Diet and trophic niche partitioning of three aerial hawking bat species in the Venezuelan Llanos[1]

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[1]YA, JN, and JM formulated the idea. YA and JN developed the study's sampling design and sound recording methodology, YA and JM developed the genetic work. YA conducted fieldwork. JM generated sequencing data and conducted molecular analyses. YA performed the statistical analyses. YA and JM wrote the manuscript. JN provided feedback and additional information on earlier and final versions of the manuscript.

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ABSTRACT

Since Neotropical insectivorous bats (IB) share feeding sites and prey, their coexistence must depend on processes promoting divergence at trophic niches. We conducted diet and activity time analyses of three coexisting IB species in Venezuelan paddies, *Molossus molossus, Eptesicus furinalis*, and *Myotis nigricans*, to understand their trophic niche partitioning. We used statistical models to understand: (1) the dietary and activity time overlap among IB species, and (2) the relationship of IB species' morphology and hunting strategies with 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, as well as in their activity periods. As expected from its larger size and fast flight, *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 the other two bat species. On the other hand, also consistent 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: coexistence, croplands, metabarcoding, insectivorous bats, activity time.

INTRODUCTION

Insectivorous bats (IB) are remarkably diverse predators with 20-45 species coexisting in syntopy in tropical forests and croplands 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). Thus, foraging strategies and diets appear 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 and/or maximum feeding activity periods among Neotropical IB species (Esbérard and Bergallo 2010; Gomes et al. 2020). These spatiotemporal differences in foraging strategies and patterns of activity time most likely led to dietary differences among IB species, particularly in relation to size, hardness, position in the air column, and flight mode of consumed prey (Segura-Trujillo et al. 2016, 2022). Regarding diet,

metabarcoding genetic analyses in IB from temperate regions have provided good evidence supporting trophic flexibility, with different degrees of dietary overlap that can only be explained through subtle spatiotemporal partitioning of food resources (Aizpurua et al. 2018; Arrizabalaga-Escudero et al. 2019; Gordon et al. 2019; Andriollo et al. 2021). In contrast, molecular-based diet analyses in Neotropical IB are still relatively scarce, usually have low taxonomic resolution, and rarely make inter-specific comparisons (but see Emrich et al. 2014, Salina-Ramos et al. 2015, Aguiar et al. 2021, and Segura-Trujillo et al. 2022).

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

DNA extractions

Bat fecal samples. DNA was extracted from ten fecal pellets of each bat using the DNasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). Sterile 1.5 mL microcentrifuge tubes containing fecal pellets and 1 mL lysis buffer solution were placed on a shaker at 30 rpm in an incubator at 55-56°C for two hours. Afterward, samples

were centrifuged for 1 min at 1000 rpm, and 100 µL of supernatants were extracted and deposited in new sterile 2 mL microcentrifuge tubes. The remaining protocol steps followed the manufacturer's instructions. To detect cross-contamination, extractions were performed in batches of 24 tubes, including two no-feces negative controls with and without water. Extracted DNA was stored at -18°C until subsequent PCR analysis.

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

PCR amplification and sequencing

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

DNA extracts were PCR-amplified in triplicate for fecal samples and once for insects. PCR reactions (volume 20 µL) contained: 10 µL of AmpliTaq Gold Master Mix (Applied Biosystems™, Foster City, USA), 0.2 µM of each primer, 5.84 µL of water, 0.04 µg of bovine serum albumin (Roche Diagnostic, Basel, Switzerland), and 2 µL of DNA diluted 1:20. The PCR protocols were: 1x: 10 min at 95 °C, 45x: 30 s at 95 °C, 30 s at 52 °C (16S-1) or 50 ºC (16S-2), 60 s at 72 °C, 1x: 7 min at 72 °C. The PCR batches

included two extraction negatives, a PCR-negative (water), and a blank (empty well). The PCR products were pooled per replicate, resulting in eight pools: six bat diets (three per metabarcode) and two insects (one per metabarcode). Amplicon pools were purified using the MinElute PCR Purification kit (Qiagen), quantified using a Qubit 2.0 Fluorometer (Life Technology Corporation), and sent to Fasteris SA (Geneva, Switzerland) for library preparation and sequencing using the MetaFast protocol (www.fasteris.com/metafast; Taberlet et al. 2018).

Bioinformatic analyses

The raw sequences were processed separately for each library using the *OBITools* package (Boyer et al. 2016). Reads were assembled with a minimum quality score of 40 and assigned to samples based on unique tag and primer combinations, allowing for two mismatches with the primer. Sequences more abundant in extraction, PCR and blank negative controls than in samples were considered as contaminants and removed. Sequences with coverage <10 reads, or with lengths outside the expected range (e.g., 76–168 bp for Arth02, Taberlet et al. 2018), or present in only one of the three fecal sample replicates were also discarded. The remaining sequences were clustered into prey MOTUs (*i.e*. Molecular Operational Taxonomic Unit) using the sumaclust algorithm with a sequence similarity of 97% (Mercier et al. 2013). Taxonomic assignment of MOTUs was achieved using the NCBI BLAST algorithm (Altschul et al. 1990), first against the 16S sequence database from local insects, and then against GenBank for the sequences that could not be assigned in the previous way. The following *ad hoc* assignment rules were applied in this study: (1) If there was a single best match: species level for similarity ≥ 98%, genus level for 95% ≤ similarity < 98%,

family/sub-family level for 92% ≤ similarity < 95%, order level for similarity < 92%; (2) If there were multiple best matches: assigned to the lowest taxonomic level subsuming all best matches (e.g. assigned to the generic level if all best matches correspond to two or more species in the same genus). Sequences that remained unassigned were classified as "unknown". A table of diet items was created by removing rare occurrences (< 0.01%) and converting the counts into presence/absence values.

