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DNA metabarcoding reveals the role of urban insectivorous bats in pest suppression

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Abstract

Urban parks and other green spaces can function as critical refuges and habitat corridors for bats across fragmented urban landscapes. Although urban environments have received comparatively little attention in bat ecology, bats provide valuable ecosystem services in cities, including the suppression of arthropod pests. Slottsskogen is one of Gothenburg's oldest and largest parks, which actively promotes biodiversity conservation. Faecal DNA metabarcoding allowed identification of prey species, offering high taxonomic resolution compared to traditional methods that rely on manual visual identification of food items in faecal samples. In this study, we investigated four bat species, *Cnephaeus nilssonii*, *Pipistrellus nathusii*, *P. pygmaeus*, and *Vespertilio murinus*, and detected 191 Operational Taxonomic Units of arthropod prey in their diet, including several agricultural/forestry pest species and disease vectors, primarily dipterans. We identified distinct dominant species assemblages in the diet composition of *P. pygmaeus* varied between spring and summer, likely due to seasonal turnover of prey rather than prey preference. Overall, this study identifies the role of insectivorous bats in pest suppression and disease vector control, with important implications for urban environments.

Introduction

More than 60% of the world's 1,500 bat species are insectivores (Simmons and Cirranello 2026) and play a crucial role in pest control by consuming vast quantities of arthropods each night while foraging over agricultural fields, urban gardens, parks, water bodies, and forests (Kunz et al. 2011, Tuneu-Corral et al. 2023). Understanding dietary variation is essential to assess how bats contribute to broader ecosystem processes, including arthropod pest suppression in agricultural systems, which has been extensively studied and quantified in economic terms (Cleveland et al. 2006, Federico et al. 2008, Kunz et al. 2011). For instance, it has been estimated that the Brazilian free-tailed bats (*Tadarida brasiliensis*) contribute up to 1.7 million USD annually in arthropod pest suppression to cotton production in Texas (Cleveland et al. 2006). However, the importance of bats and the ecosystem services that they provide in urban environments have been largely understudied (Russo et al. 2023).

Rapid urban expansion continues to replace natural habitats (Angel et al. 2005), and its effects on bats are highly species-specific (Russo and Ancillotto 2015). While some species adapt and even thrive in urban areas, others experience declines due to habitat loss, fragmentation, and pollution (Fischer et al. 2015, Russo and Ancillotto 2015). Nevertheless, urban green spaces, such as parks, play a crucial role in maintaining bat populations (Moretto et al. 2023). Old trees with cavities and large crowns provide roosting sites, while vegetation and the presence of water bodies promote insect abundance (Moretto et al. 2023). These green spaces also increase habitat connectivity, especially when connected tree canopies help bats navigate urban landscapes and reduce light and noise pollution (Moretto et al. 2023). In turn, bats deliver valuable ecosystem services such as mosquito suppression, reducing the risk of mosquito-borne diseases such as West Nile virus and malaria that pose major public health challenges in urban areas worldwide (Russo et al. 2023). In addition, urban bats feed on a range of arthropod pests, including species whose bites cause allergic reactions, damage crops and green spaces, are disease vectors, and affect stored products such as food, textiles, and household materials (Russo et al. 2023).

The variation in the diet of insectivorous bats provides critical insights into their ecological roles and behaviour as well as health of the ecosystems they inhabit. These insights in turn inform on species interactions, habitat quality, and even broader environmental changes. Different bat species exploit different ecological niches through diverse foraging strategies which shape their dietary composition (Kunz et al. 2011). For example, the brown long-eared bat (*Plecotus auritus*) often hunts within vegetation or near the ground by gleaning, consuming mainly non-flying prey such as blowflies (Diptera: Calliphoridae) (Rydell 1989b). In contrast, the northern bat (*Cnephaeus nilssonii*) primarily captures small dipterans in flight, while flying in open spaces 2–20 m above the ground by aerial hawking (Rydell 1989b). Despite their sympatric distributions, *P. auritus* and *C. nilssonii* (Rydell 1989b), they employ distinct foraging strategies. Similarly, in Japan, two morphologically similar sympatric species, Ussuri tube-nosed bat (*Murina ussuriensis*) and Ikonnikov's bat (*Myotis ikonnikovi*), exhibit contrasting strategies: *M. ikonnikovi* has a broader, more generalist diet, while *M. ussuriensis* specializes on moths (Lepidoptera) and other prey likely captured via gleaning (Heim et al. 2021).

Bat foraging activity and dietary patterns are strongly influenced by seasonal fluctuations in prey availability and sex-specific factors. In temperate regions, insect abundance declines sharply during winter, leading several insectivorous bat species to hibernate to conserve energy, and activity resumes in spring as insects become plentiful, allowing bats to rebuild energy reserves (Wilson and Mittermeier 2019). Mating typically occurs in late summer or early autumn and females delay fertilization until spring (Wilson and Mittermeier 2019). Females form maternity colonies in late spring or early summer to rear young when prey availability is highest (Heim et al. 2016). Activity remains high through summer to support both foraging and reproduction, intensifying again in late summer and early autumn as bats build fat reserves in preparation for winter as insect prey availability declines (Stawski and Geiser 2010, Wilson and Mittermeier 2019). Males often forage over larger areas to avoid dominant females, resulting in a more diverse diet (Mata et al. 2016, Wilkinson and Barclay 1997), whereas females often forage at the same sites during pregnancy and lactation (Wilkinson and Barclay 1997), likely because familiarity with these areas enhances foraging efficiency and reduces energetic costs (Rydell 1989a). Females additionally select higher-calorie or specialized prey to meet reproductive energy demands (Mata et al. 2016, Racey and Entwistle 2000, Wilkinson and Barclay 1997). For example, Mata et al. (2016) identified a higher consumption of large and migratory moths by female European free-tailed bats (*Tadarida teniotis*) during pregnancy and lactation. In addition, morphological (Lisón et al. 2014), behavioural (Schuchmann et al. 2012), and physiological differences (Levin et al. 2013) between sexes further influence habitat use and prey choice (Mata et al. 2016).

Traditionally, bat diet has been examined by visually identifying partially digested prey remains, such as insect fragments, plant material, or pollen, etc., under a microscope in gut contents or faeces (Alviola et al. 2023, Bernard 2002, Rydell 1989b, Rydell et al. 1996), which is often challenging and provides limited or biased information, especially if food items are well-digested (Rabinowitz and Tuttle 1982). Molecular approaches such as DNA metabarcoding offer a more efficient and comprehensive way to characterize diet, providing much higher taxonomic resolution of prey species (de Sousa et al. 2019, Ingala et al. 2021, Ling et al. 2023, Mata et al. 2019, Swift et al. 2018, Tiede et al. 2020). In DNA metabarcoding analysis, sequencing reads are typically clustered into Operational Taxonomic Units (OTUs) for downstream analyses (Liu et al. 2020). OTUs represent groups of sequences clustered based DNA sequence

similarity (Taberlet et al. 2018), and serve as proxies for taxonomic species when species boundaries are unclear (Liu et al. 2020). This approach is especially valuable for “dark taxa” such as arthropods, a hyper diverse but poorly described group (Hartop et al. 2024). Limited reference databases and cryptic morphology often constrain arthropod taxonomic resolution. By characterizing these taxa through OTUs, metabarcoding allows for quantification and comparison of community diversity, even when species remain undescribed (Hartop et al. 2024). These molecular insights not only highlight biodiversity patterns but provide a basis for understanding the ecological roles of key taxa.

Here, we leverage faecal DNA metabarcoding to determine the diet of four widespread bat species, the Northern bat (*Cnephaeus nilssonii*), Soprano pipistrelle (*Pipistrellus pygmaeus*), Nathusius’ pipistrelle (*P. nathusii*), and Parti-coloured bat (*Vespertilio murinus*) in Slottsskogen Park, Gothenburg, Sweden. Knowledge of bat diet in the park will offer insights critical for conserving bat populations and guiding habitat management, while increasing the understanding of the ecosystem services bats provide in urban areas. Given that there are no existing arthropod studies from the park, this study also offers valuable insight into the arthropod diversity present in the park.

Materials and Methods

Study area

Slottsskogen Park was established in 1874 and spans over 135 hectares, making it one of the largest and oldest parks in Gothenburg city centre, in southwestern Sweden. The park hosts the Gothenburg Natural History Museum and Slottsskogen Zoo, both of which actively promote biodiversity and its conservation. The zoo is ungated and freely accessible to the public. A large part of Slottsskogen is made up of natural forest and has several ponds that host a variety of wildlife, such as many species of waterbirds and migratory birds. These varied habitats are actively managed by the park to maintain biodiversity. Sampling was carried out around four pond sites: Fågeldammen, Lilla dammen, Ödledammen, and Stora dammen (Fig. 1).

