Can an introduced gecko act as biological pest controller? Exploring the diet of *Tarentola mauritanica* in Madeira Island

Beatriz Martins

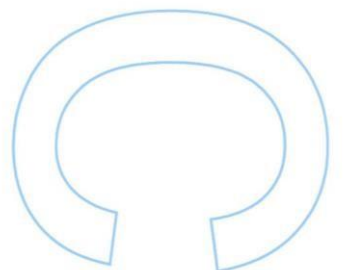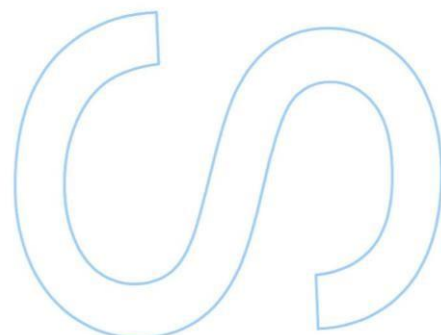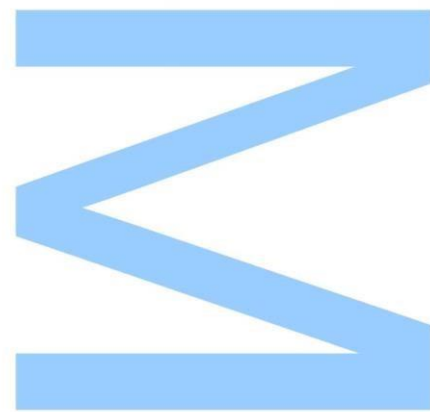MSc in Biodiversity, Genetics and Evolution
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FCUP | i
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island
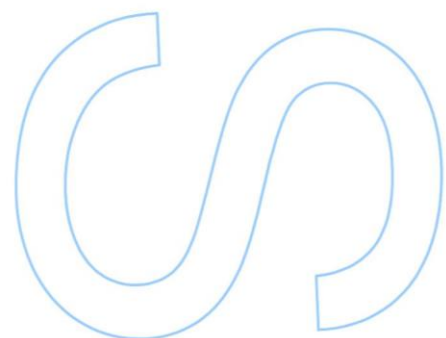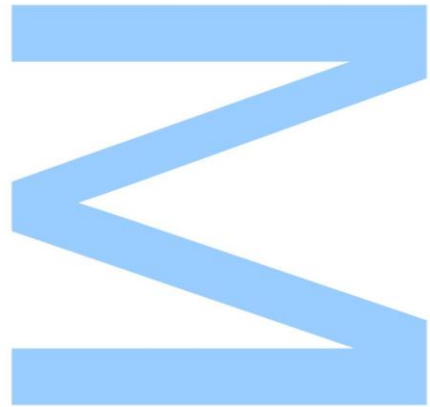
Ackowledgments

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FCUP | ii
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Abstract

Biological invasions are key drivers of biological change in island ecosystems. The Moorish gecko, *Tarentola mauritanica*, is a relatively recent introduction in Madeira Island, where it was first reported in 1993. The species' synanthropic habits and its predisposition for long-distance dispersal has contributed to its expansion across Madeira's southern coast and its arrival to the nearby island of Porto Santo. Trophic studies offer unique ecological insights, particularly important for insular systems, where predator-prey networks often include numerous endemic species. Next generation sequencing (NGS) is an emerging technique increasingly used in ecology. However, the use of NGS for investigating the diet of reptiles is rather scarce with very few studies using this approach for *Tarentola* geckos. Moreover, Madeira Island harbors a wide diversity of agricultural crops, many of which are impacted by arthropod agricultural pests, which may serve as prey to *T. mauritanica*. Metabarcoding was used to analyze fresh faecal pellets from six localities across the southern coast of Madeira to uncover the invertebrate diversity and richness present in each locality. Furthermore, it was investigated how this introduced gecko might be affecting the endemic arthropods of Madeira and assessed if this species might be consuming agricultural pests and arthropods that may act as vectors of human diseases. The results revealed 250 different Operational Taxonomic Units in the diet of *T. mauritanica*, with 47% of them belonging to invertebrates classified as introduced species, and 6% classified as endemic species to Madeira. Hence, based on the proportion of sample counts obtained, the introduced *T. mauritanica* does not seem to have a great impact on endemic arthropods in Madeira, but it does seem to play a role in agricultural pest control, however further studies would be helpful to make more consistent conclusions. This study highlights the importance of trophic studies for monitoring introduced species in islands, considering their potential effects on the spread of agricultural pests, and on the conservation of endemic species, emphasizing the potential of metabarcoding to assess interspecific relationships in a non-invasive way. Moreover, this study emphasizes the importance of metabarcoding diet analyses as an indirect way of uncovering unknown species records, especially the cryptic ones such as invertebrates.

Keywords

FCUP iii
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Resumo

As invasões biológicas são os principais motores das mudanças biológicas nos ecossistemas das ilhas. A osga-moura, *Tarentola mauritanica*, é uma introdução relativamente recente na Ilha da Madeira, onde foi relatada pela primeira vez em 1993. Os hábitos sinantrópicos da espécie e a sua predisposição para dispersão a longa distância contribuíram para a sua expansão pela costa sul da Madeira e a sua chegada até à ilha vizinha, Porto Santo. Os estudos tróficos oferecem perceções ecológicas únicas, particularmente importantes para sistemas insulares, onde as redes predador-presa geralmente incluem numerosas espécies endémicas. O sequenciamento de nova geração (SNG) é uma técnica emergente, cada vez mais usada em ecologia. No entanto, a utilização de SNG para investigar a dieta de répteis é bastante escasso, com muito poucos estudos usando esta abordagem para o género *Tarentola*. Além disso, a Ilha da Madeira alberga uma grande diversidade de culturas agrícolas, muitas das quais afetadas por artrópodes pragas agrícolas, que podem servir de presa à *T. mauritanica*. O metabarcoding foi utilizado para analisar amostras fecais frescas em seis localidades da costa sul da Madeira, para descobrir a diversidade de invertebrados presente na dieta das espécies. Além disso, investigou-se como esta osga introduzida pode estar a afetar os artrópodes endémicos da Madeira e avaliamos se esta espécie pode estar a consumir pragas agrícolas e artrópodes que podem atuar como vetores de doenças humanas. Os resultados revelaram 250 unidades taxonómicas operacionais diferentes na dieta da *T. mauritanica*, com 47% destas pertencentes a invertebrados classificados como espécies introduzidas e 6% classificados como espécies endémicas na Madeira. Assim, com base na frequência de ocorrência, a espécie introduzida *T. mauritanica* não parece ter um grande impacto nos artrópodes endémicos da Madeira, mas sim ter um papel no controlo de pragas agrícolas, no entanto, estudos adicionais seriam vantajosos para tirar conclusões mais consistentes. Este estudo destaca a importância dos estudos tróficos para o monitoramento de espécies introduzidas em ilhas, considerando os seus potenciais efeitos na disseminação de pragas agrícolas e na conservação de espécies endémicas, enfatizando o potencial do metabarcoding para avaliar relações interespecíficas de forma não invasiva. Além disso, este estudo enfatiza a importância das análises de metabolismo da dieta como uma forma indireta de descobrir registos de espécies desconhecidas, especialmente as crípticas, como os invertebrados.

Palavras-chave

SNG, Osga-Moura, Metabarcoding, Espécies Introduzidas, Ilha da Madeira, Pragas Agrícolas, Dieta.

FCUP

Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

iv

Index

FCUP | v
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

List of Tables

FCUP vi
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Supplementary Table 7: Multilevel pairwise comparison among localities. Df stands for degrees of freedom, SS for sum of squares, F Model for F-statistic, R2 for R-squared, and p. adjusted sig for adjusted *p-value*.

List of Figures

FCUP viii
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

FCUP ix
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

List of Abbreviations

BOLD – Barcode of Life Data System

FOO – Frequency of Occurrence

GLM – Generalized Linear Model

HTS – High-Throughput Sequencing

IUCN – International Union for Conservation of Nature

LC – Least Concern

NGS – Next Generation Sequencing

OTU – Operational Taxonomic Unit

PEAR – Paired-End Read Merger

RRA – Relative Read Abundance

SVL – Snout-Vent Length

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

1

Introduction

Firstly, in order to get a revision of the bibliography available on this topic, it is presented a contextualization of introduced species and their potential benefits or harmful effects, without forgetting a review over biological control. Secondly, both key aspects of this research are approached: the sampling location (Madeira Island) and the model species (*Tarentola mauritanica*). Then, an overview is given on agricultural pests and vector-borne human diseases that are known to be present in Madeira. Lastly, the technique used to obtain an overview of the diet of this species (metabarcoding) is described, finalizing with the research goals of this Master thesis.

