequence of the high productivity of rice fields in artificial wetlands, in which the elevated abundance represents an almost unlimited food resource, leading to low competition and high trophic niche overlap (Krüger et al. 2012).

**Bat activity time**

Consistent with our hypothesis, *M. molossus* exhibited low similarity in activity time compared with *M. nigricans* and *E. furinalis*, which in turn had medium to high activity time similarity among them. *Molossus molossus* is the first to appear in sound recordings, followed closely by the near-simultaneous emergence of *M. nigricans* and *E.*

494 *furinalis*. Our observations and sound recordings, along with other studies indicating that
495 open-space bats forage before sunset (Esbérard and Bergallo 2010; Roemer et al.
496 2019), suggest that *M. molossus* is exploiting the insect community in a different
497 temporal window compared to the two vespertilionid species. The medium to high
498 overlapping times at activity peaks, occurring mainly between *M. nigricans* and *E.*
499 *furinalis*, suggest that competition between these bats might be at play, and that the
500 coexistence of the two species should be favored by divergence at trophic niche axes
501 other than diet composition and activity time.

502 **Concluding remarks**

503 The coexistence between *M. molossus*, *M. nigricans* and *E. furinalis* possibly
504 reflects differences in dietary preferences and the timing of hunting periods. *Molossus*
505 *molossus* shows a preference for relatively larger prey (e.g. Coleoptera and
506 Lepidoptera), that tend to fly fast and that exhibit a medium to high degree of
507 sclerotization, with both onset and peak of hunting activities occurring significantly
508 earlier than in the two other bats. Thus, divergence at these two trophic niche
509 dimensions should reduce inter-specific competition and enhance coexistence between
510 *M. molossus* and the vespertilionids, which agree with niche theory predictions (Finke
511 and Snyder, 2008). On the other hand, although *M. nigricans* exhibits a significant
512 preference for Diptera and other prey that tend to fly slowly and that are weekly
513 sclerotized, it shows relatively high dietary and temporal overlaps with *E. furinalis*. This
514 suggests that coexistence between these species is not favored by divergence at the
515 two trophic niche dimensions studied here but perhaps to the spatial structure of habitat
516 for hunting. In fact, these vespertilionids exhibit some aerodynamic differences that

517 make probable a differential use of space, both horizontal and vertical (Amador et al.
518 2020). Illuminating these aspects through integrative analyses will be key to unravel the
519 selective forces that contribute to the close coexistence and high diversification of bats
520 in tropical environments.

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720 **Table 1**. Percentage of prey by taxon (Pi) in the diet of three species of aerial hawking bats at North-
721 western Venezuela's rice crops. Pi = (∑Ni/N total) × 100, where ∑Ni is the total number of prey items for
722 taxon i found in the diet and N total is the total number of prey items across all taxa in the diet

