The diet of the house mouse in three protected islands in Italy: results from DNA metabarcoding

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Abstract:
Islands are globally recognized biodiversity hotspots but remain highly vulnerable to invasive species. Among these, the house mouse (*Mus musculus*) is a widespread invasive rodent, known to impact native island ecosystems significantly. While extensive research has been conducted on its diet and ecological effects in oceanic islands, data from the Mediterranean region are scarce. This study provides a first overview of the diet of house mice from three Italian islands (San Domino, Ventotene, and Pantelleria) using a DNA metabarcoding approach. By analysing gut content and faecal samples, we identified 172 Amplicon Sequence Variants (ASVs), including 78 invertebrate, 46 plant, and 3 vertebrate ASVs. Similarly to what emerged from previous studies on oceanic islands, invertebrate consumption was dominated by Lepidoptera, with Noctuidae and Nymphalidae as the most represented families, while plant consumption was primarily composed of Fabaceae and Poaceae. However, no evidence of predation on native vertebrates was found and the only vertebrate sequences found were attributable to human food, indicating possible human-mice interactions on inhabited islands. Ventotene, the only rat-free island at the time of sampling, exhibited the widest niche breadth, suggesting that the absence of mammalian competitors may influence mouse feeding behaviour. These findings highlight the need for a context-specific approach when assessing rodent impacts in the Mediterranean and confirm the possible negative effects of mice on arthropods island populations.

Keywords: diet, Mediterranean, house mouse, invasive rodents, island conservation.

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ABSTRACT

Islands are globally recognized biodiversity hotspots but remain highly vulnerable to invasive species. Among these, the house mouse (*Mus musculus*) is a widespread invasive rodent, known to impact native island ecosystems significantly. While extensive research has been conducted on its diet and ecological effects in oceanic islands, data from the Mediterranean region are scarce. This study provides a first overview of the diet of house mice from three Italian islands (San Domino, Ventotene, and Pantelleria) using a DNA metabarcoding approach. By analysing gut content and faecal samples, we identified 172 Amplicon Sequence Variants (ASVs), including 78 invertebrate, 46 plant, and 3 vertebrate ASVs. Similarly to what emerged from previous studies on oceanic islands, invertebrate consumption was dominated by Lepidoptera, with Noctuidae and Nymphalidae as the most represented families, while plant consumption was primarily composed of Fabaceae and Poaceae. However, no evidence of predation on native vertebrates was found and the only vertebrate sequences found were attributable to human food, indicating possible human-mice interactions on inhabited islands. Ventotene, the only rat-free island at the time of sampling, exhibited the widest niche breadth, suggesting that the absence of mammalian competitors may influence mouse feeding behaviour. These findings highlight the need for a context-specific approach when assessing rodent impacts in the Mediterranean and confirm the possible negative effects of mice on arthropods island populations.

KEYWORDS: invasive rodents; island conservation; Mediterranean; house mouse; diet.

INTRODUCTION

Islands frequently host unique endemic species with limited ranges or breeding sites (Bellard et al., 2017, 2021; Holmes et al., 2019; Russell and Kueffer, 2019). In fact, despite covering only 6.7% of the Earth's land surface, islands are home to approximately 20% of global biodiversity (Kier et al., 2009; Sayre et al., 2019). Invasive rodents are among the most significant threats to island biodiversity, as they have invaded around 80% of the world's archipelagos (Atkinson, 1985; Capizzi et al., 2014; Harper and Bunbury, 2015; Holmes et al., 2019; Moore et al., 2022; Nance et al., 2023). These invasions have had devastating consequences for native species, including documented cases of extinctions (Hilton and Cuthbert, 2010; Bellard et al., 2016a, 2016b; Nance et al., 2023).