1. Introduced species and Biocontrol

The introduction of species represents a major biodiversity threat at a global scale, as it is also one of the main causes of animal extinctions (Clavero & García-Berthou, 2005; Early et al., 2016; McGeoch et al., 2010).

Urbanized communities have been facing this major wildlife management issue since they became a consistent target to introduced species (Adams, 2016). This is mostly due to the correlation between human spread and urbanization that leads to the expansion of global trade (Kraus, 2009). When the effects on ecosystems, native wildlife and humans become significantly negative after the introduction of a species, it is then considered as invasive, as its introduction is no longer irrelevant nor beneficial (Adams, 2016).

The last decades have been marked by an exponential increase in the rate of introduced herpetofauna, as a result of the introduction of many amphibian and reptile species to locations outside their native ranges (Stringham & Lockwood, 2018). The introduction of invasive herpetofauna species may lead to a more serious issue, not only on an economic level, but also on native species, for instance in terms of food availability, predation, spread of diseases and parasites, among others (Kraus, 2009, 2015).

These irreversible ecological consequences are especially worrying in islands (Butterfield et al., 1997; Powell et al., 1990). Islands carry unique geographical nature and biota, with high numbers of endemic species, especially the remote ones (Whittaker et al., 2017). Organisms on isolated islands are more vulnerable to new introductions, due to the low level of past evolutionary pressures. In addition, the extinction of island endemics may be attributed to the introduction of species (Wilson, 1992). In fact, 90% of documented mammal and bird extinctions in the past 500 years have been recorded on islands, at a global scale (MacPhee & Flemming, 1997; Manne et al., 1999).

FCUP 2
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Moreover, many of these introduced species are candidates for classical biological control, especially those established in conservation areas where they may threaten native species (Bale et al., 2008). Biological control is a pest management strategy, that occurs naturally in the wild and has been exploited by humans to suppress the number of pest species in agriculture, forestry and greenhouse horticulture (Bale et al., 2008; Ferron & Deguine, 2005). This control is based on the use of an organism to reduce the population density of another organism, so that the pest and natural enemy remain in the agroecosystems at low densities (Bale et al., 2008; Hoddle, 2002; Louda et al., 1997). Specifically the biological control of arthropods can be defined as the study and use of parasites, predators and pathogens for the regulation of pest densities (DeBach & Rosen, 1991). The intensification of agriculture over the years accompanied by the increase of international trade of plant species resulted in the introduction of new pest species, many of them chemical resistant, leading to uneconomic production costs, loss of biodiversity, among others (Bale et al., 2008). Therefore, biological control has sometimes been introduced to combat arthropod species that have developed resistance to insecticides. In some cases, natural enemies help significantly in reducing the population of pests, but in others additional methods, such as the use of chemicals and implementation of physical barriers, are required to achieve adequate levels of control (Bale et al., 2008).

However, the success of biocontrol should take into consideration the further conditions: 1) the strong searching ability of control agents to locate prey and identify areas of high pest density; 2) should have higher potential rates of increase than their prey; 3) control agents should be able to survive even at low density of pests; 4) the target pest should be the preferred prey of the natural enemy; 5) released agent should not attack non-target species; 6) similarity of climates between collection and release sites, otherwise biological control will fail. Although the success of biological control is not guaranteed, and some scientists have contradictory points of view upon the fundamental theories supporting biocontrol (Gutierrez et al., 1993; Pimentel, 1963), there are several successful examples with the implementation of this strategy. The first major success of the modern era was reported in California, United States, in 1880, where the control was achieved in less than two years and was repeated in other parts of the world against the same insect pest (Hoddle, 2002). In this case, the ladybird *Rodolia cardinalis* revealed to be the most effective agent against cottony-cushion scale *Icerya purchasi* on citrus crops (DeBach, 1964). Although the main examples focus on arthropods as biological control agents (De Clercq et al., 2011), there are some studies using laboratory experiences supporting evidence of the positive impact of reptiles on the control of both

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

3

crop pests in agricultural landscapes and vector-borne human diseases, such as dengue (Canyon & Hii, 1997; Monagan Jr. et al., 2017).

Usually, advantages and limitations of biocontrol are expressed comparing to pesticides. The most notable advantage of this approach is the maintenance of the normal interaction existing between natural enemies, that actively and progressively reduces the abundance of a given prey. Since the latter is uncommon to develop resistance to the biological control agent, this brings many profits in a long term. On the other hand, the main limitation of this method is the time required for the predator to get established and adapted to the new environment and starting to suppress pest populations (Bale et al., 2008).

While this is an alternative widely recognized for its benefits, the development costs associated with it are sometimes described as high, since the success of biological control depends on extensive preliminary measures, and of studies to gain a comprehensive understanding of the biology and ecology of the pest. Furthermore, the environments from which they originated and subsequently colonized, or in which they will be released constitute key aspects for the success of biocontrol (Bale et al., 2008).

2. Madeira Island

The Madeira archipelago is an autonomous region of Portugal located in the Atlantic Ocean, near the coast of North Africa, at 560 km from Rabat, Morocco (Aguin-Pombo et al., 2007) (Figure 1). Only Madeira and Porto Santo, the two largest islands of the archipelago, are currently inhabited. On the opposite, there are more than fifty other uninhabited smaller islands, including the Desertas and Selvagens, two island clusters in the south of the archipelago (Aguin-Pombo et al., 2007; Wetterer et al., 2007).

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island
4
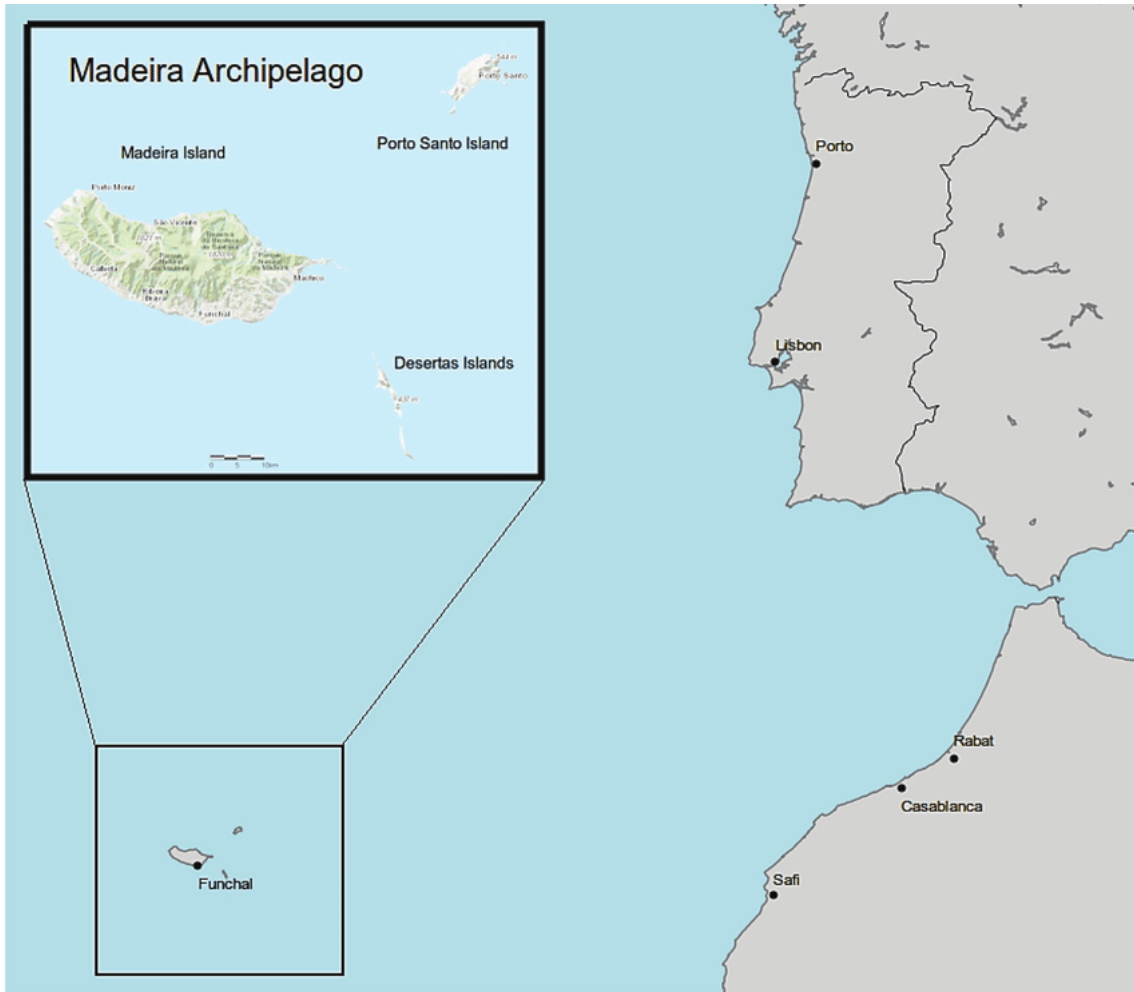
Figure 1: Map of Madeira Archipelago and its islands (Retrieved from Díaz et al. (2018)).