| Taxa | *Molossus molossus* $N_{BATS}$ = 71 $N_{PREYS}$ = 213 | *Myotis nigricans* $N_{BATS}$ = 44 $N_{PREYS}$ = 130 | *Eptesicus furinalis* $N_{BATS}$ = 21 $N_{PREYS}$ = 61 |
|---|---|---|---|
| **Order Coleoptera** | | | |
| **Suborder Adephaga** | | | |
| **Carabidae** | | | |
| Unknown Carabidae | 2.35 | 0.00 | 6.56 |
| Cicindelinae | 0.00 | 0.00 | 1.64 |
| Dytiscidae | 0.47 | 0.00 | 0.00 |
| **Suborder Polyphaga** | | | |
| Unknown Polyphaga | 2.35 | 1.54 | 3.28 |
| Unknown Cucujiformia | 0.94 | 0.00 | 0.00 |
| Elateridae: Agrypninae (*Aeolus* sp) | 13.62 | 3.08 | 4.92 |
| Ptilodactylinae | 2.35 | 6.15 | 8.20 |
| **Curculionidae** | | | |
| Unknown Curculionidae | 0.47 | 0.00 | 1.64 |
| Scolytinae | 0.00 | 0.77 | 0.00 |
| Erirhininae*: Lissorhoptrus sp* | 5.63 | 1.54 | 4.92 |
| Scirtidae | 4.69 | 0.77 | 0.00 |
| Scarabaeidae: Dynastinae | 0.47 | 0.00 | 0.00 |
| Hydrophilidae | 0.94 | 1.54 | 0.00 |
| Heteroceridae | 0.00 | 6.92 | 4.92 |
| Tenebrionidae | 0.47 | 0.00 | 1.64 |
| Chrysomelidae | 0.94 | 0.00 | 1.64 |
| Throscidae | 0.00 | 0.77 | 0.00 |
| Zopheridae: Colydiinae | 0.47 | 0.77 | 0.00 |
| **Order Diptera** | | | |
| Unknown Diptera | 0.47 | 0.00 | 1.64 |
| **Suborder Nematocera** | | | |
| Unknown Nematocera | 0.00 | 1.54 | 0.00 |
| Sciaroidea | 0.00 | 1.54 | 1.64 |
| Tipuloidea: Limoniinae | 18.31 | 26.15 | 22.95 |
| Tipuloidea: Pediciidae | 0.47 | 0.00 | 0.00 |
| Culicoidea: Culicidae | 0.47 | 6.15 | 4.92 |
| Chironomoidea: Ceratopogonidae | 0.47 | 0.00 | 0.00 |
| Psychodoidea: Psychodinae | 0.00 | 3.08 | 0.00 |

| | | | |
|---|---|---|---|
| **Suborder Brachycera** | | | |
| Unknown Muscomorpha | 0.00 | 1.54 | 0.00 |
| Calyptratae | 0.47 | 0.00 | 0.00 |
| Cyclorrhapha | 0.00 | 3.08 | 1.64 |
| Drosophilidae | 0.47 | 0.77 | 0.00 |
| Stratyiomidae | 0.00 | 0.77 | 0.00 |
| | | | |
| **Order Lepidoptera** | | | |
| Unknown Lepidoptera | 0.47 | 0.00 | 0.00 |
| **Suborder Glossata** | | | |
| Rhopalocera | 2.35 | 0.00 | 0.00 |
| Obtectomera | 6.57 | 0.77 | 0.00 |
| **Crambidae** | | | |
| Unknown Crambidae | 2.35 | 3.85 | 0.00 |
| *Rupela albinella* | 4.23 | 1.54 | 0.00 |
| *Diatraea saccharalis* | 0.47 | 0.00 | 0.00 |
| *Eoreuma loftini* | 0.00 | 0.77 | 0.00 |
| **Noctuidae** | | | |
| Unknown Noctuidae | 0.94 | 0.00 | 0.00 |
| *Spodoptera frugiperda* | 3.29 | 0.00 | 0.00 |
| *Leucania*sp. | 0.47 | 0.00 | 0.00 |
| *Condica sutor* | 0.47 | 0.00 | 0.00 |
| Hesperiidae: Eudamidae | 0.00 | 0.00 | 1.64 |
| Geometridae | 0.00 | 0.77 | 0.00 |
| Saturnidae | 0.47 | 0.00 | 0.00 |
| Erebidae | 0.47 | 0.00 | 0.00 |
| | | | |
| **Order Orthoptera** | | | |
| Unknown Orthoptera | 1.88 | 0.77 | 3.28 |
| **Suborder Ensifera** | | | |
| Gryllidae | 1.88 | 0.77 | 0.00 |
| | | | |
| **Order Hemiptera** | | | |
| Unknown Hemiptera | 0.94 | 0.00 | 0.00 |
| **Suborder Heteroptera** | | | |
| Panheteroptera | 0.47 | 0.00 | 0.00 |
| Belostomatidae | 0.94 | 0.00 | 1.64 |
| Lygaeidae | 0.47 | 0.00 | 0.00 |
| Pentatomidae: *Oebalus ypsilongriseus* | 0.47 | 0.00 | 0.00 |
| **Suborder Auchenorrhyncha** | | | |
| **Cicadellidae** | | | |
| Unknown Cicadellidae | 1.41 | 2.31 | 3.28 |

