



## Insights into the habitat associations, phylogeny, and diet of *Pipistrellus maderensis* in Porto Santo, northeastern Macaronesia

Eva K. Nóbrega<sup>1</sup>, Nia Toshkova<sup>2</sup>, Angelina Gonçalves<sup>3</sup>, André Reis<sup>4,5</sup>, Elena J. Soto<sup>6</sup>, Sergio Puertas Ruiz<sup>7</sup>, Vanessa A. Mata<sup>8,9</sup>, Catarina Rato<sup>8,9</sup>, and Ricardo Rocha<sup>10</sup>

<sup>1</sup>Department of Animal Biology, Plant Biology and Ecology,  
Autonomous University of Barcelona, 08193 Catalonia, Spain

<sup>2</sup>National Museum of Natural History, Bulgarian Academy of Sciences, 1000 Sofia, Bulgaria

<sup>3</sup>Department of Biology, Faculty of Sciences of the University of Porto, 4099-002 Porto, Portugal

<sup>4</sup>Instituto Superior de Agronomia, Universidade de Lisboa, 1349-017 Lisbon, Portugal

<sup>5</sup>Escola de Ciências e Tecnologia, Universidade de Évora, 7000-671 Évora, Portugal

<sup>6</sup>Faculty of Biology, University of Murcia, 30003 Murcia, Spain

<sup>7</sup>Pyrenean Institute of Ecology, Spanish National Research Council (IPE-CSIC), 50059 Zaragoza, Spain

<sup>8</sup>Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO), InBIO Laboratório Associado,  
Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

<sup>9</sup>BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO,  
Campus de Vairão, 4485-661 Vairão, Portugal

<sup>10</sup>Department of Biology, University of Oxford, OX1 3SZ, Oxford, UK

**Correspondence:** Eva K. Nóbrega (eva.keltanen@hotmail.com)

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**Abstract.** Around 60 % of all bat species occur in islands, and nearly one in four is an insular endemic. Bats are often the only native terrestrial mammals in oceanic islands, and despite increasing anthropogenic pressures, little is known about the distribution, natural history, and population status of most insular bat populations. The sub-tropical archipelago of Madeira is composed of the volcanic islands of Madeira, Porto Santo, and Desertas and is home to the Macaronesian endemic *Pipistrellus maderensis*, to the endemic subspecies *Nyctalus leisleri verrucosus*, and to *Plecotus austriacus*. *Pipistrellus maderensis* is known to both Madeira and Porto Santo, whereas the other two species have only been recorded in the former. However, no bats have been recorded in Porto Santo for over 15 years, raising fears that bats are probably extinct in the island. In July 2021, we conducted an island-wide acoustic survey using AudioMoth passive acoustic recorders, leading to the detection of *Pipistrellus maderensis* in 28 out of the 46 sampling sites (60 %). The species' activity was strongly associated with artificial water sources, and genetic samples from six captured individuals revealed that the populations of *Pipistrellus maderensis* in Porto Santo and Madeira have a close phylogenetic affinity. Furthermore, using DNA metabarcoding, we found that the species feeds on a wide variety of insects, including several economically important pest species and disease vectors. These findings emphasise the need to target more conservation and research efforts towards extant island bat populations and the potential ecosystem services they provide.

## 1 Introduction

The unique size, isolation, and microclimatic/geomorphological conditions of oceanic islands often lead to significant evolutionary divergence and high rates of endemism (Emerson, 2002; Kier et al., 2009). They often act as evolutionary reservoirs for lineages that have disappeared elsewhere and, as such, are crucial for the fate of biodiversity in the Anthropocene (Russell and Kueffer, 2019; Nori et al., 2022). However, historical and ongoing human-induced environmental degradation have resulted in acute changes to island biotas (Nogué et al., 2021). Consequently, ca. 50% of the planet's terrestrial vertebrates are island dwellers (Leclerc et al., 2018), and most recorded extinctions since the expansion of Europeans have occurred in islands (Blackburn et al., 2004; Fernández-Palacios et al., 2021; Matthews et al., 2022).

Of over 1400 currently recognised bat species, ca. 25% are island endemics (Conenna et al., 2017). Of these, over 60% of the bats currently assessed by the International Union for Conservation of Nature (IUCN) are species formerly classified as “microbats”, i.e. laryngeal echolocating bats (Conenna et al., 2017; Frick et al., 2020). Similarly to their mainland counterparts, some island-dwelling microbats are seed dispersers and pollinators (Ramirez-Francal et al., 2021). However, most species are insectivores and potentially play pivotal roles in the suppression of arthropods, including agricultural pests and mosquitoes (Kemp et al., 2019). More than one-fourth of these laryngeal echolocating bats are assessed as CR, EN, or VU by the IUCN Red List, largely due to the additive and often synergistic effects of habitat loss and fragmentation, as well as the impacts of invasive species (Rodríguez-Durán et al., 2010; Conenna et al., 2017).