Sample collection

Fieldwork was conducted in three phases: 1) spring (May 2024), 2) summer (July 2024), and 3) autumn (September 2024) to obtain bat faecal samples at four sites in the park (Fig. 1). Fieldwork began at sunset, and lasted 3–4 hours in spring and summer, and around five hours in autumn. Bat capture and handling followed guidelines approved by the American Society of Mammalogists (Sikes and Animal Care Use Committee of the American Society of Mammalogists 2016). The sex, age (juvenile vs. adults), forearm length, and reproductive status (pregnant or lactating) of each captured individual were noted. Individuals were photographed from dorsal, central, and lateral views. To obtain faecal samples, captured bats were placed in breathable cloth bags (Ingala et al. 2021), with a paper plate placed inside the bag, until defecation occurred. Faecal pellets were then collected using sterilized forceps from the paper plates in the bags. The pellets were stored in sterile sampling vials containing 80% molecular grade ethanol as preservative. Gloves were worn during the entire process and both gloves and forceps were sterilized, using Sodium hypochlorite between samples (de Silva Wijeyeratne and Gweon 2025), followed by ethanol to remove any chlorine residue. Within 24 hours of collection, the faecal samples were stored at -20°C or lower to prevent the degradation of DNA until further processing (Liu et al. 2020). A total of four bat species and 33 individuals were sampled across seasons (Table 1), with faecal samples collected from 32 individuals.

Sampling was carried out as a part of Gothenburg Natural History Museum’s monitoring and data collection programme, under the permit of the museum (Permission NV-09707-22) by the Swedish Environmental Protection Agency (Naturvårdsverket). The study was vouchered by specimen of *P. nathusii* and tissue samples of *P. pygmaeus* and *C. nilssonii*, all available under the general accession number 2025–23519 together with the faecal samples on Gothenburg Natural History Museum.

DNA extraction and amplification

DNA extraction was performed on 32 faecal samples using QIAamp® PowerFecal® Pro DNA Kit (MO BIO Laboratories, Qiagen Co., California, USA) following manufacturer's protocol with slight modification for low DNA concentration. DNA was extracted from 1–3 pellets (i.e., 1 pellet = 1 replicate; maximum of three extraction replicates) per sample, resulting in 91 DNA extracts and two negative controls. Since each faecal pellet only weighed ~ 5–10 mg, the concentration of DNA obtained was very low. Therefore, the protocol was modified to use only 40 µL of Solution C6 for DNA elution, to increase DNA concentration of each sample. The DNA extracts were stored at -20°C until the first polymerase chain reaction (PCR) amplification was completed. A two-step PCR approach (initial PCR and index PCR) was used to amplify and prepare DNA metabarcoding libraries for sequencing.

During initial PCR, mitochondrial DNA was amplified using the arthropod general cytochrome oxidase subunit 1 (COI) primers fwhF2-R2n (Vamos et al. 2017). PCR reactions were carried out in final volumes of 10 µL, including 8 µL of Master Mix (i.e., 5.4 µL of MiQ water, 1 µL of 10x Kapa Taq Buffer B, 0.6 µL of 25 mM $MgCl_2$, 0.2 µL of 10 mM dNTP mix, 0.3 µL each of 10 mM forward and reverse primers, and 0.2 µL of 5U/µL Taq DNA Polymerase), and 2 µL of template DNA. PCR master mix was prepared using the KAPA Taq PCR kit (Roche, KAPA Biosystems, Basel, Switzerland). The following PCR cycling conditions were followed: initial denaturation at 95°C for 3 min, 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 10 min. Amplification success and quality was checked by visualizing PCR products in 2% agarose gels stained with GelRed. We performed three PCR replicates per DNA extract and eight PCR negative controls.

Library preparation

Only one PCR replicate per sample (DNA extract) was chosen for library preparation, specifically the replicate showing the brightest band on an agarose gel (i.e., 91 first-PCR products). Among these selected initial-PCR products, those with bands that were visibly much brighter than the DNA ladder were diluted with water to a 1:4 ratio. These products were tagged with 10-bp Nextera Unique Dual (UD) indices containing short barcodes, along with standard P5 and P7 Illumina adapters, for sample identification in the next PCR amplification step, also known as index PCR. Index PCR reactions were in 20 µL reaction volumes,

which consisted of 10 µL of 2X KAPA HiFi HotStart ReadyMix (Roche, KAPA Biosystems, Basel, Switzerland), 4 µL of index primer (readymade mix of forward and reverse primers), and 6 µL of diluted PCR product. Cycling conditions of the index PCR were as follows: initial denaturation at 98°C for 2 min, followed by 8 cycles of denaturation at 98°C for 20 s, annealing at 55°C for 20 s, extension at 72°C for 15 s, and a final extension at 72°C for 2 min. Index PCR products were then run on 2% agarose gel to check for the success of indexing, indicated by an increase in amplicon size. Then, indexed PCR products were cleaned with AMPure XP Beads (Beckman Coulter, California, USA) with 1:0.8 ratio (Gonçalves et al. 2024), following the manufacturer's instructions with modifications on a DynaMag 2 (Life Technologies Inc., California, USA) magnetic stand, to retain DNA fragments > 400bp and remove shorter DNA fragments, primers, and primer dimers. Primer dimers are unintended DNA fragments formed when PCR primers bind to each other instead of the target DNA. PCR clean-up success was verified via gel electrophoresis, indicated by the appearance of a singular and expected band size and position for all the PCR products.

Each Illumina library was quantified using the Qubit dsDNA High Sensitivity Kit (Thermo Fisher Scientific, Massachusetts, USA) and then pooled in equimolar amounts to obtain a final concentration of ~ 20 nM/ 65 µL. The pooled sample was checked on a gel to determine average fragment size and confirm the success of bead clean-up, and then re-quantified. A final clean-up was performed at a 1:0.765 bead ratio, and the cleaned pool was visually inspected with a gel, re-quantified, and subsequently sequenced on the Illumina NovaSeq X platform using a 10B flow cell, to obtain ~ 8–9 million 150 bp paired-end reads per PCR replicate. Sequencing was performed at the National Genomics Infrastructure (NGI; Science for Life Laboratory - SciLifeLab, Stockholm, Sweden).

Bioinformatics

Primer sequences were removed from the raw sequencing data (https://www.ebi.ac.uk/biostudies/studies/S-BSST3141) using Cutadapt (Martin 2011), and forward and reverse reads were aligned using FLASH2 (Magoč and Salzberg 2011) (--max-overlap 150 --min-overlap 10). Using VSEARCH (Rognes et al. 2016), reads with a maximum of 1 bp error (--fastq_maxee 1) were discarded. Retained reads were de-replicated into Exact Sequence Variants (ESVs), also known as Amplicon Sequence Variants (ASVs) (Callahan et al. 2017), within each sample. ESVs with only one read were removed (--minuniquesize 2), as they were likely PCR or sequencing errors; then we merged all the samples into a single FASTA file. Reads were then de-replicated, or collapsed, into ESVs across the entire dataset. Then, sequences shorter or longer than 180–220 bp, as well as sequences with read counts < 8 were removed, and the remaining ESVs were denoised with the command "--cluster_unoise". Chimeras were removed with "--uchime3_denovo". Finally, the sequences were clustered into OTUs at 99% similarity using '--cluster_size'. Then, the original reads were mapped back to OTU clusters with '--usearch_global' at a 99% identity threshold to determine their abundance in each sample (Gonçalves et al., 2024). The "LULU" (Frøslev et al. 2017) R package was then used to remove potential PCR artifacts, sequencing artifacts, and Nuclear copies of the Mitochondria (NUMTs). Finally, OTUs sharing more than 84% sequence similarity and co-occurring in over 95% of the samples (default parameters in LULU) were merged into a single unit, with their read counts combined, to minimize the impact of artificially split sequences (Gonçalves et al. 2024). See Supplementary material 1 for an illustration of data cleaning and processing steps, as well as a table demonstrating recovered read total during each step of the pipeline.