| | | | |
|---|---|---|---|
| *Graminella* sp | 1.41 | 10.77 | 8.20 |
| Cercopidae | 0.00 | 0.00 | 3.28 |
| Delphacidae: *Tagosodes orizicolus* | 0.94 | 0.00 | 0.00 |
| **Order Himenoptera**: Formicidae | 1.41 | 0.00 | 0.00 |
| **Order Blattodea** | | | |
| Blaberidae | 0.47 | 0.00 | 0.00 |
| Rhinotermitidae | 0.47 | 1.54 | 0.00 |
| Kalotermitidae | 0.94 | 0.00 | 0.00 |
| **Order Neuroptera**: Chrysopinae | 0.47 | 0.00 | 0.00 |
| **Order Megaloptera**: Corydalidae | 0.00 | 2.31 | 0.00 |
| **Order Odonata**: Coenagrionidae | 0.47 | 0.00 | 0.00 |
| **Unknown Pterygota** | 5.16 | 5.38 | 6.56 |
| **Total (%)** | 100 | 100 | 100 |
| **Mean** | 1.54 | 1.54 | 1.54 |
| **Median** | 0.47 | 0.00 | 0.00 |
| **2sd** | 2.99 | 3.70 | 3.44 |
| **Min** | 0.00 | 0.00 | 0.00 |
| **Max** | 18.31 | 26.15 | 22.95 |
| **25th percentile** | 0.47 | 0.00 | 0.00 |
| **75th percentile** | 1.41 | 1.54 | 1.64 |

## Figure Legends

Fig. 1. Map of Acarigua and Turén rice farms in Northwestern Llanos, Portuguesa, Venezuela (QGIS Development Team, 2023).

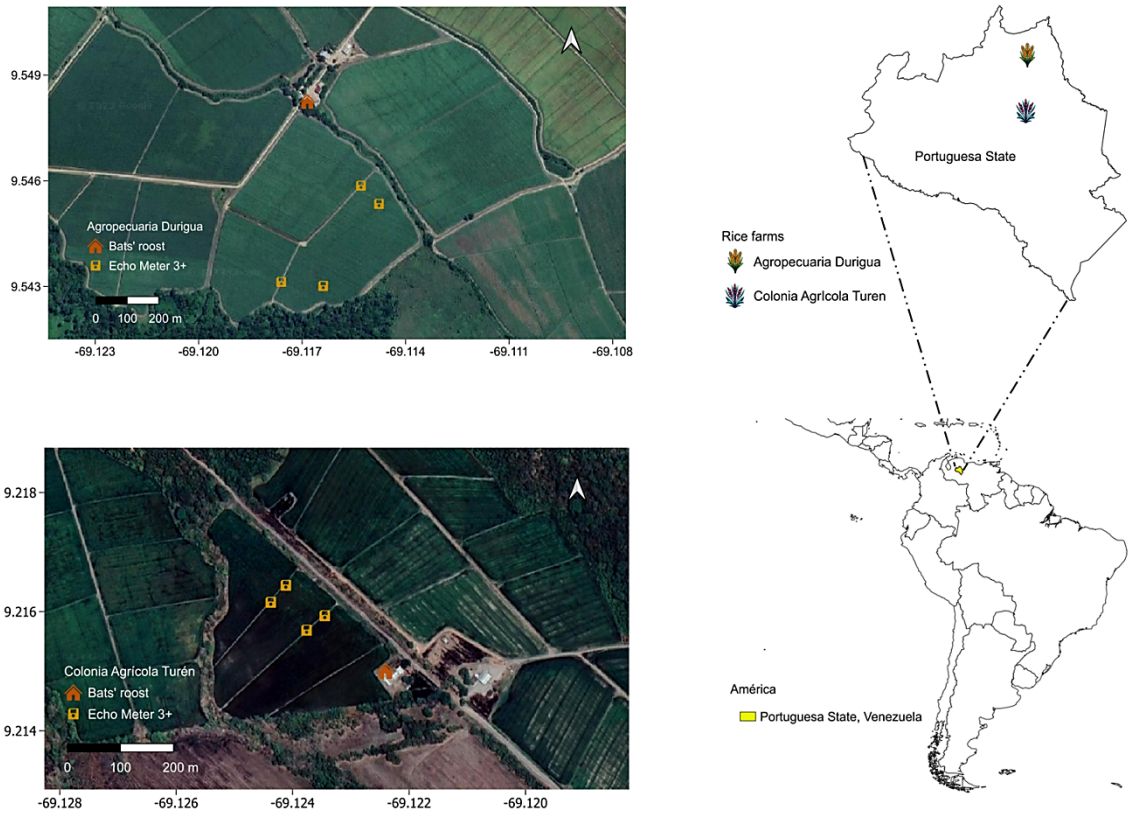
Fig. 2. Percentage of prey in the diet of three species of bats, classified by order, family and trophic grpup throughout the study. For the classification of insects into trophic groups, only the adult phase is considered. Unkn = Unknown, Phyt = Phytophagous, Zoop = Zoophagous, Sapr= Saprophagous.