Madeira's climate is conditioned by the relief, configuration, and orientation of the island (Santos, 2018). It is characterized by a very sharp relief and vigorous forms, with embedded and deep valleys, and only 8% of the area below 100 m. The highest point, Pico Ruivo, is about 1862 m above sea level. In addition to the relief, the effect of altitude, induces a local climatic differentiation, as a result of the elongated configuration of the island and its E-W orientation, perpendicular to the direction of the dominant north wind (Figueira et al., 2006). These conditions generate different air temperatures, presenting an annual average of about 20ºC at sea level (Figure 2), and levels of precipitation varying between 500 and 2000 mm (Figure 3).

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island
5
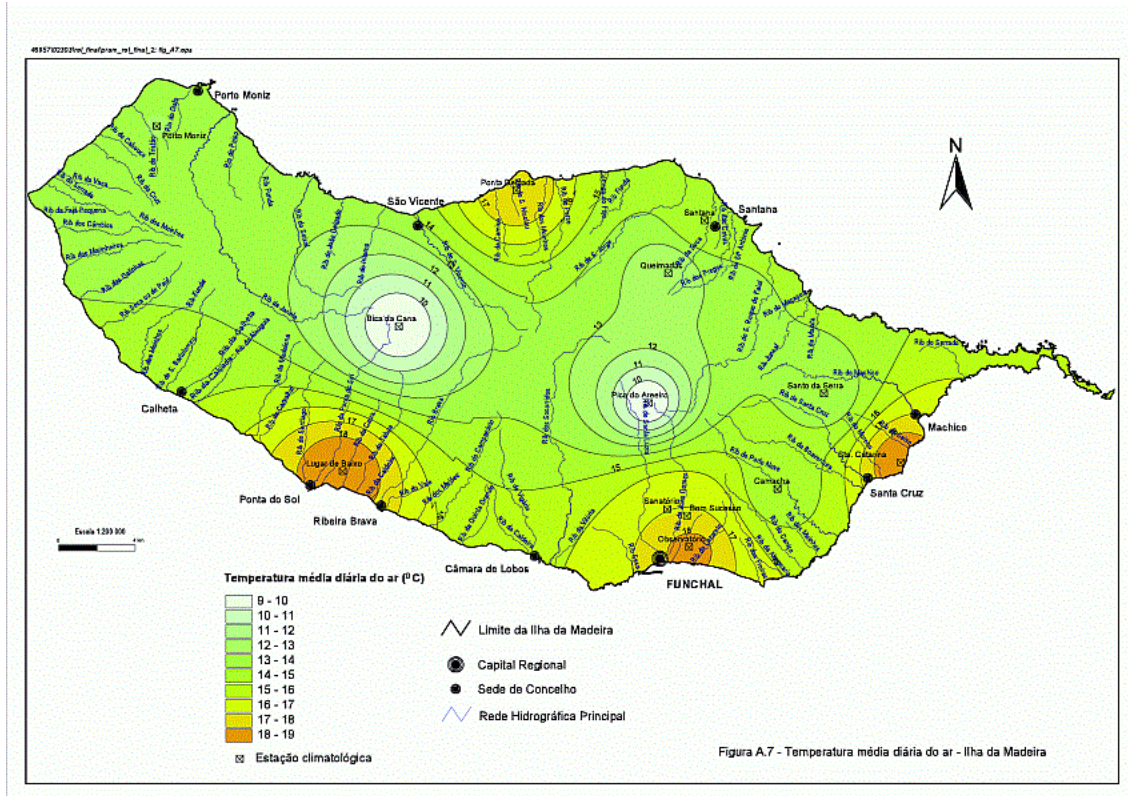
Figure 2: Average temperatures on Madeira Island. Retrieved from https://www.ocean-retreat.com/weather-and-climate/climate-of-madeira/
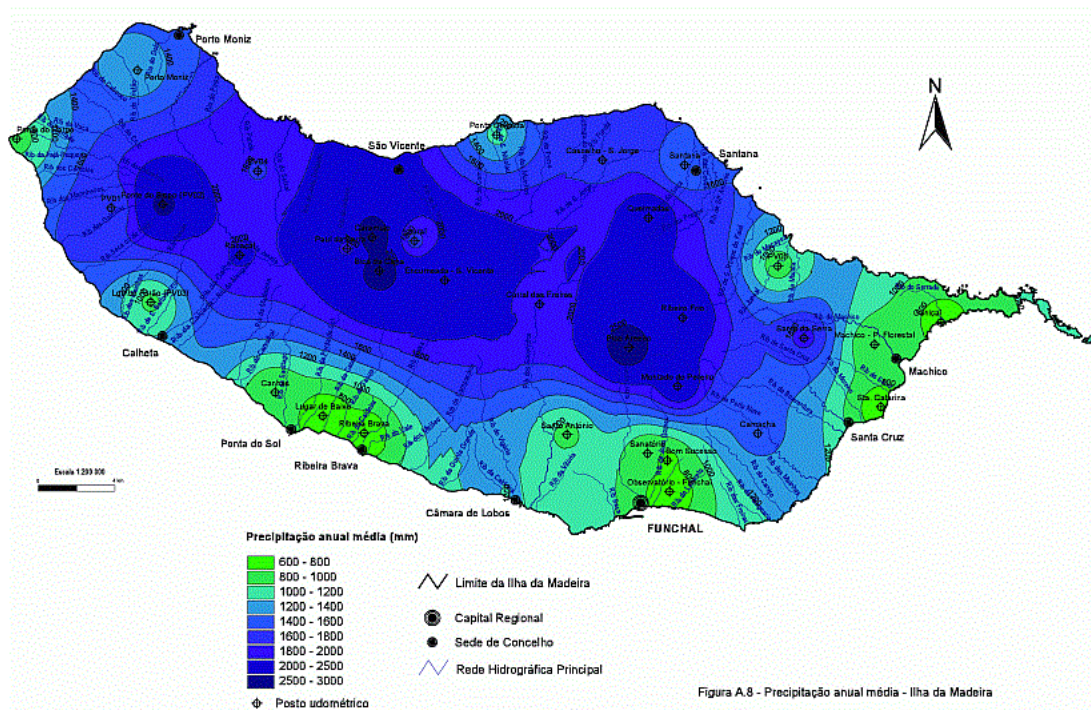
Figure 3: Average annual precipitation on Madeira Island. Retrieved from https://www.ocean-retreat.com/weather-and-climate/climate-of-madeira/