The Macaronesian biogeographical region, composed of the archipelagos of the Azores, Madeira, Selvagens Islands, Canary Islands, and Cabo Verde, is home to at least 15 bat species, including three island-restricted ones, namely the Azores noctule *Nyctalus azoreum*, the Canary big-eared bat *Plecotus teneriffae*, and the Madeiran pipistrelle *Pipistrellus maderensis* (González-Dionis et al., 2022). The latter is a relatively small bat (forearm length = 29.5–34.0 mm) likely derived from African *Pipistrellus kuhlii* that colonised the Canary Islands and the archipelago of Madeira (Pestano et al., 2003) or from a common ancestor to both species (Jesus et al., 2013). It is a synanthropic species with flexible habitat requirements, being found in the archipelagos of Madeira, the Canary Islands, and possibly Azores (Trujillo and Gonzalez, 2011; Rainho, 2022; Rocha, 2021; Rainho et al., 2002). The species seems to be more abundant at lower altitudes, occurring in a variety of natural and humanised habitats, such as native forests and agricultural and urban areas (Teixeira and Jesus, 2009; Jesus et al., 2009; Ferreira et al., 2022; Nouioua, 2022; Rocha, 2021). It feeds on a wide diversity of arthropods (Gonçalves, 2022) and is prone to roost in cliffs,

tree holes, and a variety of human-made structures such as bridges and tunnels (Rocha, 2021).

*Pipistrellus maderensis* is listed by the IUCN as vulnerable (Alcalde and Juste, 2016) and is one of Europe's most threatened bat species. Its geographic isolation and fragmented populations, typical of insular species, make it particularly vulnerable to anthropogenic stressors and natural catastrophes (Rocha, 2021). The threats to the conservation of aerial insectivorous bats such as *Pipistrellus* spp. are numerous, and since they are small, mostly nocturnal, and inconspicuous, their population declines often go unnoticed. As an extreme example, the last known Christmas Island pipistrelle *Pipistrellus murrayi* disappeared in 2009, becoming the first animal to go extinct in Australia in the last 5 decades (Woinarski, 2018). In Madeira, where it is more abundant, the population was suggested to be smaller than 1000 individuals (Cabral et al., 2005), whereas Azores might be home to fewer than 300 individuals (Queiroz et al., 2005).

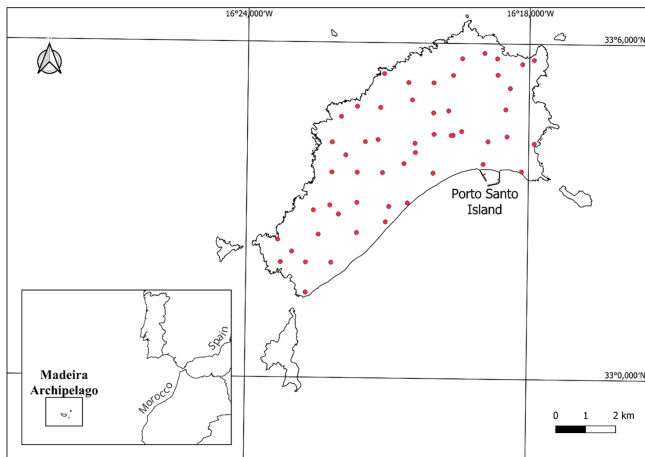
Although *Pipistrellus maderensis* were known to occur in Porto Santo (archipelago of Madeira), previous surveys failed to detect the species (Jesus et al., 2013). However, it is unclear if the method and intensity of the surveys were sufficient to prove the absence of the species in the island. Here, we combine bioacoustics, phylogenetic analysis, and DNA metabarcoding to investigate the population status, evolutionary history, and trophic interactions of *Pipistrellus maderensis* in Porto Santo. Specifically, we address the following questions.

- Is Porto Santo still home to an extant population of *Pipistrellus maderensis*? We anticipate that *Pipistrellus maderensis* still persists in Porto Santo, and we predict that considering the xeric climate of the island, bat activity is likely to be strongly influenced by artificial water sources.
- What is the phylogenetic relationship between the populations of *Pipistrellus maderensis* from Madeira and Porto Santo? We hypothesise that both populations should be closely related, and considering the relatively small distance between Madeira and Porto Santo (ca. 40 km), bat populations in both islands will likely exhibit low genetic divergence.
- Is *Pipistrellus maderensis* preying on agricultural insect pests in Porto Santo? We expect bats to prey mainly on moths and Diptera, and we predict that some of these will be agricultural pests.

## 2 Material and methods

### 2.1 Study area

Porto Santo (ca. 42 km<sup>2</sup>) is the second largest and the oldest (~ 14 million years) island of the Portuguese archipelago of Madeira, located in the Atlantic Ocean, near the coast of



**Figure 1.** Location of the island of Porto Santo, Madeira archipelago, Portugal. Sampling sites are denoted by red dots.