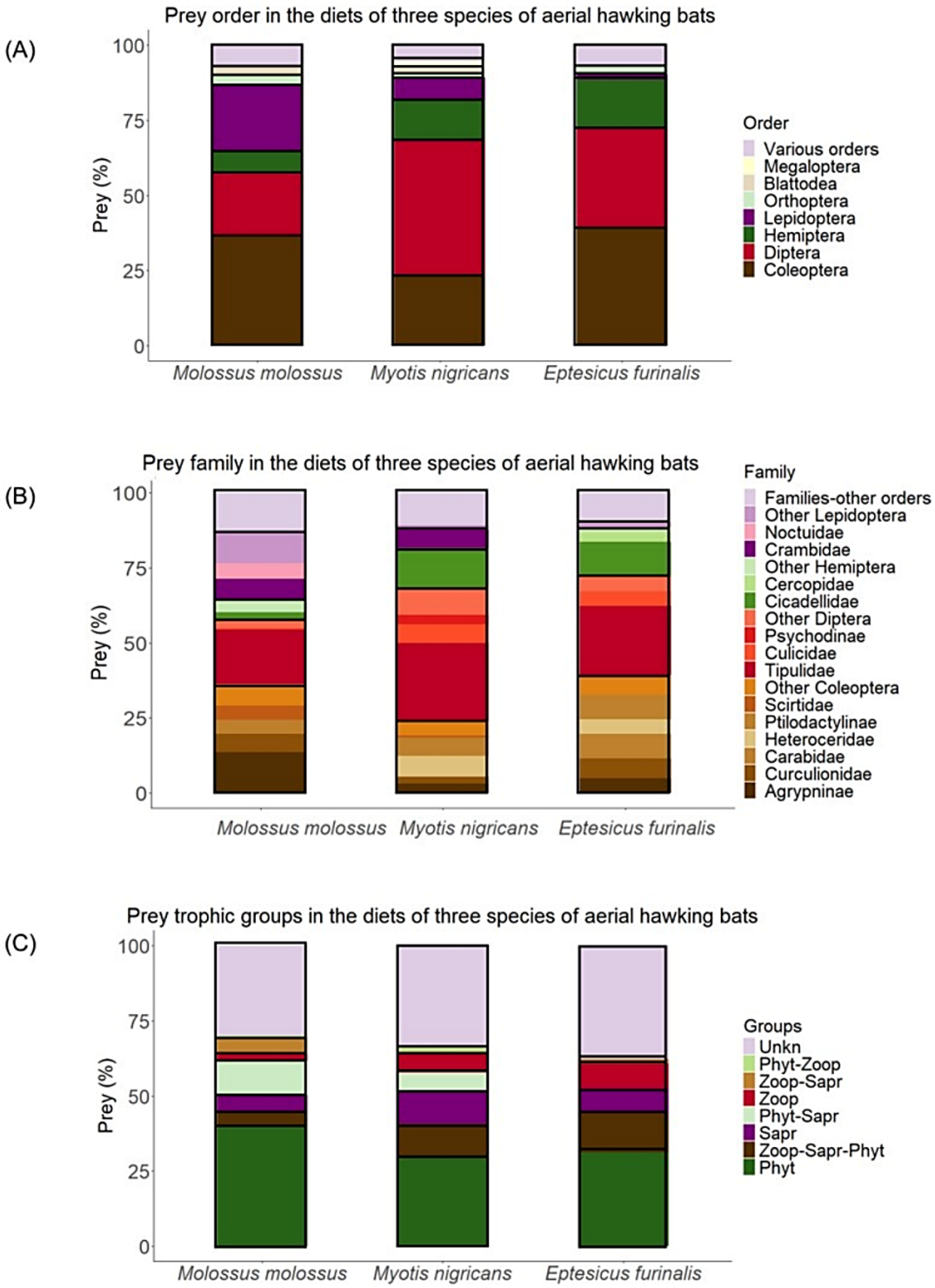
Fig. 3. Percentage of prey (%) strongly, medium, and weakly sclerotized on the diet of *M. molossus*, *M. nigricans*, and *E. furinalis*.