The house mouse (*Mus musculus*) is one of the most widespread human-associated mammals in the world (Global Invasive Species Database, 2024; http://www.iucngisd.org/gisd/100_worst.php). Its management is often challenging due to a combination of factors related to its ecology, behaviour and rodenticide resistance (Elliott et al., 2015; Capizzi, 2020; Spatz et al., 2022; Gallozzi et al., 2024). Likewise other invasive rodents, the impact of mice is especially pronounced on islands, where introduced populations have caused significant damages to native ecosystems often resulting in severely altered ecological processes (Eriksson and Eldridge, 2014; Russell et al., 2020). In fact, introduced mice are known for preying on many different island-endemic species. Invertebrates are highly impacted (St Clair, 2011; Watts et al., 2022, Norbury et al., 2023) and many plants have been observed to be potentially threatened by these rodents (Shiels and Pitt, 2014). Furthermore, predation on native reptiles has been observed as well (Wedding, 2007; Norbury et al., 2014, 2023). Moreover, the impacts of mice are usually more relevant when they are the only introduced mammals, with many examples of predation even on seabird eggs and chicks (Wanless et al., 2007; Angel et al., 2009; Bolton et al., 2014; Cuthbert et al., 2016).

The bulk of knowledge about mice impacts comes from oceanic islands. Very few data are available for the Mediterranean region and small islands are still uncovered. Furthermore, no taxonomic data about diet composition is available to date. Having a deeper knowledge about mice's dietary habit may allow us to evaluate if these rodents actually represent a threat for Mediterranean native species. The Mediterranean basin is a globally important biodiversity hotspot, with more than 5000 islands and islets hosting numerous endemic species (Vogiatzakis and Griffiths, 2008; Coll et al., 2010; Peyton et al., 2019). Mediterranean islands are highly anthropized, often serving as popular tourist destinations and with stable human settlements and activities which can provide food and shelter for synanthropic rodents (Capizzi et al., 2024). Differently from oceanic islands, where mice were recently introduced (Jones et al., 2003; Searle et al., 2009; Hardouin et al., 2010), mice have colonized the Mediterranean from the Middle East between 10,000 and 3,000 years ago (Cucchi et al., 2002, 2005; Gabriel et al., 2010). With over 300 islands, Italy is among the island-richest countries in the Mediterranean. Italian islands are home to a wide array of flora and fauna, including many insular endemics (Bonanno and Veneziano, 2016; Muscarella and Baragona, 2017; Senczuk et al., 2019; Sabatelli et al., 2023). Most of these islands are human inhabited and commensal rodents are often present, posing a possible threat to the many endemic and/or endangered taxa (Capizzi, 2020). Indeed, it should be noted that unlike in the case of rats, which have been eradicated from several mediterranean islands, eradications of mice have been achieved in the Mediterranean only once

(Capizzi, 2020). The main reason for this low success rate is the short range of activity of the house mouse, requiring capillary bait coverage of an island during eradication. However, applying high bait station density is not always possible and the use of aerial baiting may be constrained by the specifications given on the product labels, which allow their use only in bait containers (Capizzi, 2020).

Despite the persistence and widespread distribution of mice in the Mediterranean, knowledge about dietary habits and impacts in this complex yet extremely interesting environment remains poor and based on traditional methods like behaviour observation, microscopy examination of stomach content or isotopes analysis (Gasperini et al., 2024; Renaud et al., 2024). However, these methods present several limitations in capturing comprehensive food lists with precise taxon identification from fluids or soft tissues (Nielsen et al. 2018; Sato, 2025). For these reasons, in this study, we used a metabarcoding approach to provide data on house mouse diet from three Italian islands with high naturalistic value, thus representing the first available information on this topic from the Mediterranean basin based on this technique. Even though DNA metabarcoding does not allow to reconstruct the actual feeding mode (e.g., predation, secondary consumption, etc…), it is the most reliable way to obtain high sensitivity taxonomic information about the diet composition of a species, and this method is increasingly used to study invasive rodent diet (Sato, 2025). Specifically, we tested (a) if house mice have dietary habits coherent with those observed in oceanic island, thus posing a threat on native fauna and flora, (b) if there are island-specific differences in house mouse diet, and (c) if the high presence of human settlements can determine a shift towards anthropogenic food.