North Africa (Fig. 1; 38°40′44″ N, 27°13′51″ W). It has a Mediterranean xeric oceanic bioclimate, influenced by the Azores anticyclone (Rivas-Martínez, 2009). Despite some steep slopes, especially in the eastern section, the island is relatively flat, with the highest peak being Pico do Facho (517 m a.s.l.) (Kratochwil and Schwabe, 2018). Largely due to these geomorphological features, human intervention has been widespread throughout the island since the arrival of the Portuguese in the XV century, and Porto Santo’s native vegetation is nowadays restricted to small, localised patches. Non-native coniferous species (mostly pine and/or cypress) were planted in several of the island’s peaks to prevent soil erosion (Sparrus et al., 2017). Notwithstanding its small size, the island harbours a wide diversity of land-use covers – e.g. urban areas, agricultural fields, grasslands, and forests – and despite considerable environmental degradation, it is still home to nearly 250 extant endemic taxa, such as the IUCN critically endangered vetch *Vicia ferreirensis* (Carvalho and Osborne, 2011). As with numerous other oceanic islands, human arrival was accompanied by the introduction of several exotic vertebrates, among which are the domestic cat *Felis catus*, the European rabbit *Oryctolagus cuniculus*, and house mice *Mus musculus* (Borges et al., 2008; Rocha et al., 2017).

## 2.2 Bat surveys

We conducted an island-wide bioacoustic survey in June 2021. Contingent to orography, we sampled 55 randomly selected sites (generated using the *random points tool* in QGIS), spaced ca. 1 km apart throughout Porto Santo. However, only 46 sites were used in the analysis due to recorder failures – see below. In each sampling site, bats were surveyed for one night, using an AudioMoth recorder (Hill et al., 2018) placed within an appropriate waterproof box and attached to a tree trunk or a shrub. Each detector was config-

ured to record continuously from half an hour before sunset to half an hour after sunrise, at a sample rate of 250 kHz.

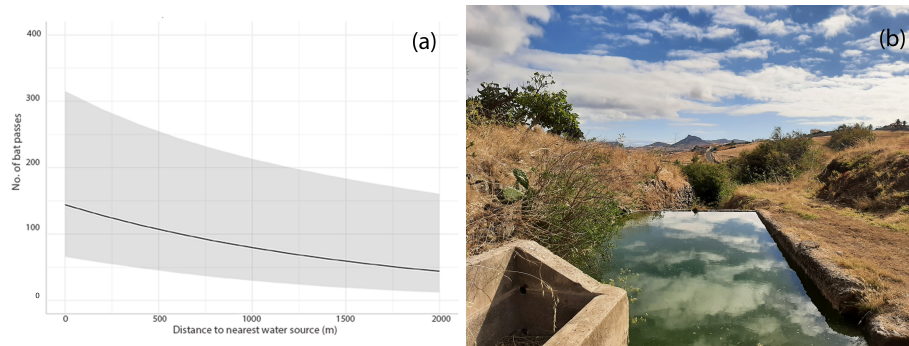
Additionally, we conducted four capture sessions using ground-level mist nets placed in potential foraging and drinking sites, such as forest trails, tunnel exits, and water ponds, and undertook targeted searches in a vast array of potential roosts – e.g. caves, abandoned houses, and underground pipelines. From each captured individual, a small wing tissue sample (< 2 mm in diameter) was collected using a biopsy punch and later preserved in 96 % ethanol. The age of each specimen was determined by examination of the extent of ossification in the epiphyses of the phalanges, and, in the case of adult females, the reproductive state was recorded by palpation (pregnant vs. non pregnant) and evidence of hair loss around the mamma and of milk leftovers/or production (lactating vs. non lactating). Additionally, whenever possible, guano pellets were collected from clean holding bags and stored with silica gel. Bat capture and handling was performed following recommendations approved by the American Society of Mammalogists (Sikes et al., 2011), and all bats were released at the capture site. No bioacoustic surveys or captures were conducted on rainy or windy days, and precipitation and wind speed were fairly constant throughout the sampling period.

## 2.3 Bioacoustic analysis

Using Kaleidoscope v. 5.3 software (Wildlife Acoustics, USA), the AudioMoth recordings in WAV format were split into 5 s recordings. If two or more bat pulses were detected in a 5 s recording, this was defined as a “bat pass”, which we used as our unit of measure for bat activity (see e.g. López-Bosch et al., 2022; Yoh et al., 2022). Kaleidoscope was programmed to detect the signals in the recordings with frequency ranges between 8 and 120 kHz and the pulse length between 1 and 500 ms. We ran the automated bat identification in Kaleidoscope Pro and manually verified the sound pulses in the sonograms. The identification of the echolocation recordings followed Teixeira and Jesus (2009).