To classify OTUs at the species, genus, and family levels, taxonomic assignments were performed using BOLDigger3 (Buchner and Leese 2020) using similarity thresholds of 98%, 95%, and 90%, respectively, and were manually verified and revised as needed to resolve ambiguous or inconsistent matches. Only OTUs assigned to the phylum Arthropoda were retained for further analysis, as numerous OTUs were either unassigned or matched non-target taxa. OTUs were classified as dietary if they belonged to the classes Insecta or Arachnida and could be identified to the order level or lower. When multiple OTUs assigned to the same higher-level taxon, their unique OTU identifiers were retained to maintain taxonomic distinction for downstream analyses (e.g., otu286 and otu297 both assigned to the family Cecidomyiidae were labelled as Cecidomyiidae_otu286 and Cecidomyiidae_otu297) following Gonçalves et al. (2024). Reads detected in extraction and PCR negative controls were removed from sample counts, and dietary OTUs representing less than 1% of total dietary reads per sample were excluded (Gonçalves et al. 2024) to reduce potential false positives from laboratory or sequencing cross-contamination, while still retaining low-abundance taxa that may represent genuine but infrequent prey, thereby providing a more complete picture of bat dietary diversity. In addition, the prey OTUs identified to species level were later classified as known pest or disease vectors based on literature review.

Results

Arthropod prey diversity

We obtained a total of 319,568,985 sequence reads and 191 haplotypes from the 32 faecal samples of *Cnephaeus nilssonii*, *Pipistrellus nathusii, P. pygmaeus*, and *Vespertilio murinus*. *Cnephaeus nilssonii* was formerly known as *Eptesicus nilssonii* (Coroiu 2016). A diverse diet composed of multiple arthropod orders and families was found for the four bat species (Fig. 2, Table 1). In total, 191 OTUs of 12 orders, 56 families, and at least 132 genera, and 155 species (Supplementary material 2) were identified in the faecal pellets of the four species. Since 36 of the 191 OTUs could not be identified to species level, we used the term 'OTU' rather than 'species' when describing the diet, as each OTU represents a unique prey taxon.

Table 1
List of species and the number of individuals (n) captured

Season/month	*Cnephaeus nilssonii*	*Pipistrellus nathusii*	*Pipistrellus pygmaeus*	*Vespertilio murinus*
Spring/May	-	-	6	2
Summer/July	17*	1	6	-
Autumn/September	-	-	1	-

Note: * One of the 17 *C. nilssonii* individuals did not defecate, thus faeces from 16 individuals were sampled·

Out of the 191 prey items identified, most belonged to class Insecta (99.48%), followed by Arachnida (0.52%). Diptera (60.21%; mostly non-biting midges and mosquitoes), Lepidoptera (15.71%; moths), Coleoptera (6.81%; beetles), and Hemiptera (5.76%; true bugs) were the most frequently detected prey orders in the diet of bats (Fig. 3). By family, Chironomidae (Diptera) was the most consumed (27.75%), followed by Tortricidae (Lepidoptera; 7.85%), Limoniidae (Diptera; 6.81%), Ceratopogonidae (Diptera; 3.14%), and Miridae (Hemiptera; 3.14%). The most frequently detected prey OTU in the diet was *Cheilotrichia cinerascens* (Diptera; 16.41%), followed by *Chironomus plumosus* (Diptera; 10.28%), *Dicranomyia modesta* (Diptera; 6.85%), *Plutella xylostella* (Lepidoptera; 5.99%), *Benthalia carbonaria* (Diptera; 4.44%), *Molophilus griseus* (Diptera; 5.99%), and *Cydia splendana* (Lepidoptera; 3.05%).

We found that most of the detected prey OTUs (170 of 191) were present at very low relative abundance, each contributing less than 1% of total reads, revealing a highly uneven diet dominated by a few frequently consumed prey taxa, and a long tail of low-abundance taxa, which included *Ectobius lapponicus* (Blattodea; forest cockroach), *Zygiella x-notata* (Araneae; missing-sector orb weaver), and *Lasius fuliginosus* (Hymenoptera; jet black ant), among others.

Individual bats consumed a varied number of prey OTUs (Fig. 4), i.e., the number of prey OTUs per individual ranged from 3–24, with an average of 11.81 ± 5.51 (mean ± SD), indicating considerable dietary breadth among individuals.

Presence of arthropod pests or disease vectors

Of the 191 prey OTUs detected, 155 were identified to species level. 12.90% of 155 OTUs were pests or disease vectors. Among them, 9.03% were found to be known agricultural/forestry pests (e.g., *Agrotis segetum*), while 3.87% of them were disease vectors (e.g., *Culex pipiens*) (Fig. 5, Table 2). Lepidoptera and Diptera were among the most frequently detected pest/disease vector orders (Fig. 6).

Table 2

List of known and potential agricultural/forestry pest species and disease vectors found in the diet of *Cnephaeus nilssonii* and *Pipistrellus pygmaeus*. The pe samples for which each prey was detected in each bat species is indicated. Sample size (*n*) of the bat species are in parentheses. No pest or disease vectors *V. murinus* and *P. nathusii* samples. References in Supplementary material 3.

Order	Family	Species	Common name	BOLD ID percentage	Status	Crop/disease	*C. nilssonii* (16)	*P. pygmaeus*		
								All (13)	Spring (6)	Summe (6)
Coleoptera	Cerambycidae	*Arhopalus rusticus*	Rust pine borer	100%	Known pest	Conifer trees	6.25%			
		Serica brunnea	Brown chafer	100%	Known pest	Plant roots	6.25%			
Diptera	Ceratopogonidae	*Culicoides pulicaris*	Biting midge	99.51%	Disease vector	Blue tongue virus (BTV)		7.79%	16.67%	
		Culicoides punctatus	Biting midge	99.51%	Disease vector	Blue tongue virus (BTV)		7.79%	16.67%	
	Culicidae	*Aedes punctor*	Woodland mosquito	100%	Disease vector	West Nile Virus (WNV)	12.50%			
		Coquillettidia richiardii	Swamp mosquito	99.51%	Disease vector	West Nile Virus (WNV), Batai virus (BATV), Tahyna virus (TAHV)	6.25%			
		Culex pipiens	Common house mosquito	99.51%	Disease vector	West Nile Virus (WNV)	12.50%	30.77%		50%
	Drosophilidae	*Scaptomyza flava/pallida*	Brassica leaf miner	100%	Known pest	Peas and some brassica crops		7.69%		
	Simuliidae	*Simulium noelleri*	Black fly	100%	Disease vector	Black-fly fever		7.69%		16.67%
Hemiptera	Aphididae	*Brachycaudus helichrysi*	English grain aphid	100%	Known pest	Stone fruits such as plum, peach		7.69%		
		Rhopalosiphum padi	Bird cherry-oat aphid	100%	Known pest	Cereal crops		7.69%		
Lepidoptera	Geometridae	*Agriopis leucophaearia*	Spring usher moth	100%	Known pest	Oak defoliation		7.69%	16.67%	
	Noctuidae	*Agrotis segetum*	Turnip moth	100%	Known pest	Root and tuber crops	6.25%			
	Plutellidae	*Plutella xylostella*	Cabbage moth/ diamondback moth	100%	Known pest	Brassica crops like cabbage, cauliflower	37.50%	7.69%		16.67%
	Tortricidae	*Cydia splendana*	Chestnut tortrix moth	100%	Known pest	Chestnut	12.50%	15.38%		33.33%
		Pandemis corylana	Chequered fruit-tree tortrix	100%	Known pest	Fruit trees	6.25%	7.69%		16.67%
		Pandemis heparana	Dark fruit-tree tortrix	100%	Known pest	Fruit trees	18.75%	7.69%		16.67%
		Rhyacionia pinicolana	Orange spotted shoot	100%	Known pest	Pine	12.50%	15.38%		33.33%
		Spilonota ocellana	Bud moth	100%	Known pest	Fruit trees	6.25%			
		Zeiraphera isertana	Mottled oak tortrix	100%	Known pest	Conifer, fruit trees, oak	12.50%	30.77%	33.33%	33.33%

Diet composition of different bat species

The sample size (n) was highly varied among different bat species (Table 1). The bat species with the highest number of samples was *Cnephaeus nilssonii* (n = 32), with a total of 81 prey items (OTUs) representing 11 orders and 33 families, belonging to class Insecta (98.76%) and Arachnida (1.23%) (Fig. 7). In

Pipistrellus pygmaeus, a total of 132 prey items were identified across three seasons, representing nine orders and 41 families. For *Vespertilio murinus*, 19 prey items were identified, corresponding to four orders and seven families, and in case of *Pipistrellus nathusii*, only three prey items were identified (Fig. 7). All prey species belonged to class Insecta for the latter three bat species (Fig. 7).