MATERIALS AND METHODS

The study area consists of three islands in Italy: San Domino (Tremiti Archipelago), Ventotene (Eastern Pontine Archipelago) and Pantelleria (Figure 1). All of them are intensely human inhabited but still represent important sites for biodiversity conservation. Particularly, Ventotene lay inside a protected marine area, while San Domino and Pantelleria are part of two national parks (Gargano NP and Pantelleria NP, respectively). Different seabird species breeds in the study area and have been observed to be negatively impacted by invasive rodents: the Scopoli's shearwater (*Calonectris diomedea*) is found in all the three islands, and Ventotene and San Domino host the endangered Yelkouan's shearwater (*Puffinus yelkouan*) (Capizzi et al., 2024). A significant number of endemic plants (e.g., *Centaurea diomedea* in San Domino, *Helichrysum errerae* and *Lymonium cosyrense* in

Pantelleria, *L. pandatariae* in Ventone) and invertebrates (e.g., *Stenostoma cossyrense*, *Gryllotalpa cossyrensis* and *Acheta pantescus* in Pantelleria) is also found (Pasta and La Mantia, 2013; Muscarella and Baragona, 2017; Galié et al., 2019). Moreover, Pantelleria hosts valuable populations of protected reptiles and mammals, such as the horseshoe whip snake (*Hemorrhois hippocrepis*), the ocellated skink (*Chalcides ocellatus*) and the North African white-toothed shrew (*Crocidura pachyura*) (Corti et al., 2006; Angelici et al., 2009).

The subspecies of the house mouse present in the study area corresponds to *Mus musculus domesticus*, sometimes referred to by its specific designation *Mus domesticus* (e.g., Loy et al. 2019). Mice were caught between 2022 and 2023 by using Sherman traps baited with peanut butter. For each sampling session, 60 total traps were located in six 10-traps transects. The transects were set to cover the three main habitats present on the islands (i.e., Mediterranean scrub, forest and urban areas), thus guaranteeing a double replicate for each habitat type. For each island, we collected samples in two separate 5 night-long sessions: one in autumn/winter and one in spring/summer to collect as much of seasonal variability. All the trapping activities were authorized jointly with the monitoring and biosecurity activities of the projects LIFE PonDerat (Ventotene), LIFE Diomedee (San Domino) and "Conservazione della Biodiversità del Lago Bagno dell'Acqua" (Pantelleria). A total of 41 mice were collected and stored in 96% pure ethanol (see Supplementary Materials for details). Gut content from stomach and faecal samples from intestines were collected from each mice in the lab. To avoid overrepresentation of the remnants of prey hard parts, we excluded those individuals with empty or less than a quarter full stomach (Le Roux et al., 2002). Following this procedure, DNA was successfully extracted from 10 mice per island using Zymo Quick-DNA Fecal/Soil Microbe Microprep kit according to provider's instructions. The extracted DNA was then used to amplify target genes via PCR. Three markers were used in this study: 16S rRNA for invertebrates, 12S rRNA for vertebrates and P6-loop region of the chloroplast trnL gene for plants. The primers used are reported in Table 1. Nextera adapters were added to the primers (FW: TCGTCGGCAGCGTCAGATGTGTATAAGAGACA, RV: GTCTCGTGGGCTCGGAGATGTGTATAA). For each marker, all samples from the same island were pooled together after amplification to obtain a DNA concentration between 5 and 20 ng/µl, required for sequencing. Amplicons were then shipped to an external service (BMR Genomics) where an indexing PCR was performed using the Nextera XT Kit and the products were sequenced with Illumina MiSeq using Paired End 2x300 bp sequencing strategy.

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For each marker, raw sequences were quality checked using *FastQC* (Andrews, 2010). Primers and Illumina adapters were removed from the raw sequences using the *cutadapt* software (Martin, 2011). The trimmed sequences were then exported into an R environment where we used the *dada2* package (Callahan et al., 2016). Sequences with a quality score less than or equal to 2 were discarded and chimeras were removed with *removeBimeraDenovo* function using the default method "consensus". The remaining forward and reverse reads were merged (function *mergePairs*) and taxonomic assignation was performed for each unique sequence present in the data, defined as Amplicon Sequence Variant (ASV). The ASVs identify and differentiate sequences based on single-nucleotide differences, offering a more precise view of the taxonomic composing of the diet. Custom reference databases for the three genetic markers used in this study were generated using *CRABS* software (Jeunen et al., 2023). In order to include the widest range of possible taxa, the reference databases were generated by downloading from NCBI all the available sequences and taxonomy information belonging to all the taxonomic classes found on the studied islands. After that, an in-silico PCR was performed to extract the amplicon region of the primer set and a taxonomic lineage for each sequence in the reference database was generated using the command *assign_tax*. Subsequently, we dereplicated the reference databases with the method *uniq_species*, which allows to retain all unique sequences for each species in the database. The databases were then cleaned up and exported in FASTA format, suitable for *dada2* in R. Due to the reduced length (~150 bp) of our sequences, the *dada2* default minimum bootstrap confidence ≥ 50 was chosen for assigning taxonomic levels (Edgar, 2018). To avoid including mice's own DNA in the analysis, the ASVs assigned to family Muridae were dropped. For those not assigned to family level but belonging to either Mammalia or Rodentia, a BLAST was performed and, if matching with Muridae sequences, they were removed as well. Sequences matching with the plant genus *Arachis* were dropped as well, being potentially related to bait consumption inside the trap. Then, the frequency of the remaining ASVs was calculated for each island. As the frequencies of invertebrate, plant and vertebrate ASVs are the results of separate PCRs and could not be compared with each other, the niche breadth was assessed for each of these taxonomic category separately for each island by using the standardized Levins index (B) (Levins, 1968). Niche overlap among the three studied islands was also estimated using the Pianka index.