## 2.4 Bat–environment relationships

Landscape-scale land-use metrics were acquired from 25 ha spatial land-use maps based on CORINE Land Cover 2018. The major land-use types in Porto Santo were defined as forest, agriculture, grassland, non-vegetated areas, and built-up areas (Table S1 in the Supplement). Previous assessments investigating the effect of landscape-scale land-use metrics on the activity of *Pipistrellus maderensis* at three different sizes (250, 500, and 1000 m) revealed consistent responses across scales (Ferreira et al., 2022). Thus, considering the small extension of Porto Santo (maximum length of ca. 9 km) and so to minimise the spatial overlap between neighbouring buffers, we used QGIS v. 3.28.0 to calculate the area of each land-use type inside buffers of 250 m, centred in



**Figure 2.** Relationship between bat activity (number of bat passes) and distance to the nearest water source (a); artificial water point where intense bat activity was detected (b).

each sampling site. Additionally, we used Google Earth complemented with field validation to determine the distance between sampling sites and the closest water sources (Table S2).

The effects of land-use type on bat activity (number of bat passes per night) was assessed using generalised linear mixed models (GLMMs) with a negative binomial distribution. As severe collinearity between predictor variables can undermine model inference (Dormann et al., 2013), prior to GLMMs, explanatory variables were centred and scaled ( $x = 0$ ,  $\sigma = 1$ ). We, therefore, quantified collinearity using variance inflation factors (VIFs), and variables with  $VIF \geq 3$  were excluded. To account for the nested spatiotemporal sampling design, sampling day was considered as a random factor in the GLMMs. A candidate model set was further constructed using all additive combinations of the four explanatory variables retained, and models were ranked based on Akaike information criterion adapted for small samples (AICc), using the MuMIn R package (Bartoń, 2020). To account for model uncertainty in multi-model inference, model averaging was used to obtain parameter estimates from the most plausible models (i.e.  $0 < \Delta AICc < 2$ ) (Burnham and Anderson, 2002). All GLMMs were conducted using R v. 4.2.1 software and the glmmTMB package (Brooks et al., 2017).

## 2.5 Phylogenetic analyses

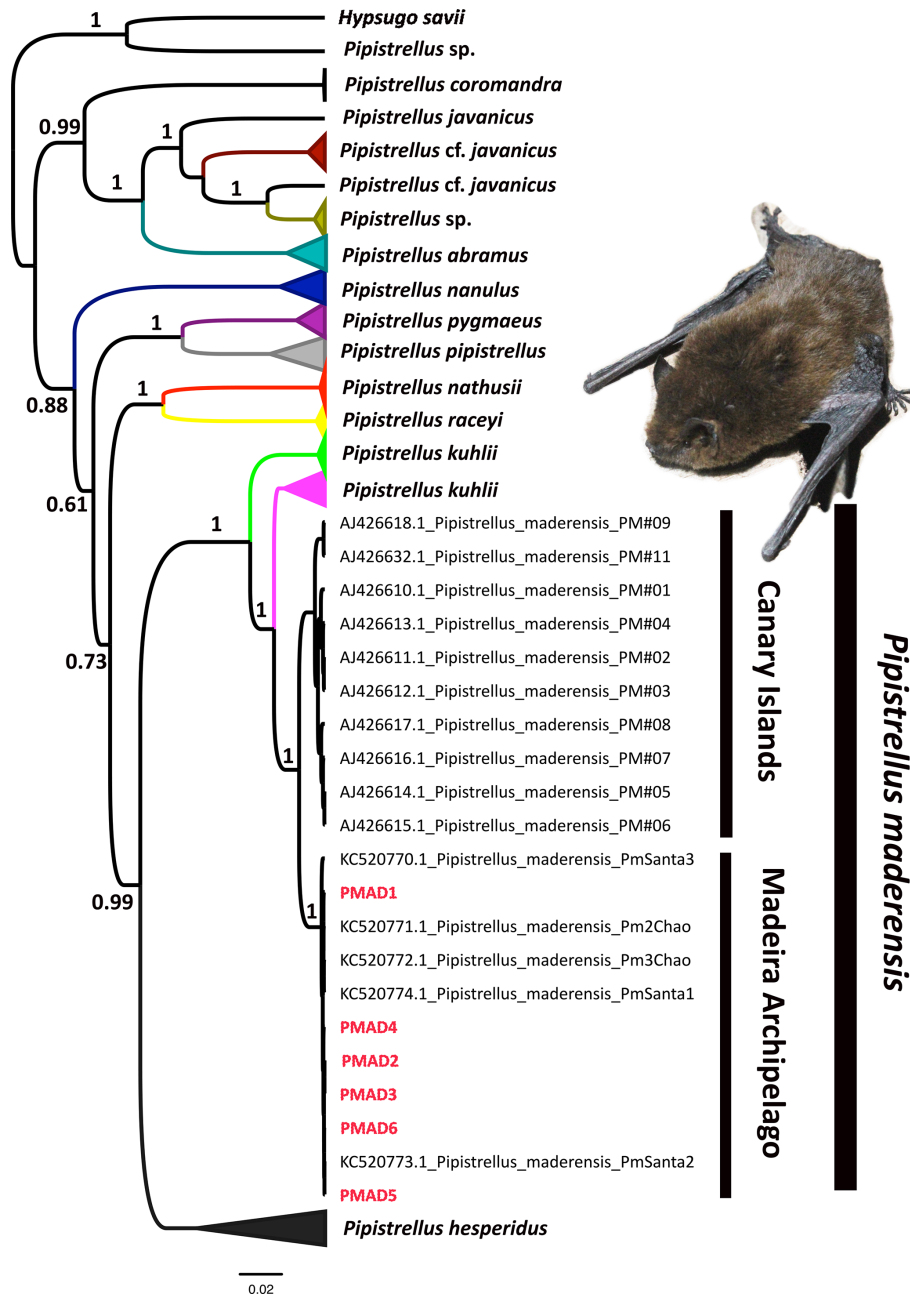
Genomic DNA was extracted from wing tissue samples of six *Pipistrellus maderensis* captured during mist netting, using the E.Z.N.A Tissue DNA Kit (Mag-Bind). A fragment of the cytochrome b (cytb) gene was amplified by polymerase chain reaction (PCR) using the primers Molcit-F (Ibáñez et al., 2006) and MVZ-16 (Smith and Patton, 1993). Amplification of the cytb fragment was carried out in a 10  $\mu$ L volume, comprised of 5  $\mu$ L of QIAGEN Multiplex PCR Master Mix (Qiagen, Crawley, UK), 0.3  $\mu$ L of each primer, 3.4  $\mu$ L of ultra-pure water, and 2  $\mu$ L of DNA extract. The PCR cycling procedure was done according to Ibáñez et al. (2006). All