Fig. 4. Percentage of prey (%) of fast, intermediate, and slow flying on the diet of *M. molossus*, *M. nigricans*, and *E. furinalis*.

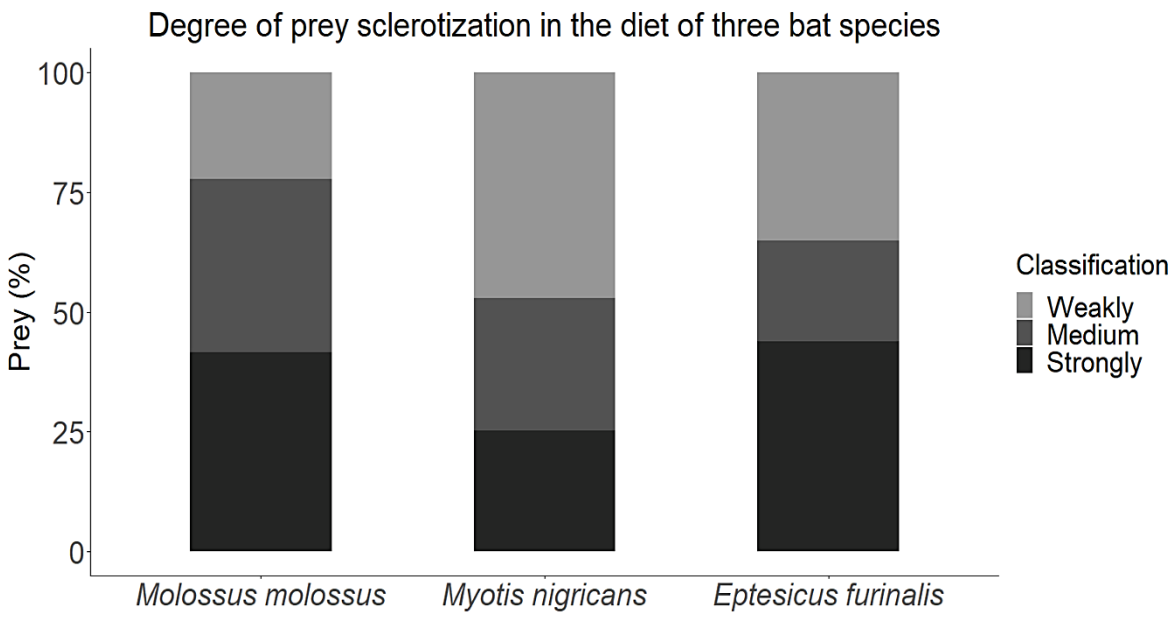
Fig. 5. Hill and Smith Principal Component Analysis (PCA hs) of dietary patterns in three bat species. The analysis includes presence-absence data of the 65 MOTUs identified in this study.