At the order level (Fig. 7), most prey belonged to Diptera (48.15%; mostly non-biting midges and mosquitoes) for *C. nilssonii* followed by Lepidoptera (20.99%; moths), Coleoptera (12.34%; beetles), and Hemiptera (4.94%; true bugs). In case of *P. pygmaeus*, most prey were identified to order Diptera (non-biting midges and mosquitoes; 68.18%), followed by Lepidoptera (moths; 14.39%) and Hemiptera (true bugs; 6.82%). Similarly, in case of *V. murinus*, most prey belonged to the order Diptera (mostly non-biting midges and mosquitoes; 84.21%), followed by Coleoptera (beetles), Hymenoptera (ants and wasps), and Lepidoptera (moths), all three at 5.26%. Lastly, all prey items were identified to the order Diptera in *P. nathusii*.

By family, in case of *C. nilssonii*, Chironomidae (Diptera) was the most consumed (23.45%) and was followed by Totricidae (Lepidoptera; 11.11%), Limoniidae (Diptera; 7.41%), and Carabidae (Coleoptera; 6.17%). Similarly, the most consumed family for *P. pygmaeus* was Chironomidae (Diptera; 30.30%), followed by Totricidae (Lepidoptera; 9.09%) and Limoniidae (Diptera; 6.82%). Again, for *V. murinus*, Chironomidae (Diptera) was the most consumed family (63.16%), followed by Limoniidae (Diptera; 10.53%). While in case of *P. nathusii*, all prey items belonged to the family Chironomidae (Diptera).

At the OTU level, the most frequently detected OTU in the diet of *C. nilssonii* was *Cheilotrichia cinerascens* (Diptera; 23.19%), followed by *Dicranomyia modesta* (Diptera; 12.58%), *Plutella xylostella* (Lepidoptera; 12.43%) and *Chironomus plumosus* (Diptera; 11.59%). While for *P. pygmaeus*, *Benthalia carbonaria* (Diptera; 10.15%), *C. cinerascens* (Diptera; 9.72%), followed by *Molophilus griseus* (Diptera; 8.09%), *Zeiraphera isertana* (Lepidoptera; 6.60%), *Monardia* sp. (Diptera; 6.51%), *C. splendana* (Lepidoptera; 5.93%) and *C. plumosus* (Diptera; 5.63%) were the most frequently detected OTUs. Furthermore, in the diet of *V. murinus*, *Tipula oleracea* (Diptera; 27.25%), *Aphomia sociella* (Lepidoptera; 20.92%), *C. cinerascens* (Diptera; 16.80%) and *Glyptotendipes* sp. (Diptera; 13.47%) were the most frequently detected. Lastly, the most frequently detected prey OTUs in case of *P. nathusii*, were *C. plumosus* (Diptera; 57.06%) and *Chironomus tentans/pallidivittatus* (Diptera; 40.53%).

Several agricultural or forestry pests and disease vectors were only detected in the diet of *C. nilssonii* and *P. pygmaeus*. Of the 67 prey OTUs identified to species level for *C. nilssonii*, 14.92% were found to be known agricultural or forestry pests (e.g., *Agrotis segetum*), while 4.48% of them were disease vectors (e.g., *Culex pipiens*) (Table 2). Similarly, in case of *P. pygmaeus*, of 107 prey OTUs identified to species level, 9.34% were found to be known agricultural or forestry pests (e.g., *Cydia pomonella*), and 3.74% were disease vectors (e.g., *C. pipiens*) (Table 2).

In this study, *P. pygmaeus* was the only bat species captured through spring, summer, and autumn, and its diet varied across seasons (Fig. 8). In spring, 68 prey items representing five orders and 20 families. Similarly, in summer and autumn, 65 prey items (nine orders and 30 families) and 16 prey items (two orders and 11 families), respectively were detected. Diptera (mostly non-biting midges and mosquitoes) was the most dominant order in all three seasons, followed by Lepidoptera (moths) in spring and summer, while Hemiptera (true bugs) in autumn. Interestingly, the most frequently detected OTUs differed between spring and summer for *P. pygmaeus*. For instance, *B. carbonaria* (Diptera; 18.25%), *M. griseus* (Diptera; 16.27%), *Z. isertana* (Lepidoptera; 12.97%), *Diadegma* sp. (Hymenoptera; 8.13%), and *C. cinerascens* (Diptera; 5.84%) were the most frequently detected prey OTUs in spring, while in summer *C. cinerascens* (Diptera; 15.57%), *Monardia* sp. (Diptera; 14.88%), *C. splendana* (Lepidoptera; 13.54%), *C. plumosus* (Diptera; 10.82%), and *Psychoda alternata* (Diptera; 6.10%) were the most frequently detected. Similarly in autumn, *Psychoda phalaenoides* (Diptera; 25%), *Bibio clavipes* (Diptera; 24.82%), *Cordyla semiflava* (Diptera; 22.14%), *Rhopalosiphum padi* (Hemiptera; 9.36%), *B. carbonaria* (Diptera; 8.02%), and *Forcipomyia ciliata* (Diptera; 4.09%) were found to be most frequently detected.

Discussion

This study of insectivorous bats and the molecular assessment of their diet in Slottsskogen park in Sweden identified that the bats exploit diverse urban arthropod communities. Altogether, 191 prey taxa were found in the diet of the four bat species studied, including several economically significant species recognized as pests in agriculture and forestry, and vectors of diseases in humans and livestock (Table 2). However, only *Cnephaeus nilssonii* and *Pipistrellus pygmaeus* were found to have highly diverse diets, which could be due to the highly varied sample size of each bat species. No statistical comparison of dietary differences between species and sexes could be made due to the limited sample size. But the mean number of prey consumed by *C. nilssonii* males was observed to be slightly higher than that consumed by females, although the differences were not significant. Nevertheless, the results demonstrate that by consuming arthropods that include several agricultural, forestry and medically important species, insectivorous bats contribute to biological pest suppression even in urban environments.

Dipterans were the primary diet component of all bat species in this study, with Chironomidae being the most frequently detected Dipteran family in our samples, similar to the findings of previous non-molecular diet studies in Sweden and abroad (Bartonička et al. 2008, Dietz and Kiefer 2016, Krüger et al. 2014, Pervushina and Gizullina 2020, Rydell 1986, Rydell 1989b, Rydell 1992). As aerial hawkers, *C. nilssonii*, *P. pygmaeus*, *P. nathusii* and *V. murinus* capture prey in flight (Dietz and Kiefer 2016, Rydell 1989b), and our samples were collected from bats foraging over ponds, environments where Dipterans are abundant. This likely explains Diptera dominance in the diet in our study. Furthermore, our results showed that the four bat species primarily preyed on flying arthropods, including Chironomidae, Limoniidae and Culicidae, consistent with aerial-hawking behaviour (Kunz et al. 2011). A molecular diet study in Sweden (Wood et al. 2025), however, reported that Ephemeroptera (mayflies) as the most frequently consumed prey by *C. nilssonii* and *P. pygmaeus*, followed by Diptera. Wood et al. (2025) suggested that the high proportion of Ephemeroptera (mayflies) in their diet may reflect their sampling in maternity colonies, which are largely composed of females. Mayflies are especially rich in polyunsaturated fatty acids, making them a valuable food source for energy-demanding breeding females (Wood et al. 2025).

Chironomids, however, are not considered agricultural or forestry pests. *Scaptomyza flava* (Brassica leaf miner) was the only known Dipteran pest (family: Drosophilidae) that we detected in the diet of bats. Furthermore, we also detected some disease vectors from the families Ceratopogonidae (e.g., *Culicoides*

pulicaris), Culicidae (e.g., *Culex, pipiens*), and Simuliidae (documented e.g., *Simulium noelleri*). *Culex pipiens*, commonly known as common house mosquito, is a globally widespread species and a known vector of West Nile Virus (WNV) in humans (Brugman et al. 2018). While no confirmed cases of WNV have been reported in Sweden as of 10 June 2026, according to the European Centre for Disease Prevention and Control, neighbouring countries have infections, suggesting a potential future risk for northern Europe, particularly under climate change scenarios (Laverdeur et al. 2025). Increasing mean temperature and altered precipitation patterns could result in an early onset of the breeding season of *C. pipiens*, and its survival, thereby increasing the risk of WNV spreading to northern Europe (Laverdeur et al. 2025).