RESULTS

A total of 606,692 raw reads for invertebrates, 456,142 for vertebrates and 550,604 for plants were retrieved from our samples. Following trimming, filtering and removing primers and chimeras, 265,673 paired-reads for invertebrates, 216,590 for vertebrates and 156,820 for plants were successfully merged and were suitable for the analyses. After dropping 19 ASVs belonging to Muridae and 2 belonging to *Arachis*, 46 ASVs were obtained for plants, 78 for invertebrates and 3 for vertebrates. Specifically, invertebrates from 5 classes, 14 orders, and 28 families were detected. Lepidoptera were the most represented order in all the three islands with Noctuidae and Nymphalidae being the most represented families. The second most common invertebrate order were Coleoptera, particularly abundant in Ventotene. Among Coleoptera, Curculionidae was the most represented family.

Plants all belonged to the taxonomic class Magnoliopsida and were distributed among 18 orders and 21 families. Fabaceae and Poaceae were frequent in all the islands, followed by Asteraceae, Euphorbiaceae, Betulaceae and Malvaceae.

The three vertebrate ASVs all belonged to domestic species: two ASVs from San Domino and Ventotene were identified as turkeys (*Meleagris gallopavo*) and one from Pantelleria as goats (*Capra hircus*). Results for invertebrates and plants are shown in Figure 2 and 3. Further details about ASVs frequency are available in Supplementary Materials.

Ventotene is the island with the widest niche breadth for invertebrates (B = 0.05), followed by Pantelleria (B = 0.01) and San Domino (B = 0.0009). A similar pattern is observed for plants, with Ventotene and Pantelleria showing B = 0.14 and B = 0.13, respectively, followed by San Domino (B = 0.08). Given the low number of vertebrate ASVs (one for Pantelleria, two for San Domino and two for Ventotene) and the likely anthropic origin (see discussion), the Levins index for this taxonomic group was not computed. The Pianka's index indicates a high overlap for the invertebrate component of the diet between the studied islands (O = 0.825-0.999), while some differences emerge for the plant component (O = 0.478-0.891). Detailed information about niche breath and overlap across the islands is available in Table 2 and 3.

DISCUSSION

Our study provides the first data on the diet of the house mouse from islands located in the Mediterranean basin. Despite the presence of some methodology limitations like differences in primer efficiency, possible contamination and low taxonomic resolution (Cuff et al. 2021; Tercel et al. 2021; Sato, 2025), the DNA-based approach allows to detect also the smallest, softer digestible items that would not be considered with morphological examination (Ingerson-Mahar, 2002; Cuff et al., 2021; Sato, 2025). This is a particularly critical issue for small mammals. Even though the low sample size, the reduced study area and the lack of numerous temporal replicas do not allow our results to be exhaustive for an accurate assessment of mice diet on Mediterranean islands, they are a valuable insight into house mouse ecology in the area. According to previous studies, a sample of 5-9 mice is sufficient to provide more than 90% of main categories of prey items (Le Roux et al., 2002). Indeed, this study provides an excellent starting point and a protocol to follow for future and more detailed assessments. It also lays the foundations for implementing evidence-based management strategies.

As expected, mice from our study area are generalist, feeding on a wide range of invertebrates and plants. Specifically, their diet appears to be composed of arthropods (mostly Lepidoptera) and large-seeded plants, like Poaceae and Fabaceae. This result perfectly matches what emerged from the only available previous studies on this topic performed on oceanic islands that showed how mice have complex, omnivorous diets but tend to prefer arthropods (especially Lepidoptera larvae), followed by seeds and other vegetative material (Smith et al., 2002; Shiels et al., 2013; Shiels and Pitt, 2014; Holthuijzen et al., 2023).