Bat activity time

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

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

Dietary richness and composition

A total of 65 insect taxa were identified in the feces of the three bat species, with identifications ranging from order and family to, in some instances, the species level (Online Resource 1 and 2). Diets included 50 MOTUs from 71 *Molossus molossus*, 31 MOTUs from 44 *Myotis nigricans*, and 22 MOTUs from 21 *Eptesicus furinalis*. Expected richness of insect taxa were 60.02 (SD = 2.89) for *M. molossus*, 36.17 (SD = 2.05) for *M. nigricans*, and 25.90 (SD = 1.89) for *E. furinalis*. Estimated completeness of the diets

were 83% for *M. molossus*, 86% for *M. nigricans*, and 85% for *E. furinalis*. Statistical exploration of these MOTUs suggested differences in the dietary composition of the bat species (Pseudo-$F_{2, 133}$ = 3.64, *P*-perm = 0.001). Specifically, the pairwise comparisons *M. molossus* vs. *M. nigricans* (pseudo-*t* = 2.39, *P*-perm = 0.001) and *M. molossus* vs. *E. furinalis* (pseudo-*t* = 1.70, *P*-perm = 0.003) were significantly different.

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

At the family level, differences were also notable among bat species (Pseudo-$F_{2, 133}$ = 3.92, *P*-perm = 0.001). Forty insect families were identified in the fecal samples, with 32 in *M. molossus*, 19 in *M. nigricans*, and 13 in *E. furinalis*. As above, the most notable differences in composition of insect families were also observed between *M. molossus* vs. *M. nigricans* (Pseudo-*t* = 2.39, *P*-perm = 0.001) and *M. molossus* vs. *E. furinalis* (Pseudo-*t* = 1.85, *P*-perm = 0.002). For *M. molossus*, favored insect families, from most to least preferred, were Tipulidae, Elateridae, Crambidae, Curculionidae, Noctuidae, and Scirtidae. *Myotis nigricans*, on the other hand, primarily favored Tipulidae, followed by Cicadelliadae, Heteroceridae, Crambidae, Ptilodactylinae, and

Culicidae; whereas *E. furinalis* preferred Tipulidae, followed by Cicadellidae, Carabidae, Ptilodactylinae, Curculionidae, and Culicidae (Fig. 2b). Some prey were assigned to genus or species level in orders Coleoptera (*Aeolus* sp., *Lissorhoptrus* sp.), Lepidoptera (*Spodoptera frugiperda*, *Leucania* sp., *Condica sutor*, *Rupela albinella*, *Diatraea saccharalis*, *Eoreuma loftini*), and Hemiptera (*Graminella* sp., *Tagosodes orizicolus*, *Oebalus ypsilon-griseus*), most of which were ingested by *M. molossus* (Online Resource 1).

No notable differences among bat species were detected as a function of prey trophic groups. The three bat species primarily feed on phytophagous insects (39.9% of diet in *M. molossus*, 31.1% in *E. furinalis*, 29.2% in *M. nigricans*, Fig. 2c). Saprophagous prey represented 10.8% in *M. nigricans*, 8.2% in *E. furinalis*, and 5.2% in *M. molossus*; while zoophagous prey accounted for just 8.2% in *E. furinalis*, 6.2% in *M. nigricans*, and only 2.4% in *M. molossus*. Regarding the secondary functional classification of the diet, *M. molossus* and *E. furinalis* exhibited four categories of phytophagous insects (anthophagous, seed-feeding, phyllophagous, and xylophagous), while *M. nigricans* displayed three categories (anthophagous, seed-feeding, and phyllophagous). Additionally, four secondary categories within saprophagous insects (detritivores and fungivores) and zoophagous insects (predators and hematophagous) were detected in the diets of all three bat species (Online Resource 3).

Prey types

Sclerotization. The primary components in the diet of the two species with the strongest bites, *M. molossus* and *E. furinalis*, were prey with a high degree of sclerotization (Fig. 3a). The results from the GLMs suggested that the lower bite force of

M. nigricans was negatively associated with highly sclerotized prey (wi = 0.63, *P* = 0.0114, CI = -0.92 to -0.12). Nonetheless, a notable similarity among the three species was observed regarding the consumption of prey with intermediate sclerotization (Fig. 3a). On the other hand, GLMs suggested that the lower bite force of *M. nigricans* was positively associated with weakly sclerotized prey (wi = 0.29, *P* =0.001, CI = 0.27 to 0.98), although this type of prey was also present in the diets of the other species with stronger bite force (Fig. 3a). Confidence intervals of these GLMs do not include zero, indicating that bite force influences prey consumption with varying degrees of sclerotization (Online Resource 4).