Lepidoptera was the second most frequently detected order found in the diet of bats in Slottsskogen Park that included *Plutella xylostella* (cabbage moth) from Plutellidae and *Agrotis segetum* (turnip moth) from Noctuidae, among several others. *Agrotis segetum* is a globally widespread pest causing substantial damage to vegetable and cereal crops (Esbjerg and Sigsgaard 2014). *Plutella xylostella* is also considered one of the most notorious pests in the world, damaging *Brassica* crops such as broccoli, cabbage and cauliflower (Sarfraz et al. 2005). Overall, these findings underscore the invaluable role of insectivorous bats in controlling arthropods that pose threats to human health and society in urban areas.

Most of the prey detected were less frequently detected, which potentially suggests that most prey species were consumed only occasionally, with bats relying primarily on a small subset of common prey. For example, *Ectobius lapponicus* (Blattodea; forest cockroach) was detected in the diet of *P. pygmaeus*, while *Zygiella x-notata* (Araneae; missing-sector orb weaver) and *Lasius fuliginosus* (Hymenoptera; jet black ant) were identified in the diet of *C. nilssonii*. Gonçalves et al. (2024) also detected two ant OTUs (Hymenoptera: Formicidae) in the diet of bats in Madeira Island (Portugal), along with three OTUs of spiders (Araneae: Araneidae and Clubionidae), one of which is *Zygiella x-notata*. Ants, cockroaches, and spiders tend to be more of opportunistic prey for bats as most insectivorous bats in the world are aerial hawkers and thus, catch prey on the wing, while flying. However, *Myotis emarginatus* (Notch-eared bat) specialises on spiders (Vallejo et al. 2019, Vallejo et al. 2025). Other European bat species such as *Plecotus auritus* (Brown long-eared bat) also consume spiders but occasionally (Razgour et al. 2011, Vesterinen et al. 2018), while some like *Myotis nattereri* (Natterer's bat) prey on spiders as an alternate food source in periods of lower insect availability (Hope et al. 2014). Additionally, the presence of spiders, ants, and cockroaches in the diet indicate that *C. nilssonii* and *P. pygmaeus*, primarily aerial hawkers, may also employ gleaning (picking up prey from surfaces) when necessary.

On closer inspection, in *P. pygmaeus*, a pronounced seasonal shift in the diet composition at the OTU level was observed. In spring, the most frequently consumed OTUs included *B. carbonaria* (Diptera: Chironomidae; non-biting midge), *M. griseus* (Diptera: Limoniidae; cranefly), and *Z. isertana* (Lepidoptera: Tortricidae; spruce bud moth), all of which are spring-emerging species whose abundance declines by summer. Conversely, the summer diet was dominated by *C. cinerascens* (Diptera: Limoniidae; cranefly), *Monardia* sp. (Diptera: Cecidomyiidae; gall midges), *C. splendana* (Lepidoptera: Tortricidae; Chestnut tortrix moth), and *C. plumosus* (Diptera: Chironomidae; non-biting midge), which are all species that emerge in late spring and peak during summer. Prey detection closely matched seasonal phenology, suggesting that *P. pygmaeus* forages opportunistically on the most abundant available resources. For example, parasitoid wasps (Ichneumonidae: *Diadegma* sp.) were consumed in spring, coinciding with their peak activity (Ulber and Nitzsche 2006), similar to the findings by Tiede et al. (2020). In summer, forest cockroaches (Ectobiidae: *Ectobius lapponicus*), found in woodland habitats and leaf litter and common in summer and autumn (Aleksanov et al. 2025), were also detected in the diet. These patterns suggest that seasonal changes in diet composition are largely driven by insect prey turnover rather than active selection by *P. pygmaeus*, which is also consistent with observations for Serotine bat (*Cnephaeus serotinus*), where seasonal dietary variation is mainly determined by prey phenology (Tiede et al. 2020).

In this study, we gain useful insight into urban bat diet and prey composition, although small sample size limits the generalization of diet patterns. We show that even with limited sampling, our data can reveal important prey taxa and offer valuable snapshots of foraging behaviour and associated ecosystem functions, particularly in urban systems that are rarely studied in detail. These findings support the need for continued monitoring within the park. Future studies should aim for larger sample sizes, repeated sampling across seasons to capture temporal variation, and integrated dietary analyses with acoustic monitoring and insect abundance surveys.

Conclusion

This molecular dietary study provides first detailed insights into the diet of insectivorous bats in Slottsskogen Park, Sweden, demonstrating the consumption of a wide range of arthropod prey, consisting mainly of insects. Among 155 prey items identified to species level, more than 10% were agricultural and forestry pests or disease vectors. The findings emphasize the important role of insectivorous bats in pest suppression services and disease vector control in urban environments. This study not only supports the role of urban bats in ecosystem services but also highlights the conservation value of urban parks as they serve as critical roosting sites and foraging grounds for bats in vast fragmented landscapes.

Using faecal DNA metabarcoding, we found that all four bat species primarily consumed prey from the order Diptera, particularly the family Chironomidae. In addition, the diet composition of *P. pygmaeus* varied between spring and summer at the OTU level, likely reflecting seasonal turnover in available arthropod prey rather than shifts in prey preference. By detecting even trace amounts of prey DNA, DNA metabarcoding allowed for the identification of a wide range of prey species. In contrast to traditional approaches, which often fail to capture fully digested prey and provide limited taxonomic detail, this molecular method offers a complete and more precise picture of bat dietary composition as well as important baseline for future research.

Declarations

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Competing interests

The authors declare no competing interests.

Author Contribution

Conceptualization: VRC; Methodology: VRC, ES, VAM; Investigation: VRC, MG; Formal analysis: VRC, RB, VAM; Visualization: VRC; Data curation: VRC; Project administration: VRC, LT, MG; Supervision: CDB, LT, MG, VAM; Funding acquisition: CDB; Writing – original draft preparation: VRC; Writing – review and editing: VRC, ES, RM, VAM, LT, MG, CDB

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Data Availability

Raw sequence data and sample information can be found at https://www.ebi.ac.uk/biostudies/studies/S-BSST3141. Supplementary information has been included within the manuscript. Voucher material is available under the general accession number 2025-23519 at Gothenburg Natural History Museum.