From a conservation perspective, even though the identification at species level of some endemic plants like *Centaurea diomedea* from San Domino and *Helichrysum errerae* from Pantelleria was not possible, they all belong to Asteraceae, a well-represented family in the diet of the mice sampled in this study. Similarly, our results also raise some concern about native arthropods diversity and community, especially regarding nymphalid butterflies, a very frequent taxon in house mouse diet. It is worth noting that one of Italy's most endangered island-endemic invertebrates, the Ponza grayling (*Hipparchia sbordonii*), belongs in fact to Nymphalidae (Lepidoptera). This species' range is now restricted to the island of Ponza (Pontine Archipelago) but it was once found also in Ventotene (Bonelli et al. 2018). Anyway, recent surveys failed in finding this species on the island (Sbordoni, 2018). Therefore, according to our results, mice could have favoured its decline and could pose a threat on the remaining populations of Ponza grayling by feeding on its larvae. Interestingly, an apparent selection for lepidopteran preys (moths and butterflies) is consistent across a wide biogeographical range investigated, since our findings confirm the results obtained on oceanic islands (Shiels et al., 2011). Accordingly, other nymphalid butterflies whose insular populations are valuable conservation targets would be at risk on invaded islands (e.g., *Coenonympha corinna*,

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Pyronia cecilia, *Hipparchia aristaeus*, *Argynnis pandora*, variously distributed across several islands in Italy) (Dapporto et al., 2017). In detail, a major concern is raised for those species with short flight periods, limited dispersal capability and/or small populations on remote islands, that have lower probability of recolonization from surrounding areas (Dapporto et al., 2017). Of course, these are totally speculative hypotheses that need to be validated through further analysis with higher taxonomic resolution. Moreover, dietary assessments alone cannot reveal if an item is found in the diet of mice following actual predation, secondary consumption or even contamination. Accordingly, it needs to be considered that detecting a taxon in the diet does not necessarily imply it is consumed in significant quantities, and even if it is, this does not automatically translate into a demographic impact on the prey species. However, the information provided here may still be considered for future conservation plans targeting native arthropods or plants.

On the other hand, a sequence of *Helicoverpa* sp. (Lepidoptera: Noctuidae) emerged from Ventotene. Some species belonging to this genus are among the worst lepidopteran agricultural pests in the world (Jones et al., 2019). Also among plants we found some introduced taxa like *Paspalum* sp. and *Oxalis* sp. which are often considered invasive (Gallardo, 2014; Gaetani et al., 2017). Accordingly, previous studies have shown that mice can feed also on non-native invertebrates and plants, sometimes acting like a natural control of many invasive species (Holthuijzen et al., 2023; Sato, 2025). Therefore, their role in controlling pest arthropods populations should not be underestimated, especially in a biogeographic context in which mice have been present for thousands of years, like the Mediterranean basin and its islands (Solano et al., 2013; Sciandra et al., 2022; Gallozzi et al., 2025). In such cases, mice can represent an important component of island ecosystems and their eradication may lead to an unexpected increase of unwanted species' populations. Anyway, even though the data at hand for Italian islands is mostly about rats, no sudden increase of unwanted invertebrate species following rodent eradications has ever been recorded (Capizzi et al., 2016).

A widest niche breadth emerged in Ventotene, especially in invertebrates. When sampling occurred, Ventotene was the only rat-free location among those included in this paper, so mice were the only terrestrial mammal present on the island (Gotti et al., 2022). It is known that, when they have no other mammalian competitor, mice tend to expand their feeding habits and increase significantly both in population and predatory behaviour (Cuthbert et al., 2016; Capizzi et al., 2024). Therefore, the absence of rats can be a possible explanation for what we observed in Ventotene. Of course, due to the low sample size we cannot exclude random effects, and this hypothesis needs to be confirmed

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through further investigation. However, acquiring information about the ecology and in particular the diet of the house mouse is considered a good practice to follow in eradication planning more and more frequently, as this may increase the chances of detecting dietary shifts and potential negative impacts on prey species as early as possible. As a matter of fact, this represents crucial information for including mice in eradications efforts, especially when the presence and effects of mice tend to be overlooked (Samaniego et al., 2024).