Figure 6. Time-density curves of bat species pairs with overlap area in gray from both farms (Acarigua and Turén) during the entire study period. Bootstrap estimates and 95% confidence intervals for the overlap coefficient, Δ, are presented on the axis-x. The overlap coefficient Δ1 is applied when the smaller sample size was less than 50, and Δ4 is used for sizes greater than 75.


9.549
9.546
9.543
Agropecuaria Durigua
Bats' roost
Echo Meter 3+
0 100 200 m
-69.123
-69.120
-69.117
-69.114
-69.111
-69.108
9.218
9.216
9.214
Colonia Agrícola Turén
Bats' roost
Echo Meter 3+
0 100 200 m
-69.128
-69.126
-69.124
-69.122
-69.120
Portuguesa State
Rice farms
Agropecuaria Durigua
Colonia Agrícola Turen
América
Portuguesa State, Venezuela


(A)
Prey order in the diets of three species of aerial hawking bats
Prey (%)
100
75
50
25
0
Molossus molossus
Myotis nigricans
Eptesicus furinalis
Order
Various orders
Megaloptera
Blattodea
Orthoptera
Lepidoptera
Hemiptera
Diptera
Coleoptera
(B)
Prey family in the diets of three species of aerial hawking bats
Prey (%)
100
75
50
25
0
Molossus molossus
Myotis nigricans
Eptesicus furinalis
Family
Families-other orders
Other Lepidoptera
Noctuidae
Crambidae
Other Hemiptera
Cercopidae
Cicadellidae
Other Diptera
Psychodinae
Culicidae
Tipulidae
Other Coleoptera
Scirtidae
Ptilodactylinae
Heteroceridae
Carabidae
Curculionidae
Agrypninae
(C)
Prey trophic groups in the diets of three species of aerial hawking bats
Prey (%)
100
75
50
25
0
Molossus molossus
Myotis nigricans
Eptesicus furinalis
Groups
Unkn
Phyt-Zoop
Zoop-Sapr
Zoop
Phyt-Sapr
Sapr
Zoop-Sapr-Phyt
Phyt