Flight speed. Flight speed of prey appeared to be closely linked to bat hunting ability in open areas. Specifically, fast-flying prey contribute largely to the diet of *M. molossus*, moderately so to *M. nigricans*, and very little to *E. furinalis* (Fig. 3b). The GLMs indicated that the diets of edge-space foragers, *M. nigricans* and *E. furinalis,* are negatively influenced by fast-flying prey (wi = 0.38, p-value = 0.002, CI = -1.26 to -0.30). Bats with larger forearm, *M. Molossus* and *E. furinalis*, showed a similar consumption of prey with intermediate flight speeds (Fig. 3b). In fact, the GLMs indicated that the diet of the species with the smallest forearm, *M. nigricans*, was negatively influenced by intermediate-flying prey (wi = 0.66, *p*-value = 0.001, CI = -2.17 to -0.59). Slow-flying prey appeared to be more associated with the edge-space foragers *E. furinalis* and *M. nigricans* (wi = 0.49, *p*-value = 0.00001, CI = 0.34 to 1.02). However, slow-flying prey were also present in the diet of *M. molossus* (Fig. 3b). Confidence intervals for these GLMs excluded zero, suggesting an impact of bat forearm length and foraging strategy on the consumption of prey with diverse flight behaviors (Online Resource 4).

Bat niche breadth

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

Bat trophic niche overlap

The highest trophic niche overlap was observed in comparisons between *E. furinalis* vs. *M. nigricans* (Pianka's index = 0.91), followed by *E. furinalis* vs. *M. molossus* (Pianka's index = 0.76) and *M. molossus* vs. *M. nigricans* (Pianka's index = 0.72). For all pairwise comparisons, the probability of observing by chance a Pianka index equal or higher than the one recorded was extremely low, indicating that the partial dietary overlaps observed among species were higher than expected by chance alone (P < 0.001, n = 10,000 simulations). This result can be visualized in the Hill and Smith PCA, which shows a clear lack of full overlap between the diet of *M. molossus* and the other two species (Fig. 4). Furthermore, these analyses indicated the presence of 24 MOTUs detected in single bat individuals, most of which were found only in *M. molossus*.

Bat activity time

Molossus molossus and *M. nigricans* represented 55.20% and 41.97%, respectively, of the sound files obtained in both farms (n = 6007). In contrast, *Eptesicus furinalis* had the lowest number of sound files during the entire study period (2.83%). The distribution of activity over time is not uniform for any of the species (Watson test =

11.1 to 241.8, all $P < 0.01$). The activity time of *M. molossus* did not overlap much with either *M. nigricans* (Δ4=0.36, CI bootstrap =0.34 and 0.38) or *E. furinalis* (Δ4=0.39, CI bootstrap=0.34 and 0.44). A medium to high overlap in activity time between *E. furinalis* and *M. nigricans* was observed throughout the study (Δ4=0.93, CI bootstrap=0.88 and 0.97) (Fig. 5). Additional tests comparing the distributions of activity over time in terms of orientation showed differences between *M. molossus* and both *M. nigricans* and *E. furinalis* (Watson and Watson-Wheeler test = 248.1 to 2312.4, all $P = 2.2e-16$). In contrast, the result of that same test in the comparison *M. nigricans* vs. *E. furinalis* was not significant (Watson and Watson-Wheeler test = 3.1, $P = 0.2$).

DISCUSSION

We investigated the diet and activity times of three bat species—*Molossus molossus*, *Eptesicus furinalis*, and *Myotis nigricans*—in an agricultural setting, employing metabarcoding of fecal samples and bioacoustics methods. These species show remarkable differences in richness, taxonomic affinity, proportions, and functional grouping of prey consumed, as well as in their activity times. In consequence, these bats differ significantly in the breadth and degree of overlapping of their tropic niche, all of which resulting from morphological and behavioral attributes related to bite force, size, and hunting abilities of these predators. Indeed, the diet and activity differences observed suggest a division of food resources among these bats that contribute to trophic niche partitioning, enhancing their coexistence in close sympatry.

Dietary richness and composition

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

In this study, differences in preference of insect orders among bat species were conspicuous. For instance, in *M. molossus*, the three main types of prey were Coleoptera (36.2%), Lepidoptera (22.5%) and Diptera (21.1%); whereas in *M. nigricans* the ranking was Diptera (44.6%), Coleoptera (23.8%) and Hemiptera (13.1%), and in *E. furinalis* was Coleoptera (39.3%), Diptera (32.8%) and Hemiptera (16.4%). The most abundant insect orders captured in the study area were Coleoptera (61%), Hemiptera (23%) and Diptera (15%) (Moreno et al. 2020). This suggests that these bats, especially *M. molossus* and *E. furinalis*, are opportunistic predators whose diet compositions reflect to a great extent the availability of potential insect prey. On the other hand, *M. nigricans*

exhibited a slightly more specialized behavior, with a marked preference for prey (Diptera) that are not the most abundant in the study area. Thus, as observed in generalist insectivorous birds, the structure of insect communities in agricultural lands likely conditions the diets of aerial hawking bats (Durst et al. 2008; Aizpurua et al. 2018; McClenaghan et al. 2019). However, the device and type of light used by Moreno et al. (2020) to collect insects in our study area are probably biased towards capturing certain taxa, so there is some uncertainty regarding the extent to which the bat diets reflect the actual insect community structure.