The Pianka's index highlighted very few differences between the studied island, mostly regarding the plant component of the mice's diet. Specifically, Ventotene and San Domino show a high niche overlap with each other (O = 0.891), while Pantelleria exhibits strong overlap with Ventotene (O = 0.732) and only a partial overlap with San Domino (O = 0.478). This probably reflects the presence of some island-specific factors (geographic location, morphology, human population density) that may influence the presence of different habitat types, plant species and resource availability. On the other hand, the high niche overlap observed for the invertebrate component of mice's diet confirms the widespread tendency of the house mouse to select lepidopterans as their preferred prey item, as already observed in previous studies (Shiels et al., 2013; Shiels and Pitt, 2014; Holthuijzen et al., 2023). Anyway, differently from what happens in oceanic islands (Holthuijzen et al., 2023), we did not find any trace of seabirds or other native vertebrates in mice's gut content/faeces and the only vertebrate sequences we found belong to goats (*Capra hircus*) and turkeys (*Meleagris gallopavo*). Both species are raised by local people on the studied islands (Gallozzi's observation). Therefore, they can be easily found by mice either in farms or as food waste inside trash bins. This is coherent with the hypothesis that, on intensely inhabited islands such the ones included in this paper, mice can exploit anthropogenic resources. This suggests that human-mouse interaction is probably playing a role in Mediterranean islands, providing sustenance and shelter for these animals. Anyway, given the low frequency of human-related items in mice's diet, it is likely that food of anthropic origin represents only a small proportion of their feeding habits, and these animals rather feed on wild arthropods and plants. Since we pooled all the individuals coming from the same island before sequencing, we cannot exclude that the small proportion of anthropogenic food may reflect the feeding habits of those individuals living in urban areas. Therefore, given the restricted home range of mice and their opportunistic behaviour (Gasperini et al., 2024), there can be significant differences in the diet of the individuals living inside and outside human-inhabited areas.

To conclude, our results provide, for the first time from small Mediterranean islands, taxonomic data about house mouse diet composition. This information can be extremely useful to identify which taxa may respond to mice removal, revealing an interesting pattern of interaction between these rodents, arthropods, plants and humans on Mediterranean islands. Unlike the case of rats, where assessment of eradication priorities is usually based on seabird communities to protect (Capizzi et al. 2010), in the case of house mouse eradication, invertebrates should be given greater consideration, given their predominance in the diet of mice. Indeed, based on our results and supported by previous studies conducted in other geographic areas (Watts et al., 2022, Norbury et al., 2023), it seems that arthropods may benefit the most from mice removal, especially when there are no other mammalian competitors on the island. However, the overall effects of such operations should be carefully evaluated also considering possible unexpected increase in population of unwanted pests. In these circumstances, the well-being of already existing species and ecosystems should be always prioritized over uncertain potential benefits for a single taxon (Ricciardi and Simberloff, 2014).

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Amplified region	Fragment length	Primer sequence 5’-3’	Reference
12S	73-110 bp	FW: TAGAACAGGCTCCTCTAG RV: TTAGATACCCCACTATGC	Riaz et al., 2011
16S	110 bp	FW: TRAACTCAGATCATGTAA RV: TTAGGGATAACAGCGTWA	Kartzinel and Pringle, 2005 Pinho et al., 2018
P6-loop	100-143 bp	FW: GGGCAATCCTGAGCCAA RV: CCATTGAGTCTCTGCACCTATC	Taberlet et al., 2007

Table 1. Primers used for this study.

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Island	Category	Levins' standardized index (B)
Pantelleria	Plants	0.13
	Invertebrates	0.01
San Domino	Plants	0.08
	Invertebrates	0.0009
Ventotene	Plants	0.14
	Invertebrates	0.05

Table 2. Levins' standardized index (B) for each island for plants and invertebrates.

INVERTEBRATES	Pantelleria	San Domino	Ventotene
Pantelleria	-	0.999	0.839
San Domino	0.999	-	0.825
Ventotene	0.839	0.825	-
PLANTS	Pantelleria	San Domino	Ventotene
Pantelleria	-	0.478	0.732
San Domino	0.478	-	0.891
Ventotene	0.732	0.891	-

Table 3. Niche overlap between the studied island expressed by the Pianka's index (O). The values range from 0 to 1. When O = 1, there is a complete overlap, when O = 0, there is no overlap.