amplified fragments were sequenced in a Sanger sequencer and deposited to the GenBank database, with accession numbers from OQ260001 to OQ260006 (Table S3).

A total of 395 cytb sequences of *Pipistrellus* sp. were retrieved from GenBank and added to the dataset. The obtained sequences were imported into the software Geneious Prime® (v. 2022.2.2 Biomatters Ltd.) where the alignment was performed using MAFFT v. 7.490 (Katoh et al., 2002; Katoh and Standley, 2013) under the default parameters. Phylogenetic analysis based on the cytb mitochondrial fragment was performed under a Bayesian inference (BI) method, using *Hypsigugo savii* as the outgroup (following Mayer and Helversen, 2001). To determine the best-fitting nucleotide model, we used ModelFinder (Kalyaanamoorthy et al., 2017) from the IQ-TREE web server (Trifinopoulos et al., 2016). The software BEAST v. 2.6.6 (Bouckaert et al., 2019) was used for the BI topology. Analyses were run twice for  $10^6$  generations with a sampling frequency of 1000. Models and prior specifications applied were as follows (otherwise by default): strict clock, coalescent with constant population size, and the JC69 nucleotide model based on ModelFinder. Convergence for all model parameters was assessed by examining trace plots and histograms in Tracer v. 1.7.1 (Rambaut et al., 2018) after obtaining an effective sample size (ESS) greater than 200. The initial 10 % of samples were discarded as burn-in. Runs were combined using LogCombiner, and maximum credibility trees with divergence time means and 95 % highest probability densities (HPDs) were produced using TreeAnnotator. Trees were visualised using FigTree v. 1.4.4 (Rambaut, 2009). Calculation of genetic distances was performed using Mega v. 11 (Tamura et al., 2021) based on Kimura's two-parameter distance (Kimura, 1980).

## 2.6 Diet analyses

DNA was extracted from pellets collected from four out of the six captured *Pipistrellus maderensis*, using the E.Z.N.A Tissue DNA Kit (Omega Bio-Tek, Norcross, Georgia, USA). For this, we followed Mata et al. (2021), except that no Inhib-



**Figure 3.** Bayesian phylogenetic tree for *Pipistrellus* spp. derived from a cytochrome b (cytb) fragment and using *Hypsugo savii* as the outgroup.

itex tablets (Qiagen, Hilden, Germany) were used. DNA was then amplified by PCR using arthropod general cytochrome oxidase subunit 1 (COI) primers fwH2-R2n (Vamos et al., 2017) modified with Illumina overhangs. Libraries were prepared following Mata et al. (2021) and finally sequenced in a MiSeq desktop sequencer (Illumina) using a MiSeq Reagent Kit v. 3 (2 × 250 bp). Bioinformatic processing of raw sequencing data was conducted using OBITOOLS (Boyer et al., 2016) and VSEARCH (Rognes et al., 2016) following Martins et al. (2022). Operational taxonomic units (OTUs)

were taxonomically assigned using the Barcode of Life Data System (BOLD) public database under BOLDigger (Buchner and Leese, 2020).