763

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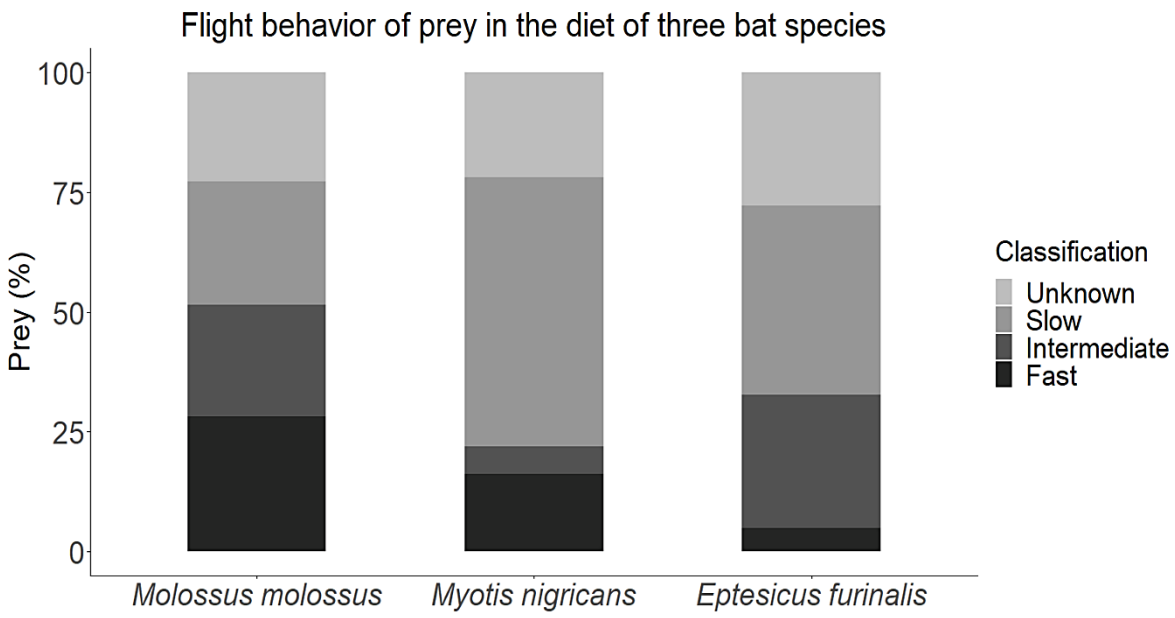
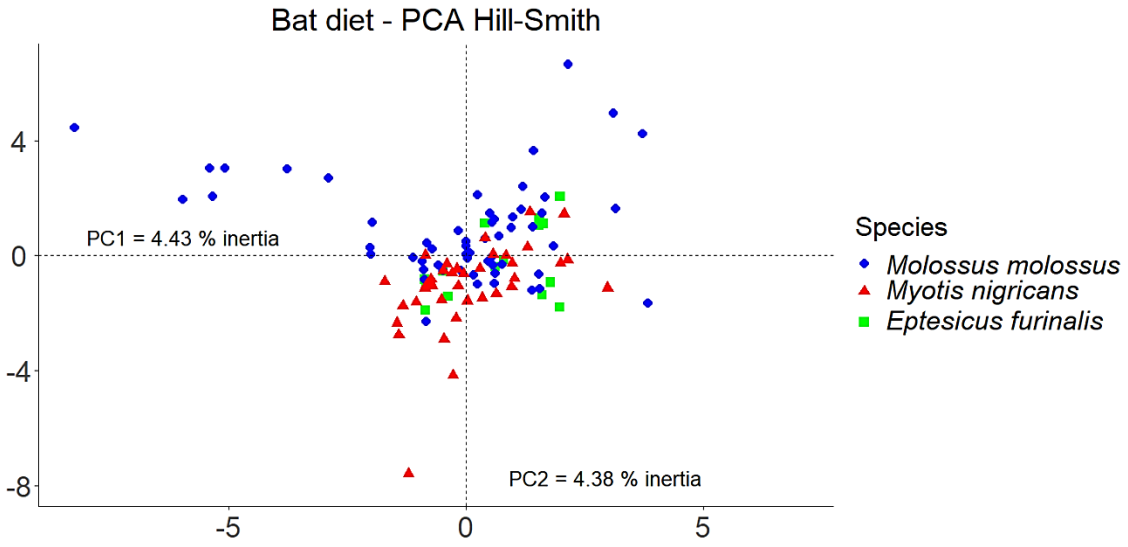
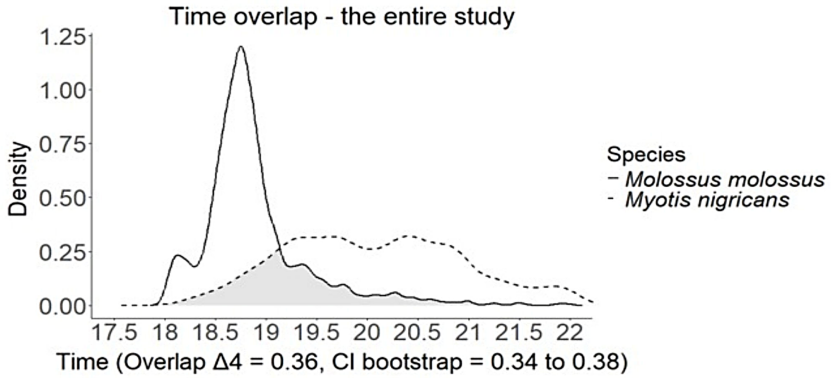
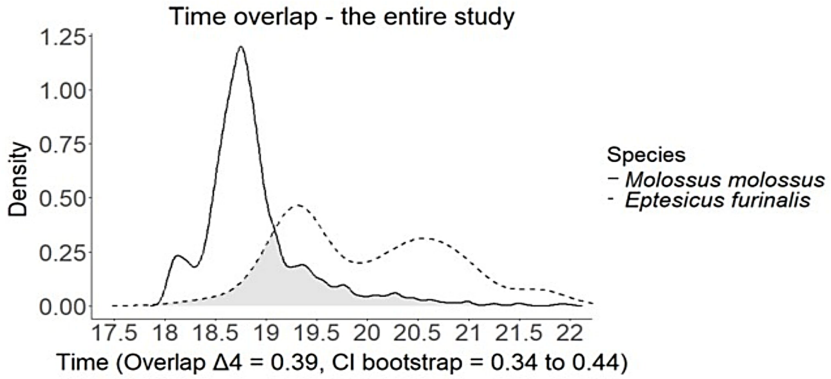