FCUP | 6
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

In addition to the observed climatic differences, the island also has a diverse spatial distribution of land use and landscape management (Figure 4), with several types of interleaved habitats in a short area, especially in the southern coast of Madeira. All these conditions contribute to the different microclimates that exist all over the island (Figueira et al., 2006).
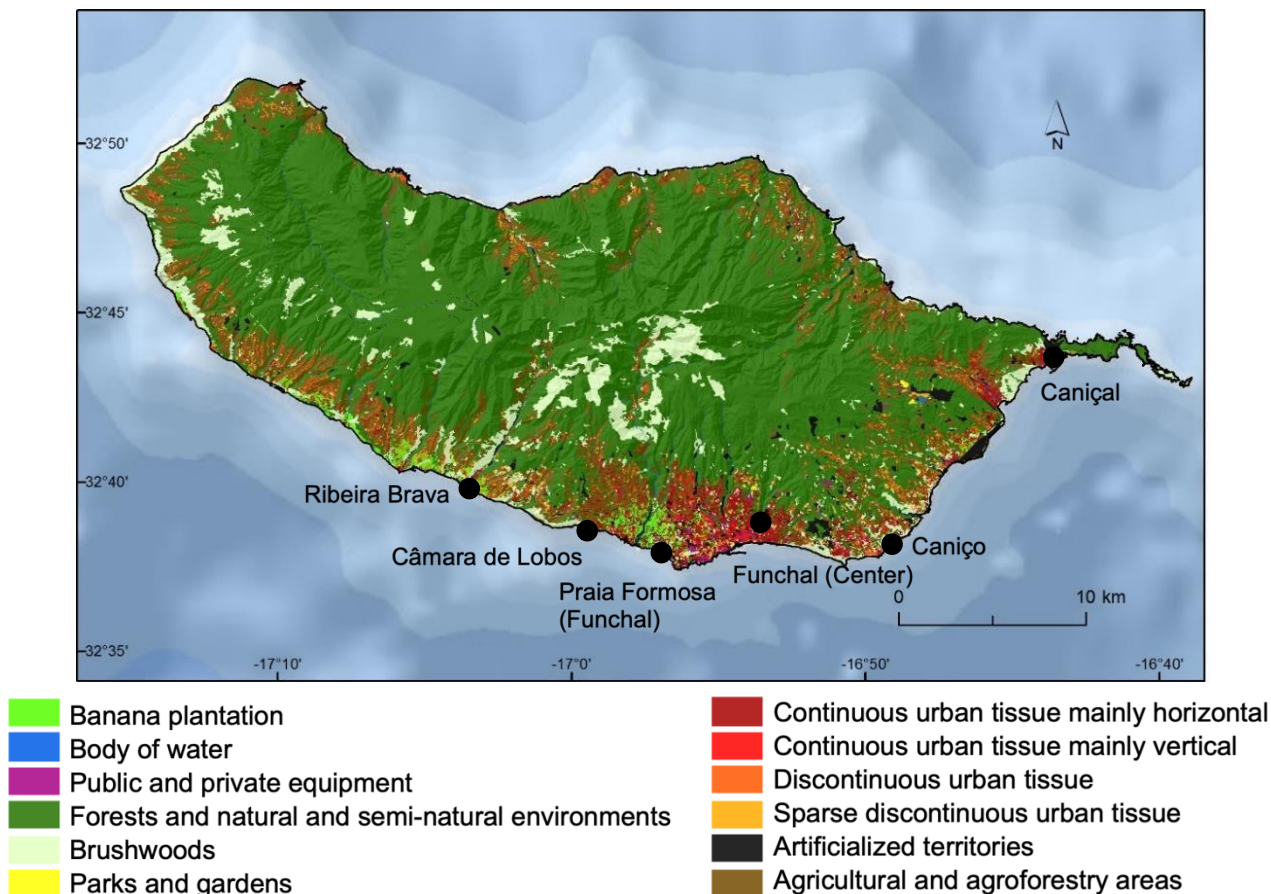
Figure 4: Spatial distribution of land use and occupation typologies (Adapted from Santos (2018)).

Moreover, the archipelago of Madeira is part of a biogeographic subregion named Macaronesia, which also includes the Azores, Canary Islands and Cabo Verde Islands. Macaronesia harbors a significant amount of species nowhere else present in the rest of the world, which means that this region can be considered as a biological hotspot (Wetterer et al., 2007). As so, introduced species represent a major obstacle in protecting endemic species (Silva et al., 2008).

Historically, Madeira was an important center for commerce since it represented a connection between Europe and the New World. With this connection, many animals and plants were introduced to the archipelago, among which were new competitors,

FCUP 7
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

predators and parasites (Wetterer et al., 2007). Actually, this archipelago was identified as exhibiting one of the highest numbers of extinct species in Europe (mostly gastropods), caused directly or indirectly by human activities (Boieiro, Aguiar, Aguiar, Borges, Cardoso, Crespo, Farinha, et al., 2014). For instance, populations of native birds were negatively impacted by the presence of species such as the black rat (*Rattus rattus*), and the domestic cat (*Felis silvestris catus*). As a result, various authors have been warning about the high vulnerability of some endemic Madeiran taxa to introduced species (Boieiro, Aguiar, Aguiar, Borges, Cardoso, Crespo, Farinha, et al., 2014; Borges et al., 2008).

Nowadays, Madeira is well-known by its rich natural heritage and beauty, as it displays a wide variety of endemic and natural areas in a great conservation state. Alongside the Selvagens archipelago, it is home to more than 7500 species of fungi, plants and terrestrial animals, which play a crucial role in terrestrial ecosystems and should be the subject to specific studies and conservation measures (Borges et al., 2008). As a matter of fact, considering all the islands of Macaronesia, Madeira Island is only behind Tenerife, in the Canary Islands, when it comes to richness (Hernández et al., 2009). This archipelago is even included in one of the most important High Biodiversity Areas at a global scale. The Laurisilva of Madeira, within the Parque Natural da Madeira (Madeira Natural Park) was classified by UNESCO as a World Natural Heritage site, highlighting once again this island's richness.

Finally, Madeira and Selvagens archipelagos are particularly diverse in arthropods, which represent about 51% of the animal taxa. Consequently, they strongly impact the overall estimates of diversity in these archipelagos, given that there are several diverse orders included in the phylum Arthropoda (Boieiro, Aguiar, Aguiar, Borges, Cardoso, Crespo, D, et al., 2014; Borges et al., 2008). Moreover, the number of endemic species of terrestrial organisms within these archipelagos is estimated to be around 1419, with 69.09% of these being arthropods. The high percentage of endemic species can be explained by all the speciation and evolutionary processes that occurred over time, mostly due to island isolation, size and topography complexity (Borges et al., 2008). Relatively to introduced arthropod fauna in Madeira, it is only represented by 28% of all the arthropods present in the island. This goes in agreement with the pattern observed in the Canary Islands, but in contrast to the Azores archipelago (Silva et al., 2008). Regarding reptiles, Madeira has one endemic species, *Teira dugesii*, and three introduced species present, one blind snake, *Indotyphlops braminus*, and two geckos, *Hemidactylus mabouia* and *Tarentola mauritanica* (Rato et al., 2021; Rato et al., 2015; Silva-Rocha et al., 2016; Silva-Rocha et al., 2018).

FCUP

Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

8

3. *Tarentola mauritanica*

Tarentola mauritanica (Linnaeus, 1758) belongs to the family Phyllodactylidae and is also known as the Moorish gecko. In general, the *Tarentola* genus is considered among the most successfully established introduced groups worldwide due to their ability to perform long distance natural and anthropogenic dispersal, outside of their native geographic range (Mačát et al., 2014; Ortiz Medina et al., 2019). In fact, human translocation and posterior colonization of new areas become so common that, some invasive geckos have now an almost cosmopolitan distribution in tropical and temperate regions (Baldo et al., 2008).

The Moorish gecko in particular, is widespread in North Africa, coastal regions of the Mediterranean Sea and Macaronesian archipelagos (Madeira, Selvagens, Canaries and Cabo Verde) (Vogrin et al., 2017). However, it has been introduced to other distant places, such as the Bahamas, North and South America, due to its frequent association to human settlements and capacity for long-distance dispersals (Carranza et al., 2000; Harris et al., 2004). In the Iberian Peninsula it is distributed mainly in the center, south and east of the territory (Cabana, 2008; Cabana & López-Bao, 2007; Paramos & Ayres, 2007; Pleguezuelos et al., 2002). Specifically, in Portugal mainland, the south is where most populations can be found, although there are several populations spread across the country (Godinho et al., 1999; Harris & Perera, 2008; Malkmus, 2004). *Tarentola mauritanica* is a recent introduction in Madeira. It was first reported by Báez and Biscoito (1993) in a small locality, Garajau, 8 km east of Funchal. Although the current distribution is still unknown, the species has been observed in several other places, as far as 20km from its initial location (Rato et al., 2021), including Porto Santo (Jesus et al., 2008).

It is found in a variety of habitats from sea-level to more than 2300 m above sea level, and it is usually associated to humanized structures (Vogrin et al., 2017).

As an introduced reptile, it might have negative impacts on native species, including predation, competition for food (such as with the endemic lizard *Teira dugesii*), spread of diseases and parasites, which are particularly alarming on islands (Mačát et al., 2014). Thus, more information on the presence of *T. mauritanica* must be gathered to evaluate the potential impacts of its introduction (Mačát et al., 2014; Ortiz Medina et al., 2019). Nevertheless, according to the only study on competition interactions over spatial niche utilization carried out so far with *T. mauritanica* (Lisicic et al., 2012), the authors concluded that the presence of sympatric populations of the Moorish gecko induced a spatial shift on *Hemidactylus turcicus*, pressing the latter to explore different micro-habitats.