Important sources of proteins and fats for bats include several phytophagous taxa within Coleoptera, Lepidoptera, and Hemiptera. Notable among them are the elaterid *Aeolus* sp. in the three bats species (Pi = 3.1% - 13.3%); the curculionid *Lissorhoptrus* sp. in *M. molossus* (Pi = 5.6%) and *E. furinalis* (Pi = 4.9%); various moths in Crambidae (*Rupela albinella* and *Diatraea saccharalis*) and Noctuidae (*Spodoptera frugiperda*, *Condica sutor*, and *Leucania* sp.) in *M. molossus* (∑Pi = 8.9%); and the cicadellid *Graminella* sp. in *M. nigricans* (*Pi* = 10.8%) and *E. furinalis* (Pi = 8.2%). Notably, the predominant prey type in the diets of these bats consists of phytophagous insects. This indicates the high abundance of these insects, their nutritional value, and the efficiency of converting them into bat body mass (García et al. 2012; Razeng and Watson 2015).

We found that Diptera represent an important fraction of the three bats' diets (21.1 – 44.6%). For instance, the giant craneflies of the subfamily Limoniinae (Tipulidae) accounted alone for 18.3% – 26.2% of the diets, more than any other food item analyzed at the family/sub-family level. The identification of Diptera and other fragile insects through morphological analysis of feces is not straightforward (Whitaker and

Philips 2012; Silva-Duarte et al. 2024), which certainly made it difficult to understand their importance as food items, and to better ascertain the drivers of food resource selection in non-molecular analyses of bat diets. Our research indicates that although Diptera have lower nutritional value compared to Coleoptera or Lepidoptera, they remain important food sources for bats due to their abundance and ease of capture in altered environments (Liu et al. 2023), a result also observed in birds (Razeng and Watson 2015; Bellavance et al. 2018; McClenaghan et al. 2019). These vertebrates may use Diptera either as substitutes for more nutritious but less available prey or as a micronutrient supplement, which could be especially important in artificial wetlands.

Types of prey captured

We predicted that *M. molossus* should consume highly sclerotized prey, while for *E. furinalis* and *M. nigricans* we suggested a higher consumption of weak ones. However, our findings partially align with these predictions, mainly due to *E. furinalis*. Indeed, the best GLMs suggest that *M. molossus* and *E. furinalis* possess bite forces suitable for consuming highly sclerotized prey, while *M. nigricans*' diet is driven by weakly sclerotized prey due to its lower bite force. Previous studies have suggested that bite force increases with skull length, which in turn correlates positively with the degree of sclerotization of consumed prey (Senawi et al. 2015; Ramírez-Francel et al. 2021). However, *E. furinalis* deviates from this relationship by having a skull length similar to *M. nigricans,* yet with a bite force almost six times stronger (Aguirre et al. 2002). Although the bite force of *E. furinalis* is slightly lower than that of *M. molossus*, it is sufficient to overcome the mechanical limitations of processing highly sclerotized items, even with a small skull. This aligns with Hedrick and Dumont (2018) finding that the dental rather

than the skull attributes are the primary focus of selective pressures related to food processing, although such a pattern seems more evident in phyllostomids than in non-phyllostomid bat families.

The best GLM confirmed our prediction that the diets of *M. nigricans* and *E. furinalis* should be negatively correlated with fast-flying prey, highlighting *M. molossus*'s ability to hunt such type prey through accelerated flight and long-range echolocation calls. Similar to swallows and swifts, molossid bats have long, narrow wings, enabling agile maneuvers during fast flights in open spaces at a lower cost compared to *M. nigricans* and *E. furinalis*, which have short, wide wings (Norberg 1986; Voigt and Holderied 2012; Matyjasiak et al. 2023). This same argument justifies the selection of the functional group edge-space foragers by the GLM to explain the higher proportion of slow-flying prey in *E. furinalis* and *M. nigricans.* The best GLM also indicated that forearm length explains the significant similarities in intermediate-flying prey consumption between *M. molossus* and *E. furinalis*. Forearm length, serving as a proxy for wing design (Thiagavel et al. 2017), suggests that *E. furinalis*' 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*, with individuals that may differ considerably in the kinds of preys consumed (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, 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. furinalis*. Our observations and sound recordings, along with other studies indicating that open-space bats forage before sunset (Esbérard and Bergallo 2010; Roemer et al. 2019), suggest that *M. molossus* is exploiting the insect community in a different temporal window compared to the two vespertilionid species. The medium to high overlapping times at activity peaks, occurring mainly between *M. nigricans* and *E. furinalis*, suggests that competition between these bats might be at play, and that the

coexistence of the two species should be favored by divergence at trophic niche axes other than diet composition and activity time.

Concluding remarks

The coexistence between *M. molossus*, *M. nigricans,* and *E. furinalis* in the Venezuelan Llanos possibly reflects differences in dietary preferences and the timing of hunting periods. *Molossus molossus* shows a preference for relatively larger prey (e.g. Coleoptera and Lepidoptera), that tend to fly fast and that exhibit a medium to high degree of sclerotization, with both onset and peak of hunting activities occurring significantly earlier than in the two other bats. Thus, divergence at these two trophic niche dimensions should reduce inter-specific competition and enhance coexistence between *M. molossus* and the vespertilionids, which agree with niche theory predictions (Finke and Snyder, 2008). On the other hand, although *M. nigricans* exhibits a significant preference for Diptera and other prey that tend to fly slowly and that are weekly sclerotized, it shows relatively high dietary and temporal overlaps with *E. furinalis*. This suggests that coexistence between these species is not favored by divergence at the two trophic niche dimensions studied here but perhaps to the spatial structure of habitats used for hunting. In fact, these vespertilionids exhibit some aerodynamic differences that make likely probable a differential use of space, both horizontal and vertical (Amador et al. 2020). Illuminating these aspects through integrative analyses will be key to unravel the selective forces that contribute to the close coexistence and high diversification of bats in tropical environments.