### 3 Results

During our bioacoustic survey we detected bats in 28 out of the 46 sampling sites (60 %; Table S2). In total, we identified 7797 bat passes of *Pipistrellus maderensis* in our recordings.

Furthermore, we mist-netted six individuals (including three juveniles and one lactating female; Fig. S1; Table S4). All captures occurred at ca. 260 m a.s.l., in forest trails near *Pico Castelo*. No other bat species were detected.

### 3.1 Bat–environment relationships

Our GLMM results indicate that the activity of *Pipistrellus maderensis* is not strongly influenced by any of the considered land-use types (forest, agriculture, grassland, non-vegetated areas, built-up areas) ( $p > 0.05$ ; Table S5). However, the number of recorded bat passes increased with decreasing distance to water sources ( $p < 0.001$ ; Table S6 and Fig. 2a). GLMM residuals were not spatially autocorrelated for most models ( $P > 0.05$ ). However, for one of the GLMMs (non-vegetated areas), the test showed spatially structured residuals ( $P < 0.05$ ; Table S7).

### 3.2 Phylogenetic analyses

The obtained cytb fragment from the sequenced *Pipistrellus* sp. had a length of 779 bp. The Bayesian phylogenetic analysis clustered the bats sampled in Porto Santo with the specimens assigned as *Pipistrellus maderensis*, with high posterior probability (Fig. 3). Specifically, the genealogy suggests that these individuals have a close phylogenetic affinity with the specimens from Madeira Island, separated by 0.04 % of genetic distance. Moreover, the bats captured in Porto Santo share the exact same cytb haplotype, differing from the sequence KC520772.1 from Madeira by a single base pair (99.8 % of similarity among sequences). This highlights the low genetic diversity of the bat populations of the archipelago of Madeira, in comparison with the Canary Islands (98.7 % of identical nucleotide sites).

### 3.3 Diet analyses

DNA material from the faecal samples collected from four individuals led to a total of 16 OTUs and 39 594 reads. In total, at least five orders, 12 families, 13 genera, and 11 species were identified to be consumed by *Pipistrellus maderensis*. Diptera was the predominant order detected, followed by Lepidoptera, Hymenoptera, Hemiptera, and lastly Neuroptera (Fig. 4). Of the identified prey, several are known agricultural insect pest species and disease vectors (Table 1).