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Figures

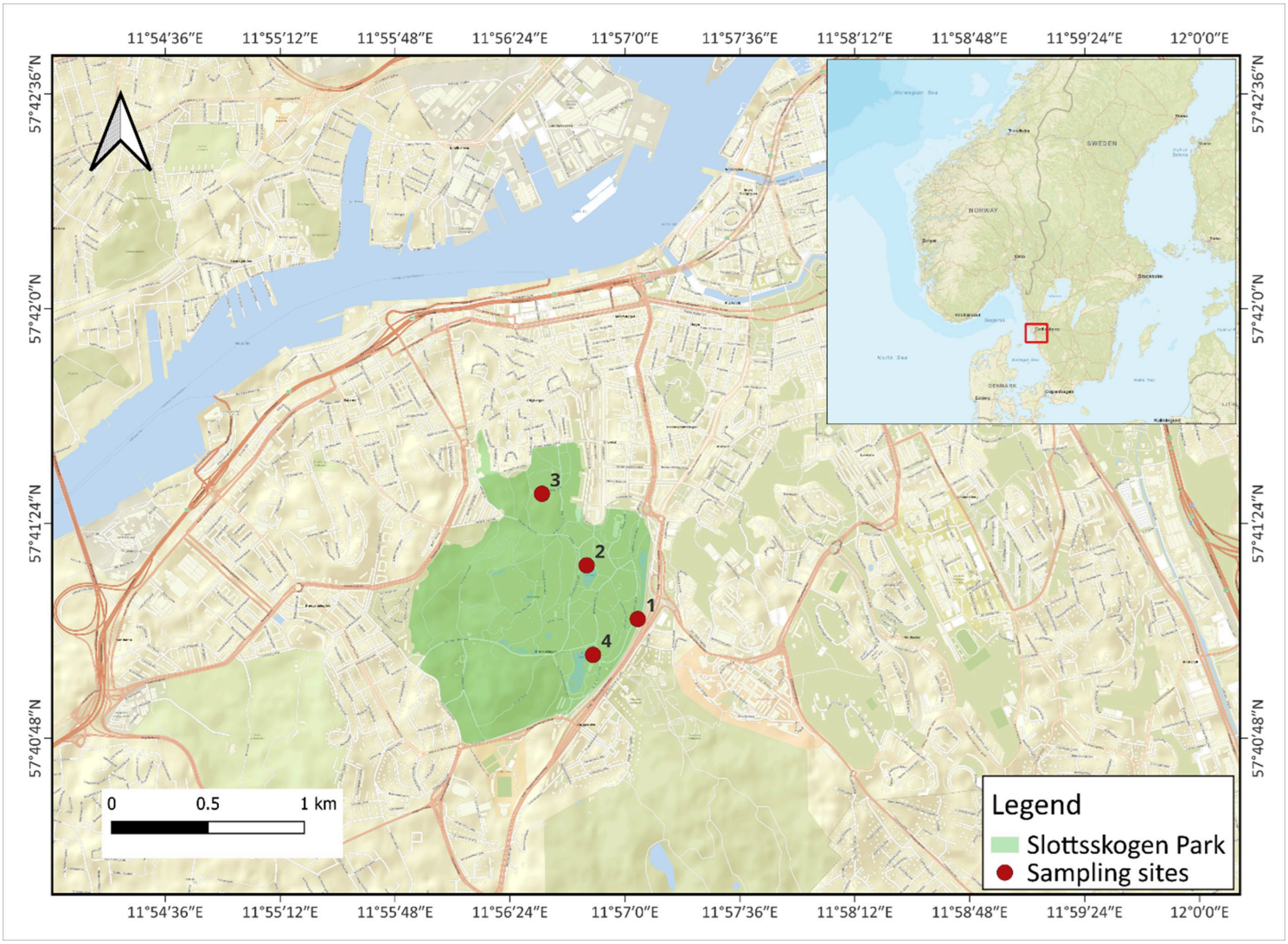


Figure 1

Map of Slottsskogen Park, located in Gothenburg, Sweden; sampling sites indicated by red dots: 1 - Fågeldammen, 2 - Lilla dammen, 3 - Ödledammen, 4 - Stora dammen. Location of Gothenburg in Sweden highlighted in red rectangle (top right)



Figure 2

Photo plate of bat species studied in Slottsskogen Park (A: *Pipistrellus pygmaeus*, B: *Vespertilio murinus*, C: *Cnephaeus nilssonii*, D: *Pipistrellus nathusii*) (Photo credit: Prahesh Chalise)

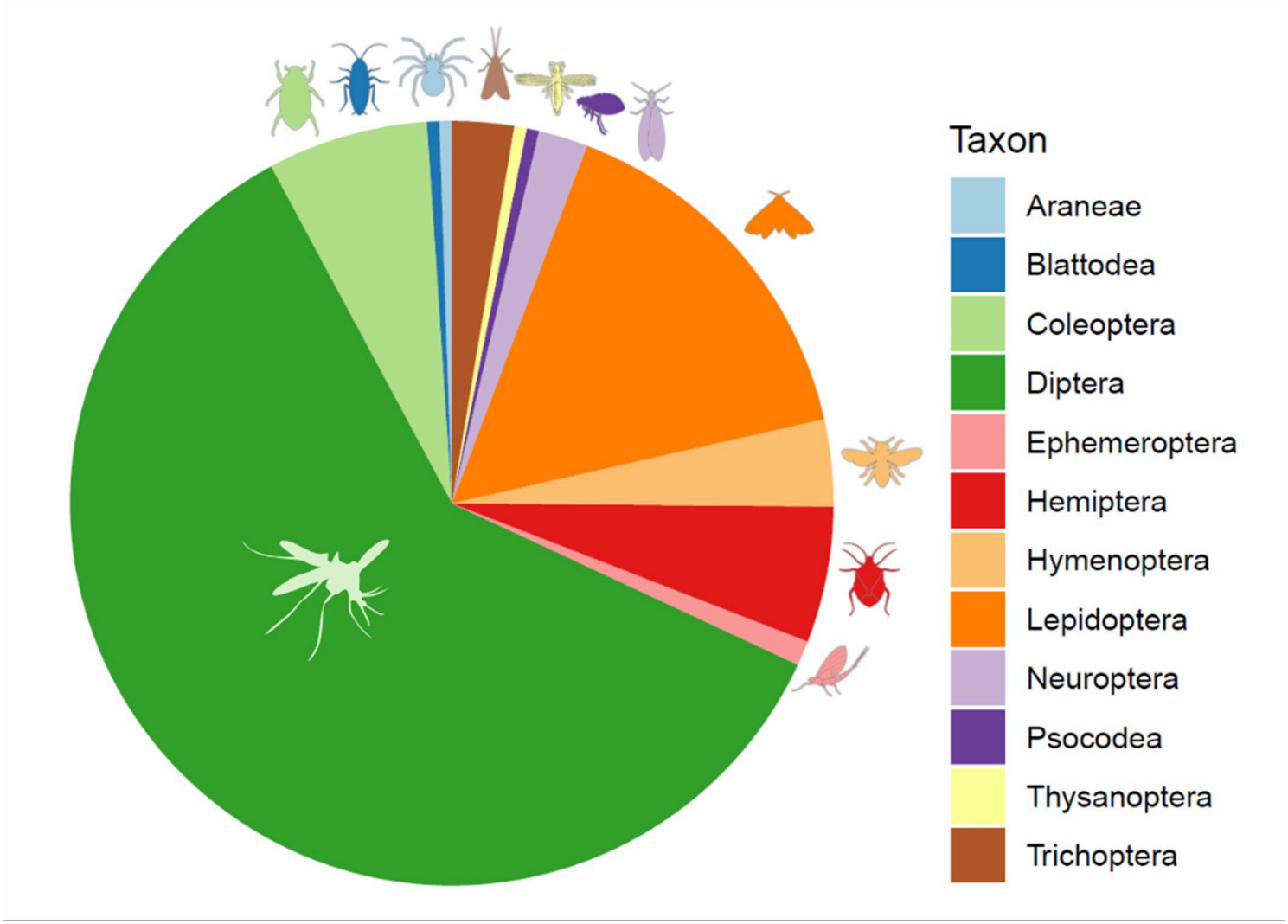


Figure 3

Overall occurrence frequency (%) of prey orders consumed by all bats (n = 32); occurrence frequency indicates the number of samples in which a prey order was observed