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Declarations

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Conflicts of interest. The authors declares no conflicting or competing interests.

Ethics approval Bat captures followed the guidelines recommended by the American Society of Mammalogists. Oficina de Diversidad Biológica (Ministerio del Poder Popular para Ecosocialismo y Aguas) provided permits to collect bats and obtain their fecal samples (Permit No. 1010-2013), to collect insects (Permit N°1011-2013), and to conduct genetic analyses on these samples (Permit N° 0241-2013 and Permit N° 059-2015).

Consent to participate. Not applicable.

Consent for publication. Not applicable.

Availability of data and material. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contribution statement. All authors contributed to the study's conception and design. YA and JN contributed to fundraising for the purchase of equipment and other materials, as well as material preparations. YA contributed to data collection and

statistical analyses. JM contributed to fundraising, genetic data acquisition and analyses. The first drafts of the manuscript were written by YA and JM. All authors contributed to the final version. All authors read and approved the final manuscript.

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Figure Legends

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

Figure 2. Percentage of prey in the diet of three species of bats, classified by (a) order, (b) family, and (c) trophic group 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.

Figure 3. Percentage of prey in the diet of three species of bats, classified by (a) degree of sclerotization, and (b) flight behavior.

Figure 4. 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 5. Time-density curves of bat species pairs with overlap area in gray from both farms 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 Δ4 is used for sizes greater than 75.

Figure 1

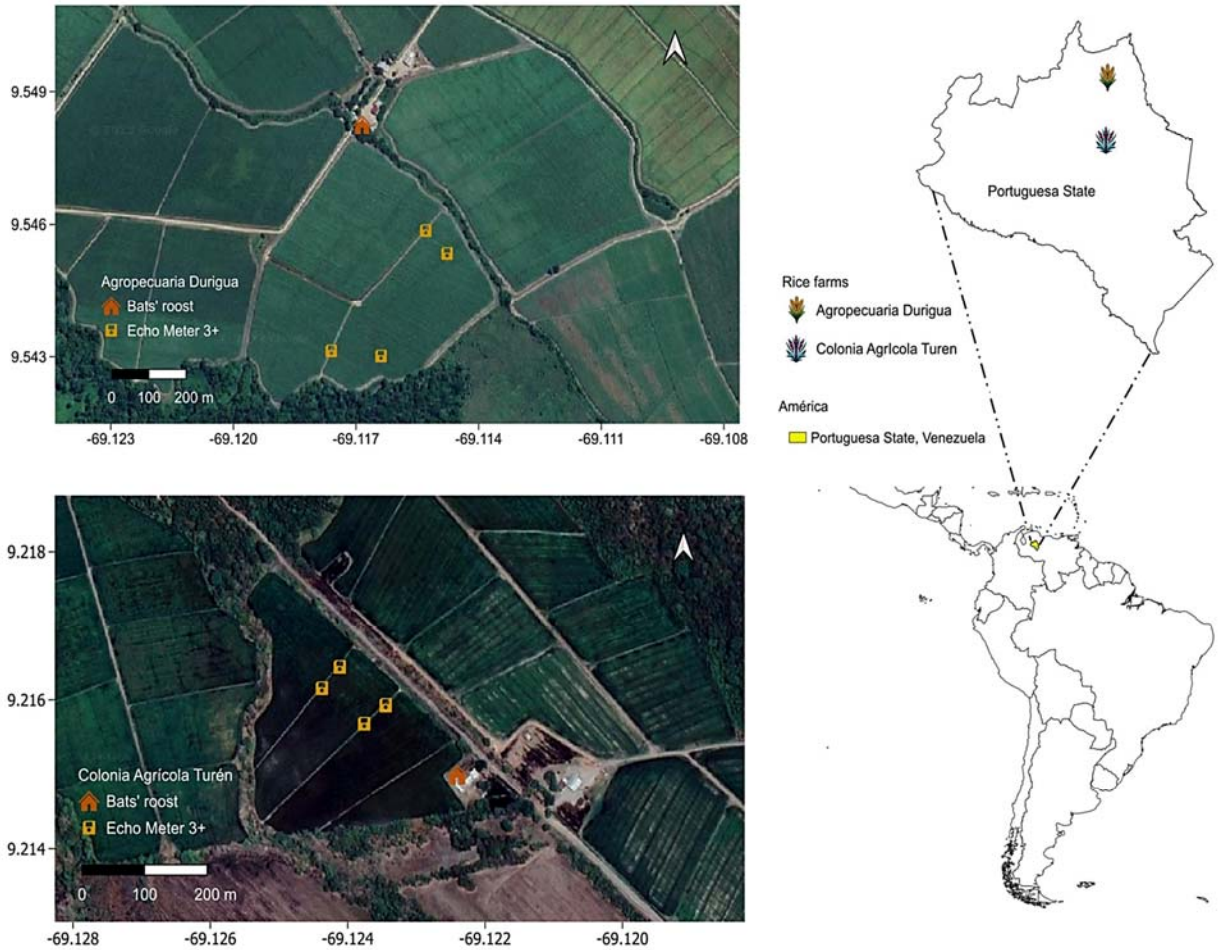

9.549
9.546
9.543
Agropecuaria Durigua
Bats' roost
Echo Meter 3+
0 100 200 m
-69.123
-69.120
-69.117
-69.114
-69.111
-69.108
9.218
9.216
9.214
Colonia Agrícola Turén
Bats' roost
Echo Meter 3+
0 100 200 m
-69.128
-69.126
-69.124
-69.122
-69.120
Portuguesa State
Rice farms
Agropecuaria Durigua
Colonia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