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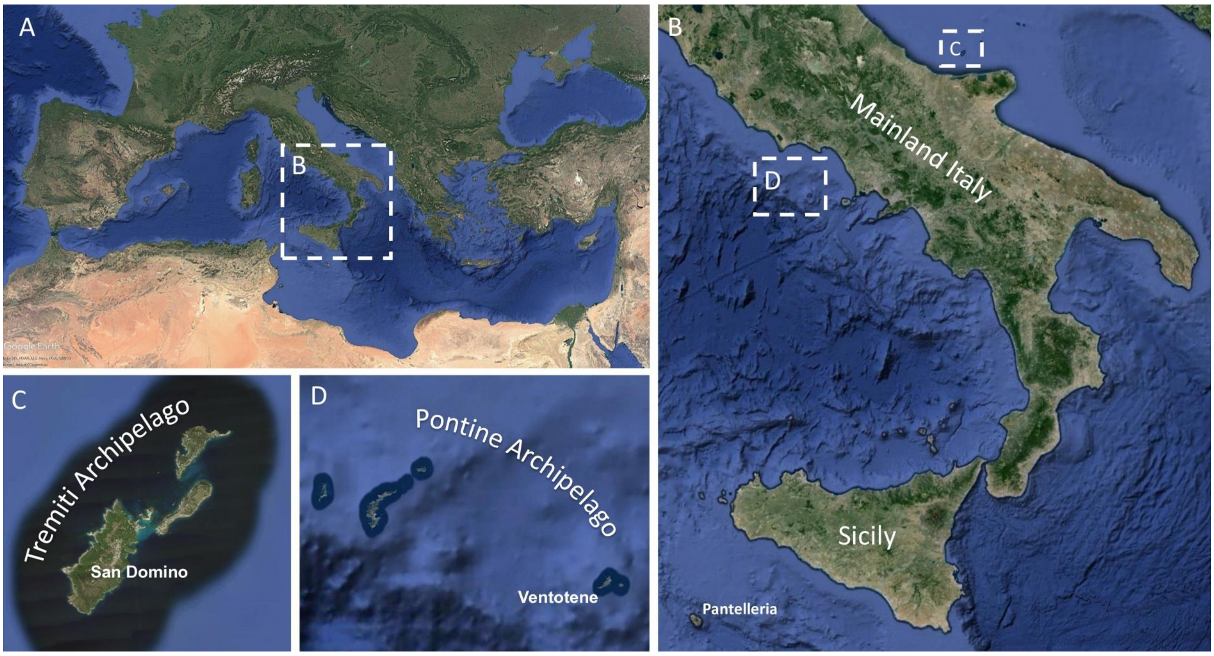

Figure 1. (A) Map of the Mediterranean basin showing the location of the study area. (B) Map of Southern Italy highlighting the location of the islands included in this study. (C) Map of the Tremiti Archipelago. (D) Map of the Pontine Archipelago. Satellite images accessed from Google Earth, 2025 Maxar Technologies.