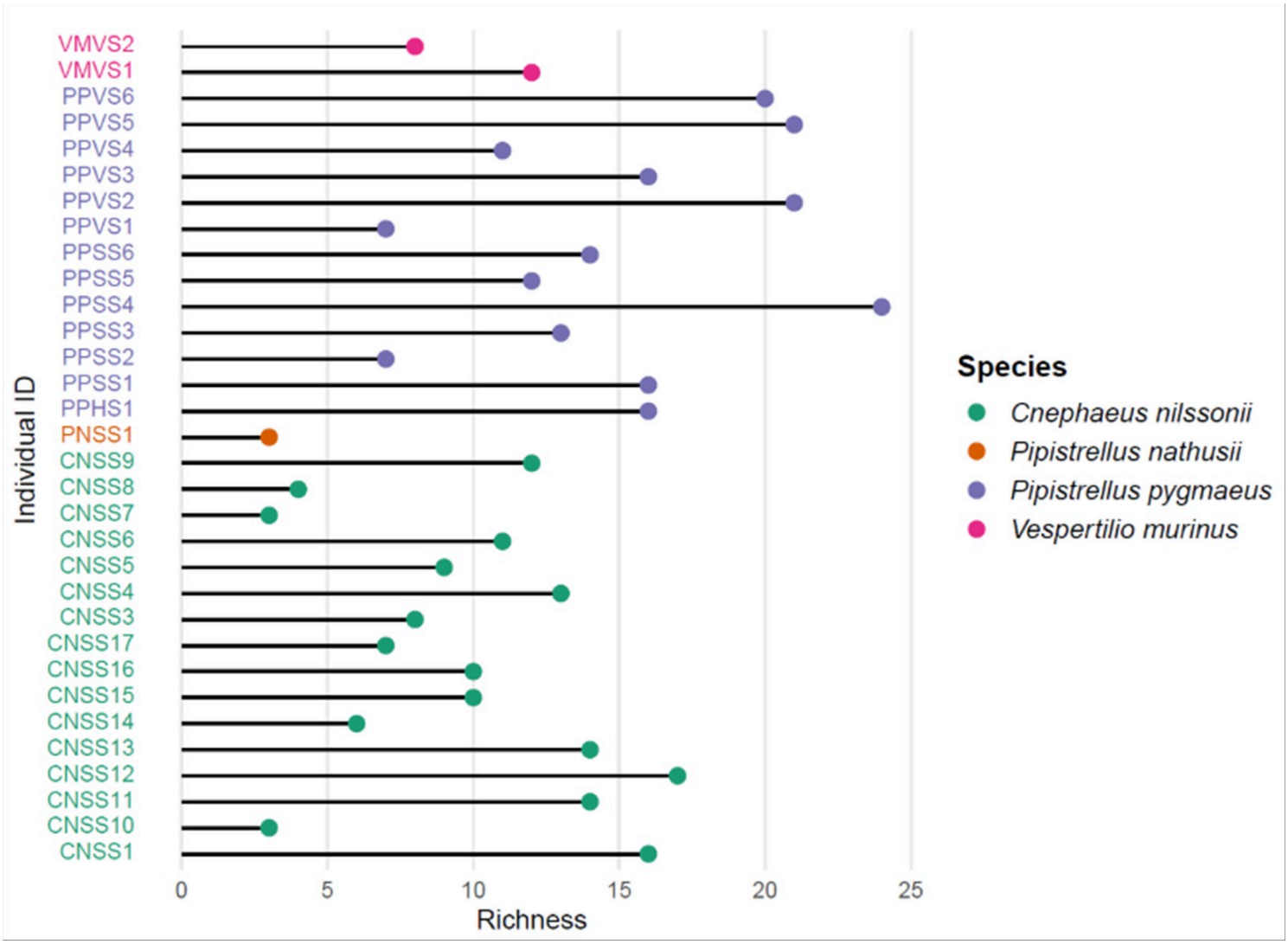


Figure 4

Number of prey OTUs (prey richness on x-axis) in each bat individual (y-axis). Note: In each individual ID: first two letters denote the bat species (CN = *Cnephaeus nilssonii*, PN = *Pipistrellus nathusii*, PP = *Pipistrellus pygmaeus*, VM = *Vespertilio murinus*); third letter denotes sampling season in Swedish (S = sommar/summer, V = vår/spring, H = höst/autumn); fourth letter is the study site (S = Slottsskogen); the numbers denote the individual number of the species

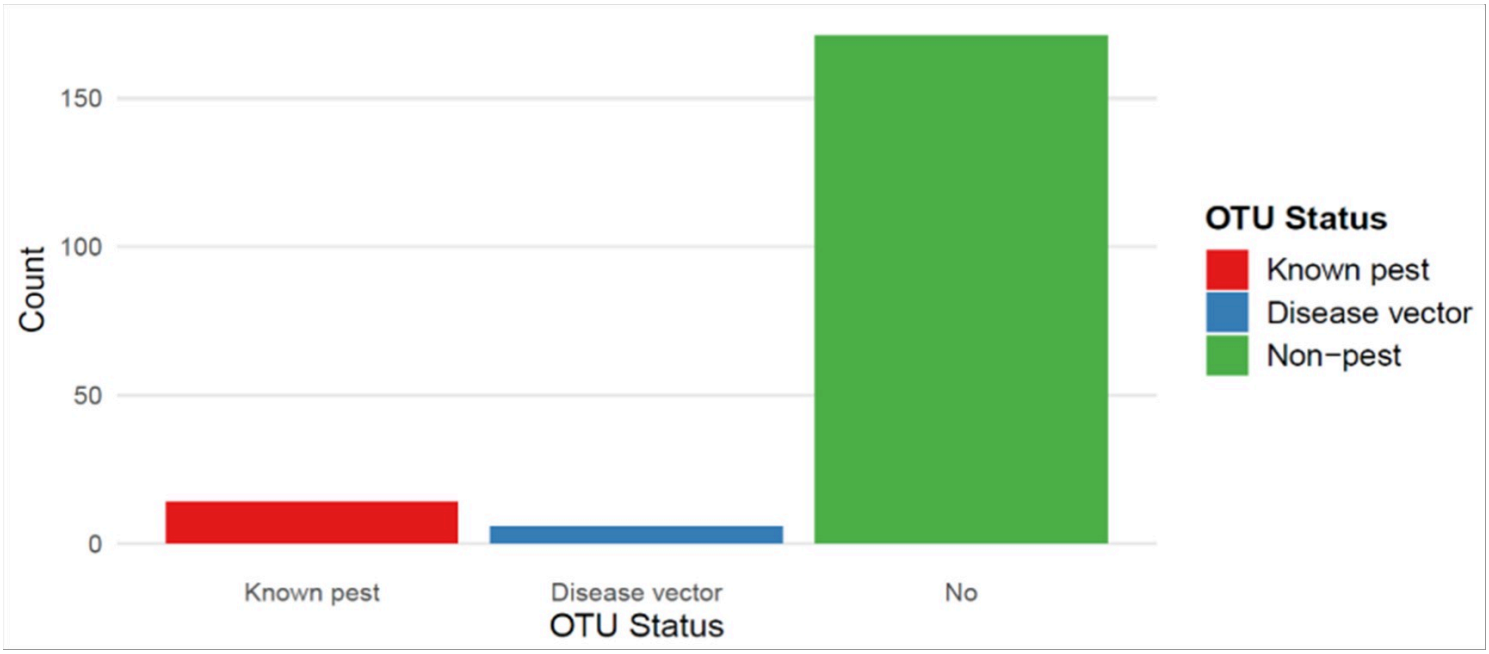


Figure 5

Distribution of OTUs (n = 191) by prey status. Note: No: OTUs recognized as neither pests nor disease vectors

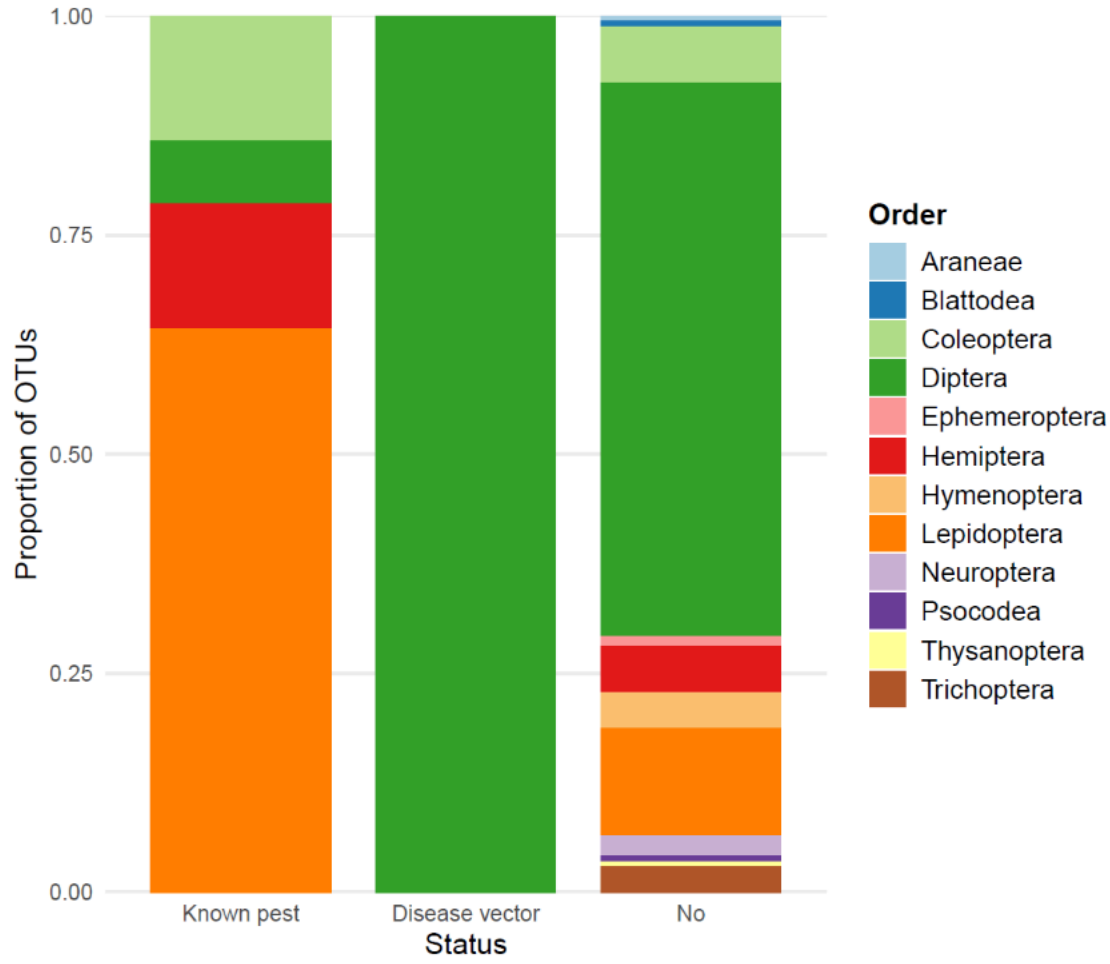


Figure 6

Taxonomic composition of OTUs by prey status. Note: No: OTUs recognized as neither pests or disease vectors