Figure 2

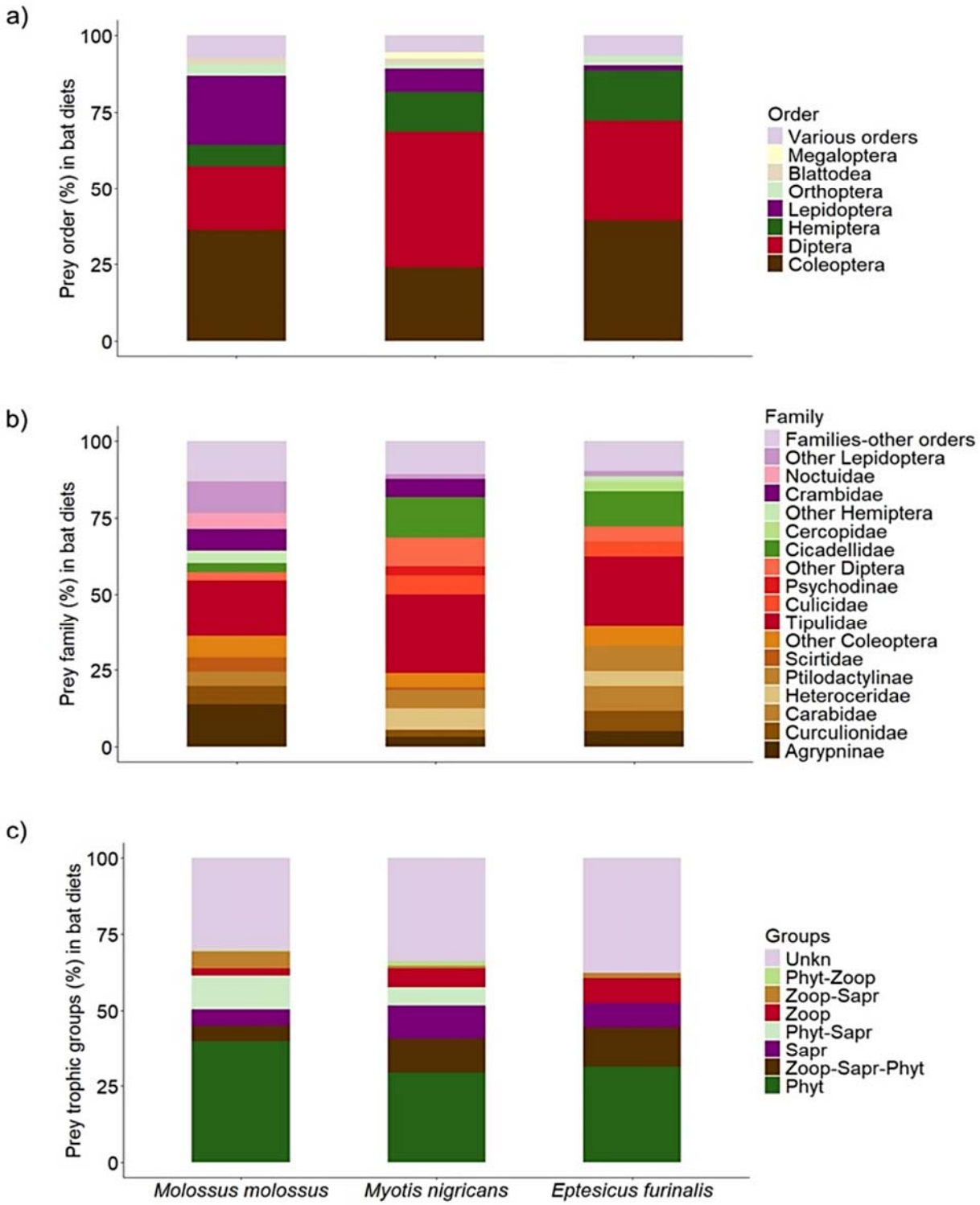

a)
Prey order (%) in bat diets
100
75
50
25
0
Order
Various orders
Megaloptera
Blattodea
Orthoptera
Lepidoptera
Hemiptera
Diptera
Coleoptera
b)
Prey family (%) in bat diets
100
75
50
25
0
Family
Families-other orders
Other Lepidoptera
Noctuidae
Crambidae
Other Hemiptera
Cercopidae
Cicadellidae
Other Diptera
Psychodinae
Culicidae
Tipulidae
Other Coleoptera
Scirtidae
Ptilodactylinae
Heteroceridae
Carabidae
Curculionidae
Agrypninae
c)
Prey trophic groups (%) in bat diets
100
75
50
25
0
Groups
Unkn
Phyt-Zoop
Zoop-Sapr
Zoop
Phyt-Sapr
Sapr
Zoop-Sapr-Phyt
Phyt
Molossus molossus
Myotis nigricans
Eptesicus furinalis