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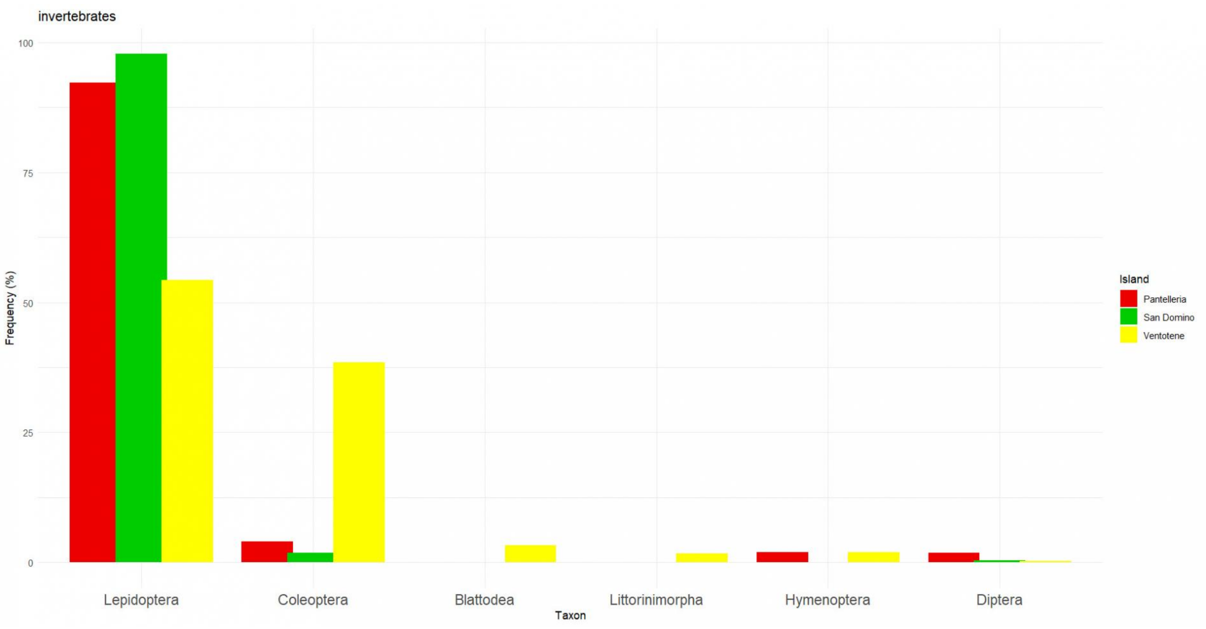

Figure 2. Frequency of the most represented invertebrate ASVs found in the three studied islands. Only ASVs with a frequency > 1% in at least one island are shown in the graph. Each island is represented by a bar of different colour.

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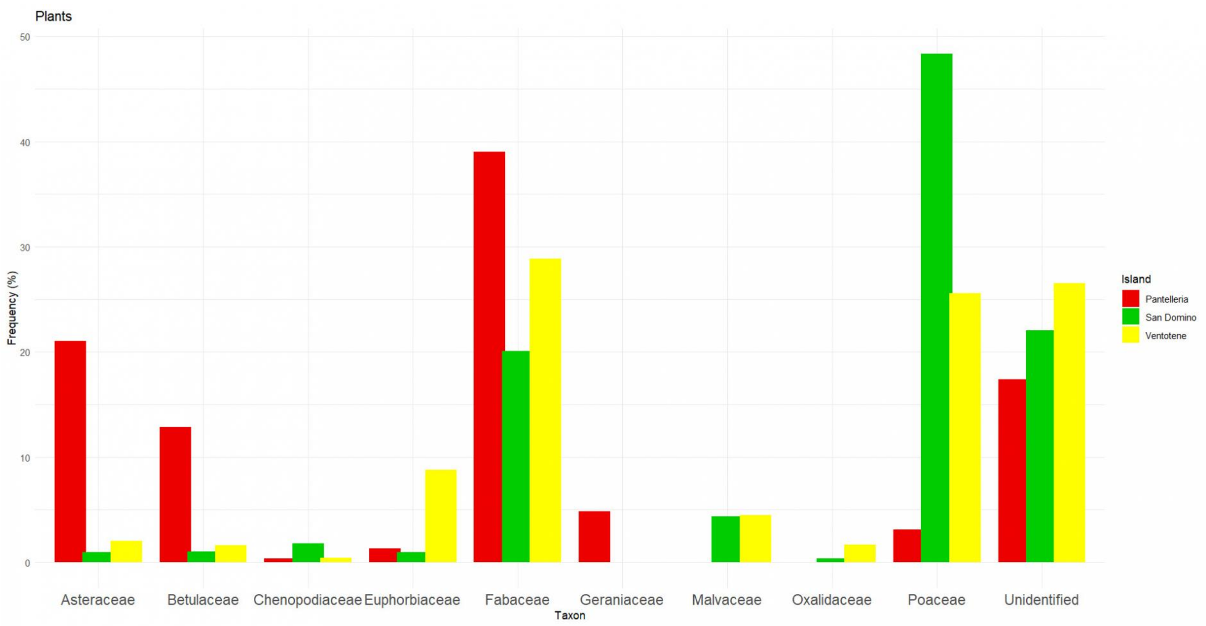

Figure 3. Frequency of the most prevalent plant ASVs identified across the three study islands. Only ASVs occurring at a frequency above 1% in at least one island are displayed. Different islands are shown with uniquely colored bars.

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