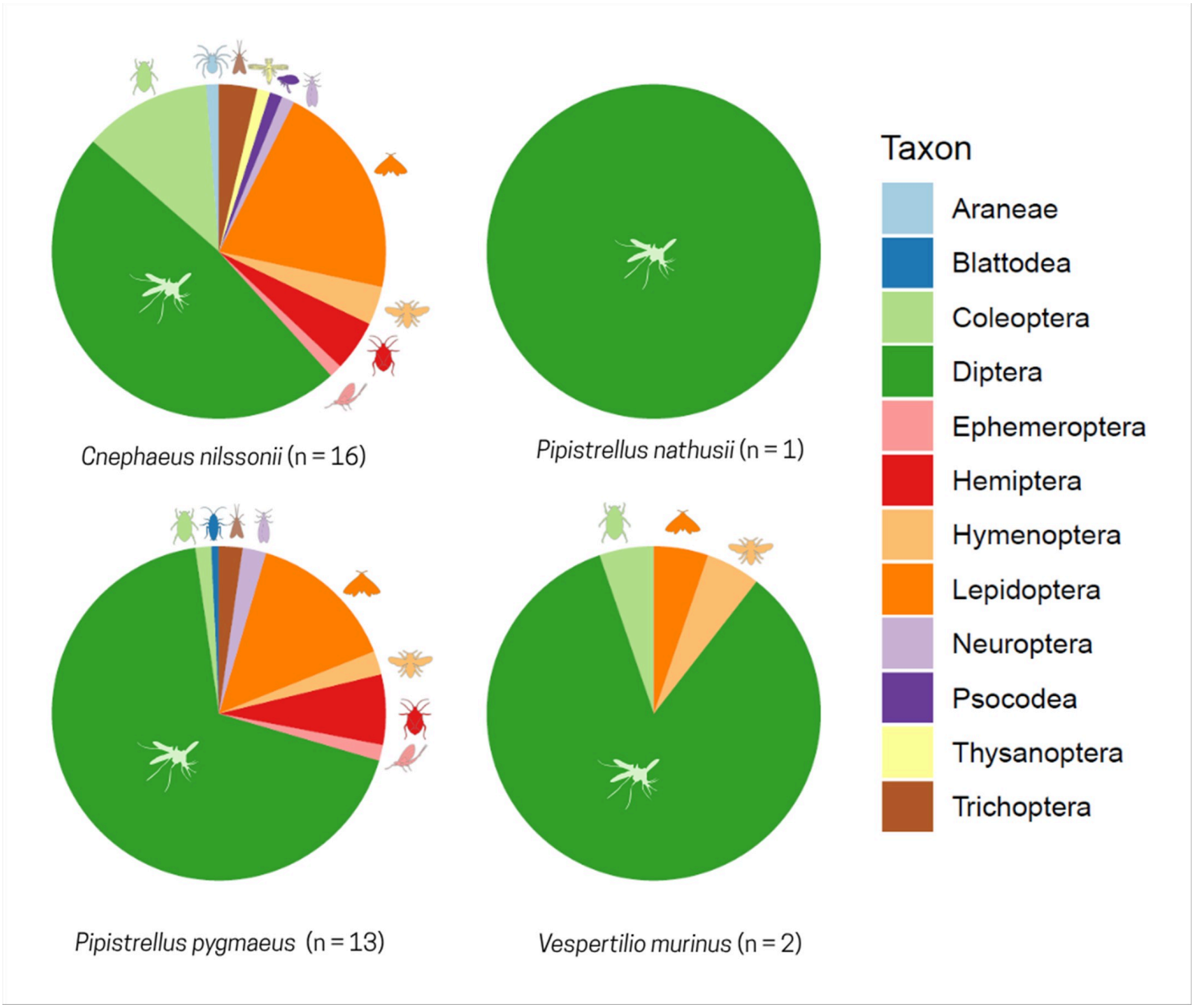


Figure 7

Prey order occurrence frequency (%) consumed by each bat species; occurrence frequency is the number of samples in which a prey order was observed

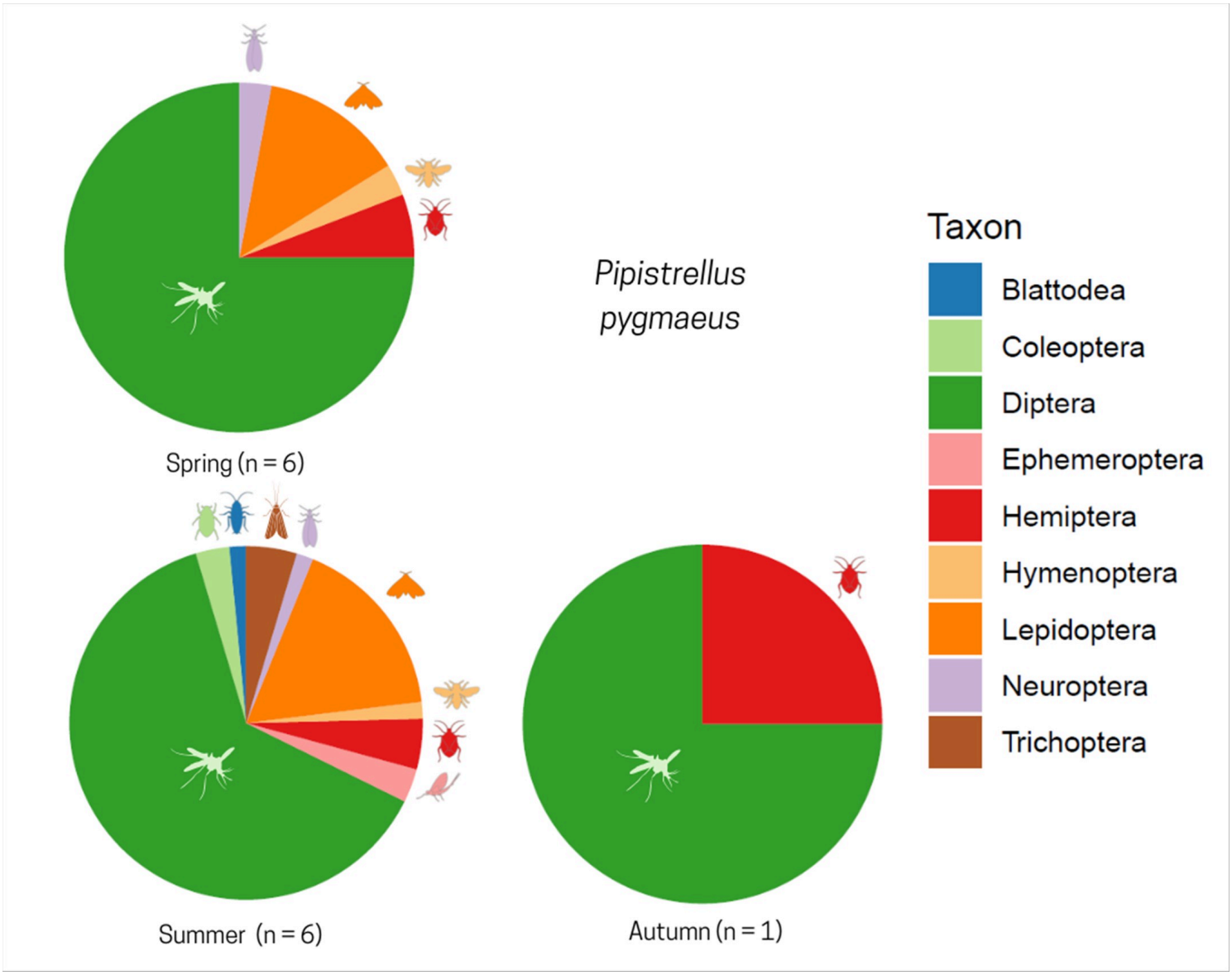


Figure 8

Prey order occurrence (%) consumed by *Pipistrellus pygmaeus* per season; occurrence frequency is the number of samples in which a prey order was observed