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

9

Dietary studies can be used as a mean to explore the impacts on native invertebrate fauna. So far, those studies were based mainly on scat morphological examinations from Spanish populations (Gil et al., 1994; Hódar et al., 2006). Here, the authors concluded that the most important groups comprising the Moorish gecko's diet are coleopterans, arachnids, hymenopterans, lepidopterans and insect larvae. On the other hand, the prey items such as oligochaetes, mites, springtails, aphids, isoptera, reptiles and fruits, were scarce (Gil et al., 1994; Hódar & Pleguezuelos, 1999).

Tarentola mauritanica is known as a voracious crepuscular mosquito predator (Martínez Rica, 1974), although some populations can be active during the day (Fulgione et al., 2019). Similarly to other geckos such as *Gekko gecko,* they are also very well-adapted to life in urban residential environments, where the contact between vector-borne diseases and human is most frequent, keeping insects, namely, mosquito populations under control (Weterings et al., 2019).

In Madeira, the prey items composing the diet of *T. mauritanica* are still unknown, as well as how the composition changes among distinct habitats across the island. Therefore, the unravelling of this species' diet is rather relevant since trophic studies offer unique ecological insights, particularly important for insular systems, where predator-prey networks often include numerous endemic species. Furthermore, diet assessments might identify the presence of additional introduced taxa not yet recorded or reveal if they are consuming species identified as agricultural pests or acting as vectors to diseases.

4. Pests and Vector-borne Diseases

In Madeira Island, the information regarding agricultural pests is generally poor. Most of the bibliography available is concentrated on specific cultures such as banana plantations and vineyards, due to its enormous social, economic and landscapes importance. Therefore, introduced arthropod pests affecting chestnuts, vineyards, and fruits such as banana and citrines are yearly reported to the island (Aguiar, 2009; Bella & Aguiar, 2020; Straw & Williams, 2013).

Madeira's characteristic mild climate and fertile soils gave enough reasons for the first settlers in the fourteenth century and their descendants after that, to successfully introduce in the island all kinds of plants from all over the world. Since most of what is consumed by its inhabitants and visitors is imported, the intense influx of plants, not also to be planted but also to be readily consumed, leads to a considerable number of introduced arthropod pests entering, and consequently permanent establishment in the island (DeBach, 1964). Moreover, another important aspect regarding the settlement of

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

10

new pests is the frequent absence of specific natural enemies and the period necessary for the indigenous enemies to adapt to the new guests promoting the rebalance of the ecosystems (Aguiar, 2009).

Banana plantations represent 20% of the local agricultural production. Main cultivars used are Dwarf Cavendish, Robusta, Grande Naine Israel and Gal. The planted area occupies approximately 850 hectares and is concentrated mainly in the southern coast, where they benefit from a temperate to subtropical environment with average temperatures ranging from 16º to 22ºC (Figure 2) and a relative humidity of 71%. The pests affecting bananas include insects, mites, nematodes, and fungi. *Cosmopolites sordidus*, *Thrips exilicornis* and *Opogona sacchari* are the insects causing more damage to these plantations in terms of quality, quantity, frequency, affected area and the efficacy of the control measures (Ribeiro et al., 2009).

The control strategies taken against banana pests and vector-borne diseases in Madeira are based exclusively on chemical treatments, however, they are still not effective against some invertebrates such as the banana flower thrips, *Thrips florum*. Therefore, the study of alternative control methods, such as natural insecticides and biological control is urgent.

Regarding vector-borne diseases, Dengue fever is the most globally widespread mosquito-borne viral disease, transmitted from an infected human to an *Aedes* mosquito, commonly *Aedes aegypti*, endemic to the tropics and subtropics, predominantly in urban and semi-urban areas (Alves et al., 2013; Auerswald et al., 2019). This species was first reported in Madeira in 2005, and an outbreak of dengue fever occurred between early October 2012 and late February 2013, resulting in over 2100 cases (Alves et al., 2013). In fact, the number of dengue fever cases has been increasing all over the world, mostly due to an increase in human population density, urbanization and climate change (Hales et al., 2002; Zuur et al., 2009).

The most densely urbanized and inhabited regions where there is a more extensive and compact distribution of areas, such as Funchal, are more suitable for the presence of the *Aedes* species. Therefore, high population and housing density, together with the high temperatures and the numerous agricultural plots (e.g. banana plantation), interspersed with family houses, can explain the predisposition of this species to the southernmost part of the island (Santos, 2018).

Mosquito populations are naturally regulated by a range of predators, generally associated with residential environments, where the contact between dengue vectors and humans is most frequent. A recent research by Weterings et al. (2019) revealed that house geckos (*Hemidactylus* and *Gehyra* spp.) have played a role in the rise of dengue

in tropical countries, especially in those where they were introduced and naturalized, since they reduce the number of spiders, known to be important predators of *Aedes* mosquitoes (Strickman et al., 1997). Therefore, predator-prey interactions drive community dynamics. Consequently, the application of environmental control measures, including the role of ecological interactions by investigating food web trophic cascades are fundamental tools in disease control (Weterings et al., 2018).

5. DNA Metabarcoding

Molecular scatology is a genetic technique that started to be used in 1990. Since then, most studies related to animal ecology have been relying on faecal samples as non-invasive genetic sources to estimate population's phylogenies, home range and population sizes (Kohn & Wayne, 1997). First DNA-based diet studies consisted in PCR amplification using taxon-specific primers. The later introduced method, Sanger sequencing, facilitated the process of isolating food DNA sequences from a multispecies mixture of faecal DNA, and the sequences were then identified through a reference database using a taxon-specific identification system based on standardized DNA region sequences (DNA barcoding). However, despite showing higher resolution, this approach was expensive and time consuming, so High-Throughput Sequencing (HTS) using Next Generation Sequencing (NGS) became the most used and efficient way for diet analyses.

DNA Metabarcoding is an emerging molecular tool towards understanding biodiversity systems that was originally developed mainly by microbiologists (Coissac et al., 2012; Sogin et al., 2006). It heavily relies on NGS methodology, capable of generating millions of DNA sequence reads. Nowadays, it is widely used for fauna and flora across the globe, assessing and monitoring of natural and restoring biological communities aboveground and belowground, as well as gene expression analysis as a measure of genetic resilience of restored populations (Williams et al., 2014).

This technique is able to match taxonomic unknown specimens to the DNA barcode (short, standardized DNA sequence) of an organism in order to identify them, performing comparisons with a library of DNA barcodes of known taxonomy (Bell et al., 2016; Deiner et al., 2017; Wilson et al., 2019). It involves the mass collection of specimens that are homogenized, the genomic DNA extracted, mass-PCR amplified for a barcode gene of interest, and sequence on machines able to separate out individual DNA molecules (Williams et al., 2014).

A high taxonomic resolution may be expected when using taxon-specific primers, and markers with shorter fragment sizes provide more accurate results regarding prey

FCUP

Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

12

detection, therefore, in faecal metabarcoding the most used barcoding markers are <300 bp (Kartzinel & Pringle, 2015). The most common region used in animal DNA barcoding is the mitochondrial Cytochrome c oxidase subunit I (COI), a particularly useful metabarcoding gene, as it is nearly universal across animals, but substitutions allow for species-level differentiation (Hebert et al., 2003), which makes large numbers of sequences accessible as a reference database for DNA metabarcoding.

Despite some lack of consensus for some groups, the large amount of sequence data in public databases, such as BOLD and GenBank, makes it beneficial to use COI markers. Furthermore, a broader taxonomic coverage and higher taxonomic resolution was observed in metabarcoding by multiple markers in comparison to single markers (Wilson et al., 2019).

A recent review by Ando et al. (2020) revealed that the number of annual publications in international journals, regarding faecal metabarcoding, has been gradually increasing over the past ten years, and more than doubled from 2017 to 2018. Moreover, it also shows that mammals are the predominant group with more than half of the annual publications. On the other hand, invertebrates are the least studied group, which might be related to the difficulties in extracting DNA from small amounts of faecal samples as well as in finding primers capable of amplifying the diet items without the simultaneous amplification of the host's DNA.

Main challenges of Metabarcoding include handling and analyzing the data generated, and apply it in practical outcomes, as well as minimizing false-positive errors in a diagnostic context due to the risk of cross-contamination and technical error, which is particularly common in many ecological studies. Another obstacle is the fact that this method only provides taxa occurrences in the samples and not their relative abundances. A final issue with this method is that the sequences recovered may be taxa-specific biased, influenced by the markers used in the amplification process. Thus, the appropriate use of controls, replication and validation is mandatory in metabarcoding approaches (Deagle et al., 2018; Piper et al., 2019).