Figure 3

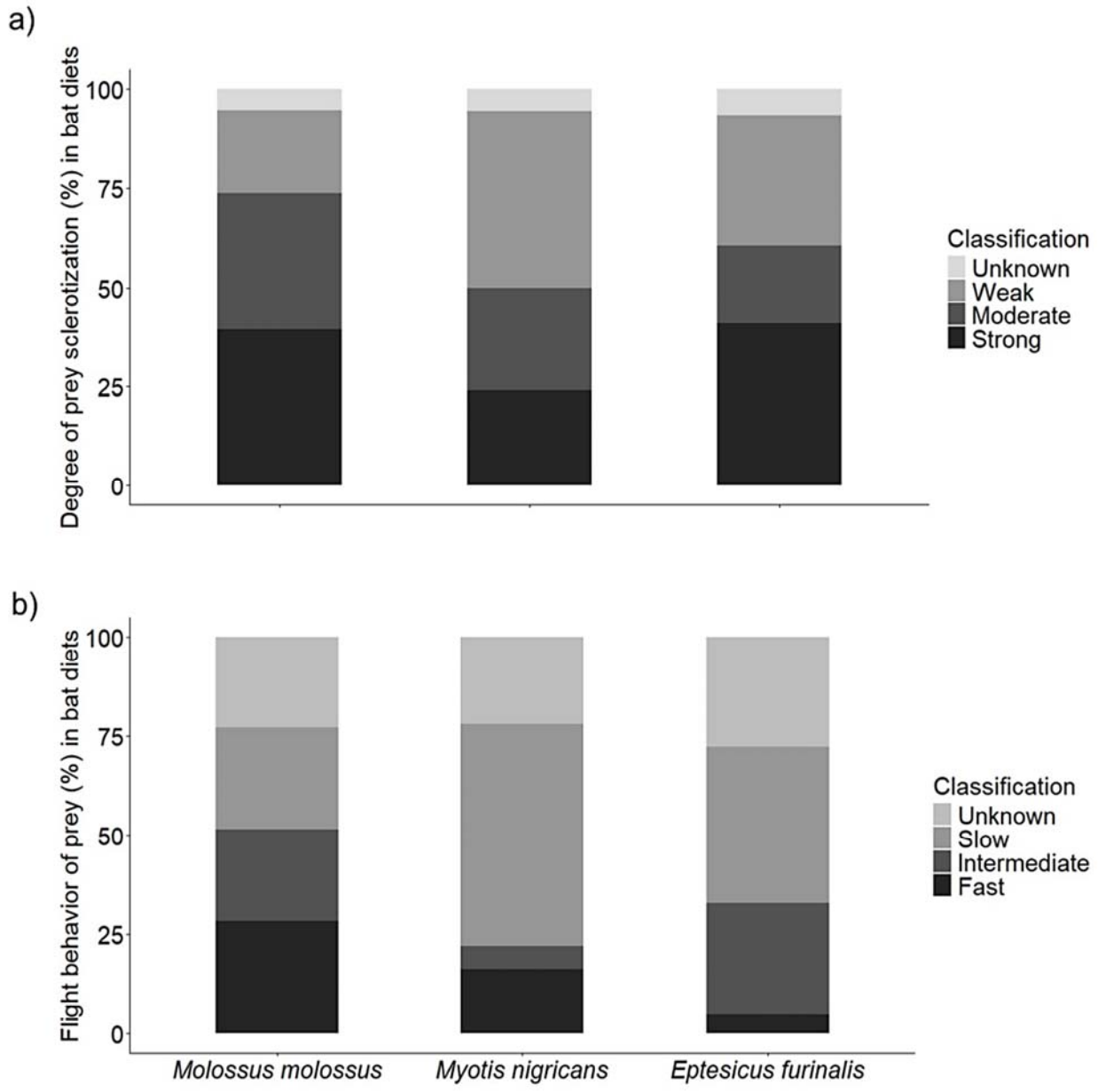

a)
Degree of prey sclerotization (%) in bat diets
100
75
50
25
0
Classification
Unknown
Weak
Moderate
Strong
b)
Flight behavior of prey (%) in bat diets
100
75
50
25
0
Classification
Unknown
Slow
Intermediate
Fast
Molossus molossus
Myotis nigricans
Eptesicus furinalis