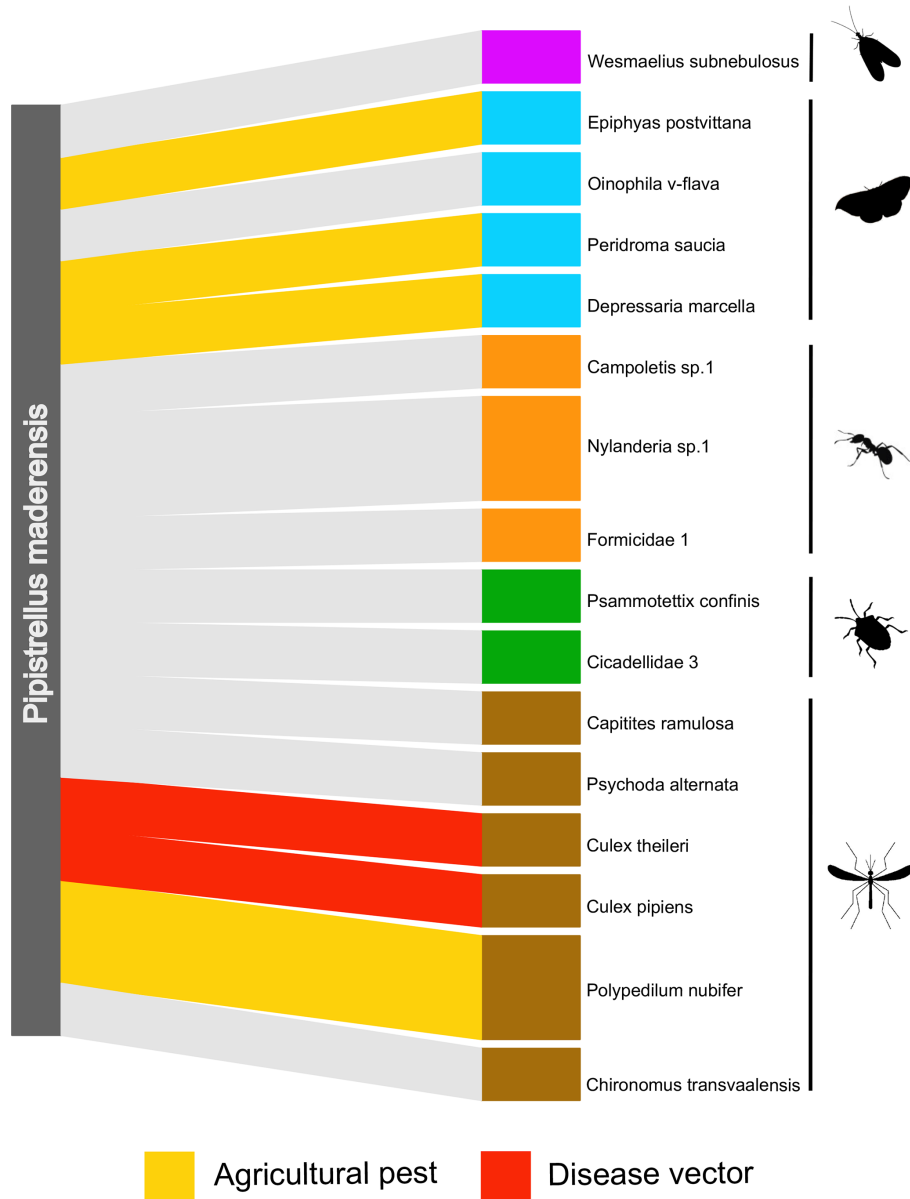
## 4 Discussion

Lack of baseline knowledge is a key constraint for the development of management strategies for the conservation of insectivorous bats and for maximising any ecosystem services they might provide. Here, we show that Porto Santo is still home to an extant population of *Pipistrellus maderensis* and provide much-needed information about their habitat affinities, phylogenetic kinship, and trophic interactions.

**Table 1.** List of known or suspected agricultural pest insects and disease vectors found in the diet of four *Pipistrellus maderensis* captured in Porto Santo, archipelago of Madeira, Portugal.

Order	Family	Species	% <sup>a</sup>	Common name	Status	Crop/disease
Diptera	Culicidae	<i>Culex pipiens</i>	25	common house mosquito	disease vector	West Nile virus (Hammer et al., 2008), Usutu virus (Fros et al., 2015), Rift Valley fever (Moutailler et al., 2008), avian malaria (Lalubin et al., 2013) (among others)
Diptera	Culicidae	<i>Culex theileri</i>	25		disease vector	West Nile virus, Rift Valley fever, Sindbis virus (Demirci et al., 2014), <i>Dirofilaria immitis</i> (Santa-Ana et al., 2006)
Diptera	Chironomidae	<i>Polypedium nubifer</i>	50		possible pest	rice (Wallace et al., 2009)
Lepidoptera	Depressariidae	<i>Depressaria murella</i>	25		possible pest	carrots (Celli, 2013)
Lepidoptera	Tortricidae	<i>Epiphyas postvittana</i>	25	light brown apple moth	known pest	horticultural plants (Thrimawithana et al., 2022), apple (Danthanarayana, 1975)
Lepidoptera	Noctuidae	<i>Peridroma saucia</i>	25	pearly underwing moth	known pest	peanuts, sunflowers, soybeans, grapevines (Alvarez et al., 2009) (among others)

<sup>a</sup> Percentage of samples for which each prey was detected.



**Figure 4.** Frequency of occurrence network displaying the operational taxonomic units (OTUs) consumed by four Madeiran pipistrelles *Pipistrellus maderensis* in Porto Santo. Different OTUs are separated by white lines, and different colours denote different taxonomic orders.

Bats are known to present marked responses to landscape features in both island and non-island ecosystems (Ancillotto et al., 2023; López-Baucells et al., 2021). In the nearby island of Madeira, *Pipistrellus maderensis* was found to be particularly associated with shrubland, agricultural areas, and Laurisilva (primary) forest (Ferreira et al., 2022). However, in Porto Santo and probably as an artefact of the small dimensions of the island, in which multiple land-use covers converge within relatively short distances, we were unable to detect these associations. Yet, consistent with previous studies (Ancillotto et al., 2019; Moretto et al., 2023; Torrent et al., 2018), we have found that bat activity was positively asso-

ciated with artificial water reservoirs such as dams and urban ponds. Water limitations and unpredictability in precipitation, typical of xeric climates such as the one characterising Porto Santo (Maestre et al., 2016), pose important challenges to insectivorous bats (Conenna et al., 2021). Our results indicate that in Porto Santo artificial water sources likely provide important drinking and foraging resources for *Pipistrellus maderensis*, potentially reducing spatiotemporal variations in food availability. However, it is important to note that our bioacoustic survey was conducted over ca. 1 week – too short of a period to be able to capture complex bat activity variations, which can be affected by multiple factors, includ-

ing lunar phase and season (Appel et al., 2021; Gorman et al., 2021).