6. Research objectives

The main goals of this project include the assessment on how the composition of the diet changes across different habitats as well as to assess if the Moorish gecko is consuming: i) endemic arthropod species of particular conservation concern; ii) economically relevant agricultural pests; iii) arthropods that may act as vectors of human diseases, such as *Aedes aegypti* transmitter of dengue fever. This study will reveal if the introduced populations of *T. mauritanica* in Madeira can, through the consumption of

FCUP 13
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

problematic arthropods, have a positive impact on human health and on agriculture, or if they may have harmful effects on the island's endemic arthropod fauna. Ultimately, the screening of this voracious insectivore gecko's diet might also reveal the presence of several invertebrate species still unknown to Madeira.

Methodology

1. Study Area

Fieldwork took place in Madeira Island, during September 2020. Data was collected in six localities across the southern coast of the island, more precisely in Ribeira Brava, Câmara de Lobos, Praia Formosa, Funchal, Caniço and Caniçal (Figure 5). Sampling was performed at night (20 PM-1 AM) in order to match with the peek crepuscular activity pattern of the species, although some populations can also be active during the day (Fulgione et al., 2019).

Geckos were captured by hand or noose and received a belly massage to release fresh pellets. All faecal samples were preserved in 96% ethanol until the extraction was performed. A total of 150 *T. mauritanica* (135 adults; 15 juveniles) pellets were collected during field work: 31 in Câmara de Lobos; 23 in Caniçal; 24 in Caniço; 23 in Funchal Center; 23 in Ribeira Brava and 26 in Praia Formosa. Also, individuals were sexed either by inspecting for the presence of hemipenes or by placing a white light against the skin, dorsally to the tail base (Atzori et al., 2007) (males: 55; females: 80). Additionally, the snout-vent length (SVL) of each individual was measured to the closest 0.01 mm using an electronic caliper, and also the weight. All measurements were taken by the same person (C. Rato) to eliminate inter-observer error. In order to build a genetic library of the available prey invertebrates, nocturnal insect specimens were collected using mercury vapor traps in each sampling site, which were then transported to the Museum of Natural History in Funchal where taxonomic identification was assessed by the colleague specialist Ysabel Gonçalves.

Sampling permits and protocols were approved by the Madeiran delegation from the Instituto para a Conservação da Natureza e Florestas (ICNF).
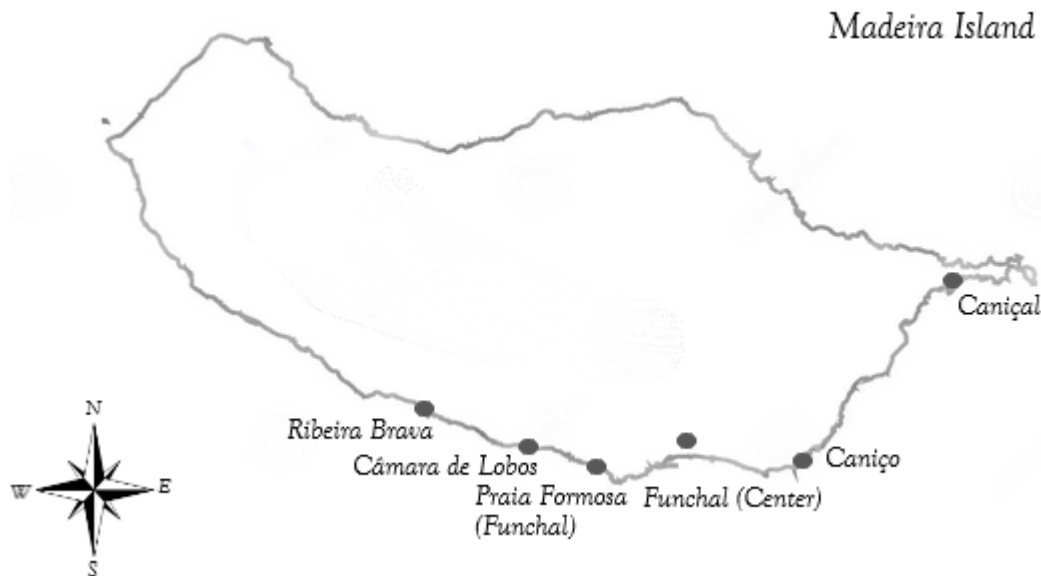
Figure 5: Madeira map representing the six sampling sites.

2. DNA Extraction and Amplification

2.1. Faecal samples

From the 150 *T. mauritanica* pellets collected in the different sites, only 142 (130 adults; 12 juveniles), corresponding to 58 males and 72 females, were chosen to be extracted, since the remaining ones had only residual amount of faecal material. First, major part of the ethanol was taken out from the tubes using appropriate micropipettes, followed by a dehydration in an incubator at 50°C to remove the remaining ethanol (Pinho et al., 2018). After that, DNA from approximately 200 mg of each *T. mauritanica* faecal sample was extracted using the Stool DNA Isolation Kit (Norgen Biotek Corp., Thorold, ON, Canada) following the manufacturer's guidelines. Two elutions of 50 µL each from each pellet, including 8 negative control samples, were obtained and stored at -20°C in 96-well plates until amplification. A short fragment (205 bp) of the mitochondrial cytochrome c oxidase subunit I (COI) was amplified by Polymerase Chain Reaction (PCR) using six N' combined Fwh2 primers (Vamos et al., 2017), which have been confirmed as the best primer pair for the study of insectivorous diets (Tournayre et al., 2020). The PCR reaction was comprised by 5 µL of QUIAGEN Multiplex PCR Master Mix (Quiagen, Crawley, UK), 0.3 µL combination of 6 Forward primers, 0.3µL combination of Reverse Primers, 3.4µL of ultra-pure water, and 2.5 µL of DNA extract from the first elution. Replicates were made performing this reaction three times per

feacal sample, in order to obtain as more information as possible from each scat. Cycling conditions used consisted in an initial denaturing at 95°C for 15 min, followed by 45 cycles of 95°C denaturing for 30s, annealing at 52°C for 45s, extension at 72°C for 20s, and a final extension at 60°C for 5min. All amplifications were checked by running the PCR products in 2% agarose gels.

2.2. Insects

Insect extractions from 56 different individuals were performed using the E.Z.N.A. Tissue DNA Purification Kit, according to the manufacturer's instructions. Tissue samples consisted in one or two legs, depending on the specimen size. With this protocol, two elutions of 100 µL were obtained, and stored at -20°C until amplification.

PCR amplifications were conducted using two different primer sets, targeting separate regions of the mitochondrial cytochrome c oxidase subunit I (COI); LC with 350bp (Folmer et al., 1994; Geller et al., 2013; Leray et al., 2013), and the B2 with 420bp (Elbrecht & Steinke, 2019) in order to create the Folmer Fragment (658bp), especially relevant for future research related with the captured insects. Thus, for each fragment and each insect species, different pairs of primers were combined depending on their amplification success (Supplementary Table 1).

PCR reactions were carried out in 10 µL volumes, that included 5 µL of QUIAGEN Multiplex PCR Master Mix (Quiagen, Crawley, UK), 0.2 µL of each primer, 3.6 µL of ultra-pure water, and 1 µL of DNA template. Regarding the LC fragment, most of the species amplified using the primer set FwhF1-NGS/Ind_C_R under the following cycling conditions: initial denaturation at 95°C for 15 min, 40 cycles of denaturing at 95°C for 30s, annealing at 46°C for 90s, extension at 72°C for 45s and final extension at 60°C for 10min. For the B2 fragment, the BF2/BR2 was the pair of primers targeting most of the samples under the following conditions: initial denaturation at 95°C for 15 min, 40 cycles of denaturing at 95°C for 30s, annealing at 48°C for 60s, extension at 72°C for 30s and final extension at 60°C for 10min. All amplifications were checked by running the PCR products in 2% agarose gels.

3. Library Preparation

This process consisted in an initial, optional, PCR Clean-Up to remove free primers and primer dimers, followed by an Indexing PCR needed to properly identify each amplified product. Next, a second PCR Clean-Up is performed to eliminate primer dimer generated by the previous step. After that, the library is quantified, normalized and pooled in equimolar volumes, transferred to the TapeStation to assess for quality control, and finally, validated through qPCR.