Figure 4

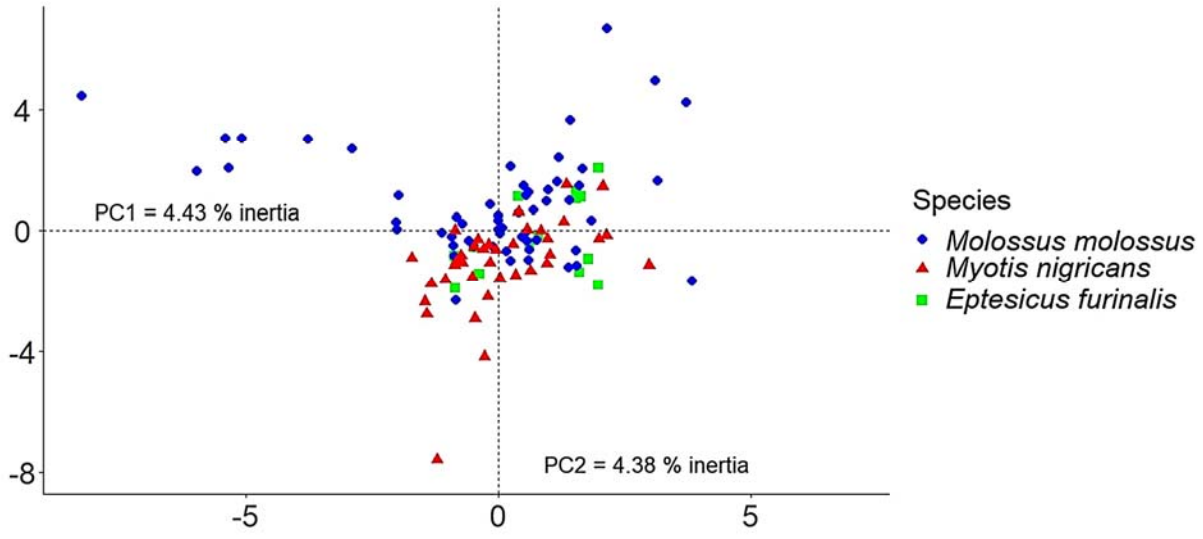
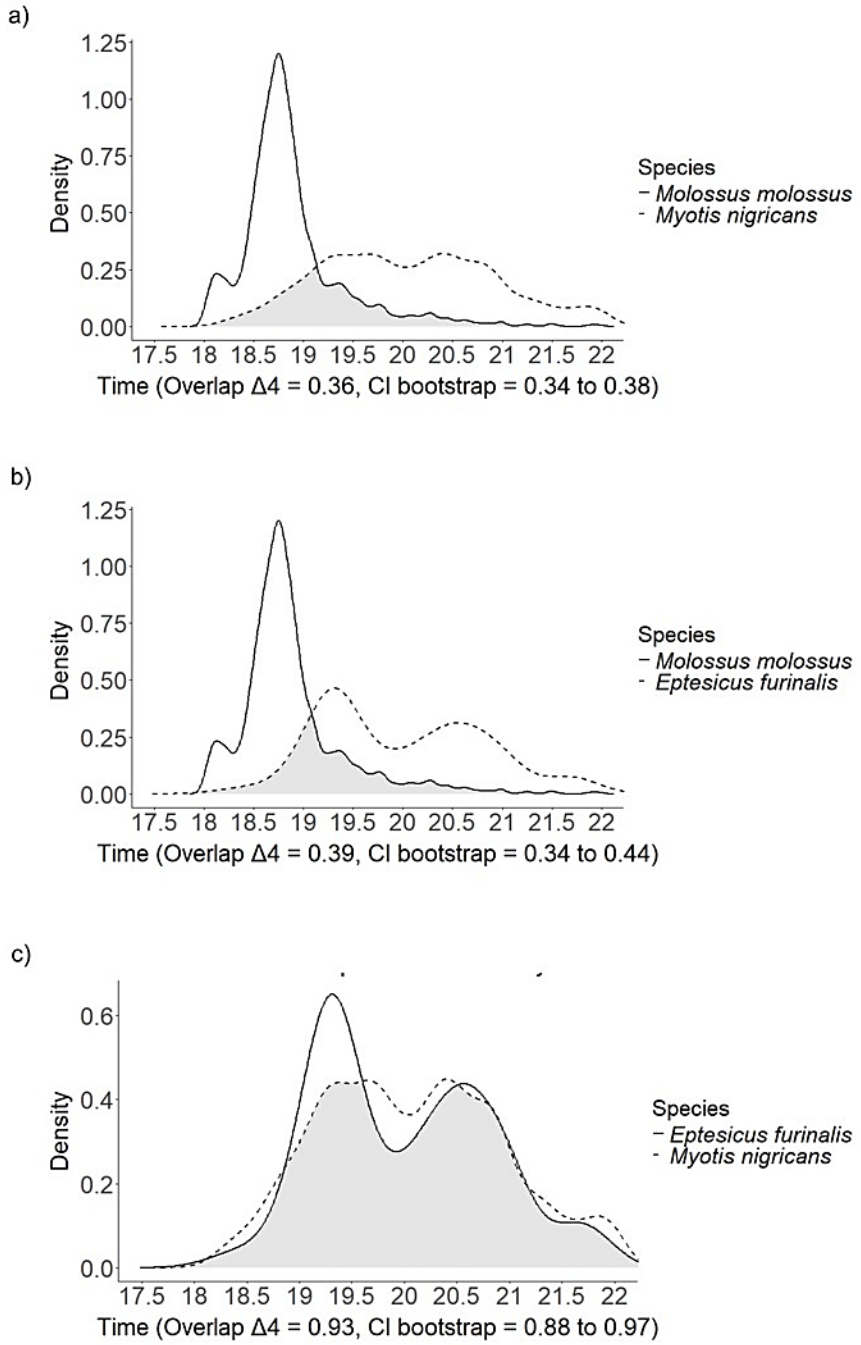

4
0
-4
-8
-5
0
5
PC1 = 4.43 % inertia
PC2 = 4.38 % inertia
Species
Molossus molossus
Myotis nigricans
Eptesicus furinalis

Figure 5


a)
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)
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
c)
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