The evolutionary history of insular species is often shaped by the geographic separation of different island populations (e.g. Recuerda et al., 2021). Previous molecular phylogenetic analyses suggested the existence of at least three lineages of *Pipistrellus maderensis* in the Canary Islands (Pestano et al., 2003) and one in Madeira (Jesus et al., 2013). Indeed, there is no haplotype sharing between *Pipistrellus maderensis* from both archipelagos, suggesting that they represent distinct evolutionary lineages, albeit not distinct enough to constitute different species (Jesus et al., 2013). Our results indicate a close phylogenetic affinity between the *Pipistrellus maderensis* from Porto Santo and Madeira (only 0.04 % genetic distance; Fig. 3), indicating that the ca. 40 km separating the two islands has not led to considerable genetic divergence between both populations.

Due to their capacity to fly, geographical barriers, such as large expanses of ocean, do not necessarily represent effective barriers to gene flow among bat populations (García-Mudarra et al., 2009). Yet, molecular studies revealed limited overwater dispersal of *Nyctalus azoreum* between some islands of the archipelago of the Azores (Salgueiro et al., 2008). Likewise, while dispersal and gene flow of *Hypsugo savii* among some of the closest islands (> 40 km) of the Canary Islands is frequent, gene flow is rare among Canarian populations of *Pipistrellus maderensis* inhabiting different islands (Pestano et al., 2003). The low genetic divergence between the populations of *Pipistrellus maderensis* of Porto Santo and Madeira suggests gene flow between both islands. However, this might also be due to a relatively recent (re)colonisation of Porto Santo, with individuals from Madeira. In fact, Porto Santo was heavily impacted by human-induced habitat change, which combined with the effects of invasive species might have led to the decline in the abundance – and possible extinction – of *Pipistrellus maderensis* and possibly other bat species, which would mimic patterns of anthropogenic extinctions observed in other vertebrate groups (e.g. Alcover et al., 2015).

DNA metabarcoding allows unprecedented resolution in the assessment of the diet of insectivorous predators. Although we were only able to sample and analyse faecal samples from four individuals, we detected prey items belonging to five insect orders, corresponding to at least 11 different species. In Madeira, a recent study identified Lepidoptera as the most consumed order by *Pipistrellus maderensis* (Gonçalves, 2022). However, Diptera was the most represented order in the four samples analysed from Porto Santo (Fig. 4). These results align with findings for *Pipistrellus kuhlii* (a sister taxa of *P. maderensis*) in the Iberian Peninsula, where Diptera has been found to make up over half of the species' diet (Goiti et al., 2003). Hymenoptera, Lepidoptera, Hemiptera, and Neuroptera were also detected in the diet of *Pipistrellus maderensis* in this study, which in combination with the identification of Coleoptera, Tipulidae, Chi-

ronomidae, and Aranea in the diet of the species in Madeira (Gonçalves, 2022) suggests that, similarly to *Pipistrellus kuhlii* (Amichai and Korine, 2020), *Pipistrellus maderensis* is likely a “selective opportunist” that feeds on a wide variety of prey, according to its availability. Despite the reduced sample size of our diet analyses, we detected at least four known or potential economically important insect pest species (e.g. *Epiphyas postvittana*, which is known to damage the fruits of multiple species; Danthanarayana et al., 1975; Thrimawithana et al., 2022) and two mosquitoes known to be vectors of human and non-human diseases (e.g. *Culex pipiens*, which can spread avian malaria; Hamer et al., 2008; Fros et al., 2015) (Table 1). The high percentage of known or potential agricultural pest species and of species of health relevance consumed by *Pipistrellus maderensis* highlights the potential role of insectivorous bats as suppressors of arthropods with negative economic and (both human and non-human) disease implications (Montauban et al., 2021; Ancillotto et al., 2022, 2023; Ferreira et al., 2023).

Insectivorous bats are often not considered as charismatic as other island vertebrates, and their conservation is neglected. The population of *Pipistrellus maderensis* from Porto Santo, despite being genetically similar to the one inhabiting Madeira, is of critical importance to the ecological balance, acting as a predator of a multitude of arthropod species. The bats were detected throughout most of the island, but the extant population appears to be small and thus particularly vulnerable to threat factors such as the destruction of roost and feeding habitats, reduction of prey due to pesticide use, or the impacts of invasive species (e.g. free-ranging cats *Felis catus*, a known predator of *Pipistrellus maderensis*; Rocha, 2015). We urge that more research is devoted to this population, which represents the sole native terrestrial mammal of Porto Santo.

**Data availability.** All DNA sequences are available on GenBank with accession numbers OQ260001 to OQ260006.

**Supplement.** The supplement related to this article is available online at: <https://doi.org/10.5194/we-23-87-2023-supplement>.

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