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island | 16

First PCR Clean-Up was performed in both faecal and insect samples, using the Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA), except for the B2 fragment, since it did not show any signs of primer dimer. This cleaning was performed using 2.8 µL of ultra-pure water, 7µL of 2x Kapa HiFi, 0.7 µL of each Index (P7/P5), and 2.8 µL of cleaned DNA. Index PCR required an initial denaturation of 95°C for 3min, following 9 cycles of 95°C for 30s, annealing at 55°C for 30s, extension at 72°C for 30s, and a final extension of 72°C for 5min. Second PCR Clean-Up was performed under the same conditions as the first one.

Succeeding the mentioned steps, all indexed samples (diet and insects) were quantified using Epoch, and Nanodrop for confirmation, followed by a normalization to 20 nM. Then, samples were pooled according to the replicates, in the case of faecal samples, and to each fragment concerning the insects. The final pool, diluted to 4 nM, was tested for quality control in the TapeStation, and the library was validated using qPCR, which is a highly effective method that only quantifies indexed PCR products. All samples were ran, simultaneously, in a MiSeq sequencer (Illumina) for 2 x 250 bp target length and approximately 39 hr, using a MiSeq Reagent Kit v2 (Illumina, San Diego, CA, USA) (Figure 6), generating an expected average of 25.000 paired-end reads per replicate, and 3.500 paired-end reads for both LC and B2 fragments.
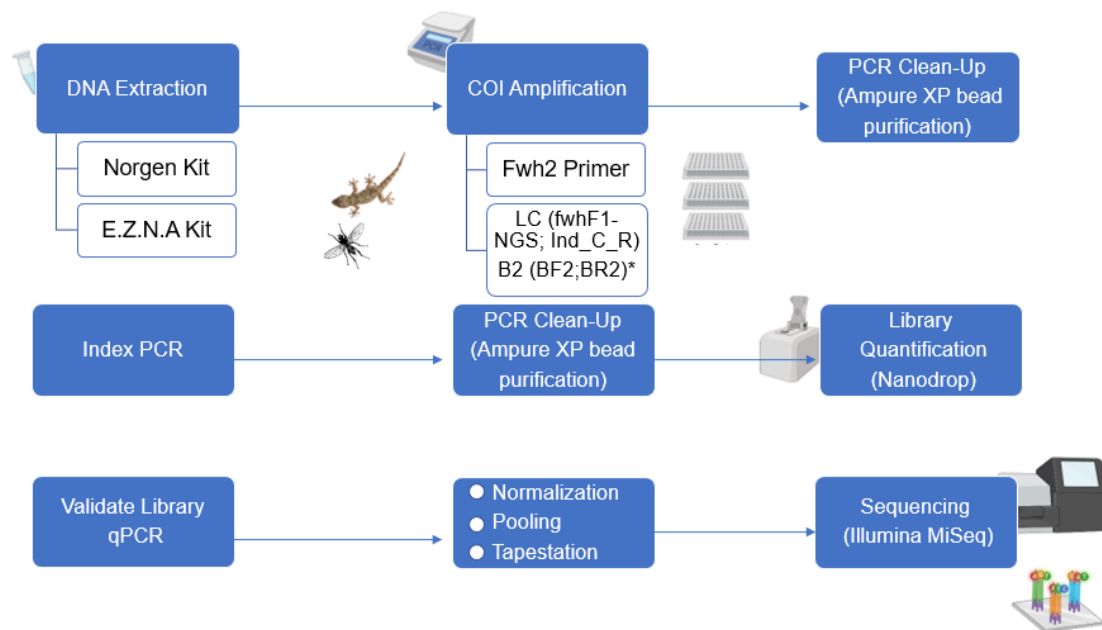
Figure 6: Scheme showing all laboratory process performed in this study. *Most used primers for these fragments (Supplementary Table 1).

FCUP 17
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

4. Bioinformatics

Next-generation sequencing (NGS) requires the ability to deal with huge sequence datasets. Both data from *T. mauritanica*'s diet and insect reference were processed using the software packages OBITools 1.2.13 (https://git.metabarcoding.org/obitools/obitools), VSearch 2.17.0 (https://github.com/torognes/vsearch), and PEAR (https://github.com/xflouris/PEAR.git), according to Pinho et al. (2018). OBITools package was designed to analyze NGS data in a DNA metabarcoding context, being able to edit and filter sequences while taking into account taxonomic annotation (Boyer et al., 2015). On the other hand, VSearch is a versatile open-source tool for processing and preparing metagenomics, including commands for pairwise alignment, clustering by similarity, chimera detection and dereplication (Rognes et al., 2016). PEAR (Paired-End Read Merger) is a highly optimized, memory-efficient and ultrafast pair-end read merger, that can merge millions of paired-end reads within a couple of minutes.

First, reads were counted and moved to a raw data file using command "grep". Forward and reverse sequences were aligned using PEAR, where base pairs with less than 26bp of overlapping quality were rejected. Then, each assembled read was assigned to its corresponding marker combination, using OBITools (command "ngsfilter"). After this step reads were once more quantified with the "grep" command, to check for successful rates of the previous procedure. Reads were then dereplicated into unique sequences (command "obiuniq"), since it is more convenient to work with sequences instead of reads. At last, sequence cleaning was performed differently according to the corresponding experiment. Diet samples were cleaned using VSearch, where sequences not having between 202bp and 208bp were discarded from the dataset, since nuclear copies, bacteria and unidentified sequences are mostly found outside this interval. Also, sequences with less than 8 reads and potential errors such as singletons resulted from PCR malfunctions were excluded.

Concerning the insects, OBITools (command "obiclean") was used to remove PCR/sequencing errors or chimeras. First, to get a statistical approach on the number of haplotypes and reads, the command "obistat" was used and a threshold established. Based on the previous observation, all haplotypes with less than 6 reads were removed from the dataset. Also, haplotypes from samples with less than 100 reads in total (samples *Agromyza sp.* and *Tipula sp.* of fragment B2 in this case) were all maintained to avoid losing any information (command "obigrep"). Then, to split the files into unique files per sample, the command "obisplit" was used. LC and B2 fragments were assembled and aligned with Geneious Prime 2021.1.1 (https://www.geneious.com).

FCUP | 18
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Both LC and B2 fragments from the insect library dataset and diet sequences were taxonomically assigned using both the Nucleotide BLAST from NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch &LINK_LOC=blasthome) and BOLD (Barcode of Life Data System) (https://www.boldsystems.org/index.php/IDS_OpenIdEngine) online databases. Blast is a program that compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches.

Besides that, the obtained dietary sequences were also compared with the insect reference samples. However, data from insect reference was not used for further analysis since in many cases, there was a mismatch between the morphological species identification with the online taxonomic databases. Additionally, the similarity percentages were higher between the generated sequences and the corresponding OTUs (Operational Taxonomic Units). The taxonomical level assignment was performed according to the following similarity percentage: on the class level if below 90%; on the family level if between 90 and 95%; and on the genus or species level if higher than 95% similarity. In case of match with multiple genus or species, OTUs were assigned taking into account the species known to be present in Madeira. After this step, any reads identified in the negative controls were subtracted from other samples (Evans et al., 2021), and each replicate was assigned to the corresponding reads and haplotypes. Only haplotypes present in at least two of the three replicates were maintained, and singletons (haplotypes with only one read) were discarded.

Following this identification, the haplotypes with high probability of occurring from lab contaminations, such as humans (*Homo sapiens*) and pig (*Sus scrofa*), were not considered for further analysis.

5. Statistical Analyses

All statistical analyses were performed in R v. 4.1.0 (R Core Team, 2021) to assess both diet richness and composition of each scat sample. Each faecal sample was assigned to its corresponding information, namely locality, sex, SVL and weight.

In order to achieve a better understanding of the data, two different treatments were executed. The first one takes into account the proportion of total reads (or relative read abundance: RRA) retained in the refined dataset, and the second encompasses sample counts (or frequency of occurrence: FOO), which represents the amount of times a prey item is present in a faecal sample.

Taxon richness was calculated based on the count of each prey taxon detected, using Microsoft Excel. Given that the data was not normally distributed (Shapiro-Wilk

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

19

normality test: W = 0.94185, *P* < 0.001), a Generalized Linear Model (GLM) with a Poisson distribution was performed, using the function "lmer" from the package lme4 v. 1.1-27.1 (Bates et al., 2014). This function was implemented to compare the richness of prey communities among the different variables. Since this model considers all variables as independent, SVL and weight were tested for correlation coefficients using the function "lm" of the stats package v. 3.6.2 (R Core Team), showing effects of correlation (*r* = 0.5675) (Nakagawa & Cuthill, 2007). They were then aggregated into a single variable, Body Mass Index (BMI), which takes both previous variables into consideration. This variable was calculated according to the (weight/SVL^2) formula, previously used for Ptyodactylus geckos by Sion et al. (2021).