Flight behavior of prey in the diet of three bat species
Prey (%)
100
75
50
25
0
Molossus molossus
Myotis nigricans
Eptesicus furinalis
Classification
Unknown
Slow
Intermediate
Fast


Bat diet - PCA Hill-Smith
PC1 = 4.43 % inertia
PC2 = 4.38 % inertia
4
0
-4
-8
-5
0
5
Species
Molossus molossus
Myotis nigricans
Eptesicus furinalis

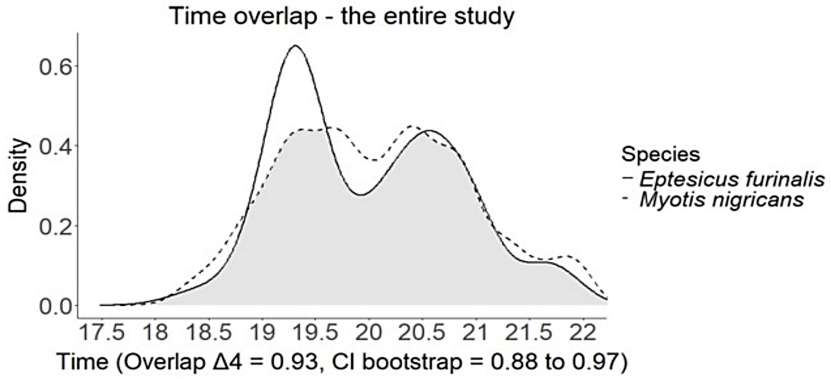
(A)


Time overlap - the entire study
Density
1.25
1.00
0.75
0.50
0.25
0.00
17.5 18 18.5 19 19.5 20 20.5 21 21.5 22
Time (Overlap Δ4 = 0.36, CI bootstrap = 0.34 to 0.38)
Species
Molossus molossus
Myotis nigricans

(B)


Time overlap - the entire study
Density
1.25
1.00
0.75
0.50
0.25
0.00
17.5 18 18.5 19 19.5 20 20.5 21 21.5 22
Time (Overlap Δ4 = 0.39, CI bootstrap = 0.34 to 0.44)
Species
Molossus molossus
Eptesicus furinalis

(B)


Time overlap - the entire study
Density
0.6
0.4
0.2
0.0
17.5 18 18.5 19 19.5 20 20.5 21 21.5 22
Time (Overlap Δ4 = 0.93, CI bootstrap = 0.88 to 0.97)
Species
Eptesicus furinalis
Myotis nigricans

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