Statistical significance of the GLM model was tested with a likelihood ratio test, using the function "anova" from the package car v.3.0-12 (Bates et al., 2007). Besides, a boxplot of the richness, obtained from the six localities, was performed to find more information on the variability and dispersion of the data. Also, a multiple comparison test using the function "glht" from the multcomp package v. 1.4-17 (Hothorn et al., 2008) was made to check among which localities the significant differences were concentrated. This function is based on simultaneous tests for general linear hypotheses and multiple comparison of means using Tukey contrasts.

The package iNEXT v. 2.0.20 (Hsieh et al., 2016) was used to perform rarefaction and extrapolation curves in order to ensure that sex, SVL, weight and sampling locations had no impact on the differences in prey taxon richness. The "iNEXT" function was run incidence frequencies for prey taxa with 250 samples, 100 knots, 1000 bootstraps and 95% confidence intervals. The "estimateD" function was then used to perform both sample size-based and coverage-based comparisons within each category, with 95% confidence intervals and 95% sample coverage (Harper et al., 2020). Despite that, rarefaction and extrapolation curves were performed for SVL, weight and locality, paired with the sex variable in order to give a better look on the possible disparities among sexes.

Due to the high number of taxa, both the analyses and the corresponding results are presented according to families. To compare family composition among the above-mentioned variables, the read count data were first converted to presence/absence using Microsoft Excel, based on the records of prey taxa in each sample. Then, a Jaccard distance matrix was calculated using the "ADONIS" function from package vegan v. 2.5-6 (Oksanen et al., 2012), to mitigate potential taxon recovery bias, and tested statistically using permutational multivariate analysis of variance (PerMANOVA). The null hypothesis of this test states that differences among the centroids in the dataset are as similar in

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

20

size as if they had been obtained under permutation, randomly allocating individual sample units to the groups (Anderson & Walsh, 2013). Concerning this study, it was used with the aim of comparing diet composition among the different sites. To achieve a reliable conclusion, PERMDISP was also performed to make sure that the differences observed in the previous test are not due to unequally dispersed values across the different groups, as it evaluates the homogeneity of multivariate dispersions (Anderson, 2006).

In this analysis, a binomial method (presence/absence) and 9999 permutations were considered, concerning the nature of the data. Pre-defined values used for size and weight effects were converted into categories. In terms of size, they were grouped by the categories Small (50.5 – 60.9 mm), Medium (60.9 – 71.3 mm) and Large (71.3 – 92.1 mm). On the other hand, the individuals were assigned to the following weight categories: Light (1.89 – 4.49 g), Middle weight (4.49 – 7.09 g), Light heavy (7.09 – 9.69 g), and Heavy (9.69 – 22.69 g). Afterwards, a Principal Coordinates Analysis (PCoA) was produced to visualize similarities or dissimilarities of the data, including possible outliers.

In order to assess possible differences among localities, a multilevel pairwise comparison was performed using the "pairwise.adonis" function from the pairwiseAdonis package v. 0.4 (Martinez Arbizu, P., 2020) . This analysis compares the six localities in pairs, checking for each pair if there are significant differences. Finally, considering the previous results, a similarity percentage analysis was performed, also using the vegan package, function "SIMPER", a test that allows to distinguish the contribution of each family between different locations.

Since there were collected and analyzed adult and juvenile individuals, it is also interesting to evaluate if there are any differences between both, even though weight and SVL information is not specified for juvenile individuals. As a result, this data received a distinctive statistical analysis. Following the pattern previously made, the analysis started performing a GLM with a Poisson distribution, using the function "lmer" from the package lme4 v. 1.1-27.1 (Bates et al., 2014), followed by a boxplot of the richness calculated by stage, to find more information on the variability and dispersion of the data. Richness and composition analyses were then performed through PerMANOVA and PerMADISP to assess if there were differences between stages. Afterwards, the iNEXT package (Hsieh et al., 2016) was once again used to perform rarefaction and extrapolation curves for this variable, in the same conditions described for the other variables. Lastly, a similarity percentage analysis was also performed to

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

21

evaluate the contribution of each prey taxa to the differences between stages. All figures were produced using the package ggplot2 v. 3.3.1 (Villanueva & Chen, 2019).

6. Calculation of primary production

Data from remote sensing, such as the normalized difference vegetation index (NDVI) is often used as proxy to quantify for primary productivity (Bailey et al., 2004). A recent study from Fernández-Tizón et al. (2020) supports the applicability of NDVI as a suitable habitat-specific proxy for insectivore prey availability for higher trophic levels during spring. Remote sensing data was downloaded from the Sentinel satellite, part of the European Space Agency's Copernicus Programme (https://scihub.copernicus.eu/). Calculation of NDVI per locality, was performed using the software QGIS 3.16 Hannover (Qgis Development Team, 2020), using the raster calculator to subtract values of the red band from the Near-infrared (NIR) band, then dividing it by the sum of the Red and NIR bands. Finally, a regression model between species richness and NDVI per locality was calculated using the "lm" function from the stats package v. 3.6.2 (R Core Team) to evaluate the level of correlation among the variables.

Results

1. Data filtering

The libraries generated around 13 million raw sequence reads, that were reduced to 10867717 counts by trimming, removal of chimeras, redundancy via clustering, and length filter application in OBITools. Contamination from different sources (12%) was observed both in the negative control extraction samples and PCR controls of faecal samples. This was solved after the application of the false positive threshold, during the bioinformatic analysis. Also, only OTUs present in at least two of the three replicates were considered. Before the threshold, 1095 haplotypes were detected from the 142 faecal samples, of which 19% was fungi, mainly ascomycota and basidiomycota. In the final dataset all analyzed faecal samples presented arthropod items in their diets. An expected amount of *T. mauritanica* was observed as well, corresponding to 8% of the counts. After the threshold application and replicates filtering, the gecko's diet final dataset consisted in 6861811 reads encompassing 250 taxa (93 assigned to species-level). When compared to Madeira's terrestrial checklist by Borges et al. (2008), as well as Aguiar et al. (2013) and also to Fauna Europaea (De Jong et al., 2014), 72 out of these 93 species are already described to Madeira Island.

Most of the Moorish gecko's diet consists of introduced species to Madeira (47%), and a quite small percentage to endemics of the island (6%) (Table 1).

Table 1: List of the 93 OTUs (species level) found in *Tarentola mauritanica's* diet, after data filtering, alongside their presence in Madeira and Colonization Status in the island (according to Borges et al. 2008 and Aguiar et al. 2013). Introduced represents taxa introduced by man that became naturalized; Endemic represents Madeiran endemics, taxa that only occur naturally in the islands of the archipelagos of Madeira and Selvagens; Native represents taxa that occur naturally in the archipelagos of Madeira and Selvagens, but can also be found in nature outside of Macaronesia; MAC represents Macaronesia endemic species; and "-" stands for species whose colonization status appear in blank. The presence and status of species *Anatrachyntis badia* and *Xystrologa grenadella* were confirmed by António Aguiar (personal communication).

OTUs	Scientific ID	Present in Madeira	Status
otu43	*Armadillidium vulgare* (Latreille, 1804)	No	
otu849	*Cameraria ohridella* (Deschka & Dimic, 1986)	No	
otu15	*Cerobasis guestfalica* (Kolbe, 1880)	No	
otu2472	*Euborellia arcanum* (Matzke & Kocarek, 2015)	No	
otu6538	*Frankliniella tritici* (Fitch, 1855)	No	
otu182	*Gryllodes supplicans* (F.Walker, 1859)	No	
otu685	*Holocnemus pluchei* (Scopoli, 1763)	No	
otu1762	*Lasius flavus* (Fabricius, 1782)	No	
otu231	*Liposcelis entomophila* (Enderlein, 1907)	No	
otu81	*Liposcelis paeta* (J.V.Pearman, 1942)	No	
otu195	*Megaselia striolata* (Schmitz, 1940)	No	
otu1835	*Oligotoma saundersii* (Westwood, 1837)	No	
otu267	*Pheidole moerens* (Wheeler, 1908)	No	
otu3384	*Plagiolepis pallescens* (Forel, 1889)	No	
otu5644	*Plecia nearctica* (Hardy, 1940)	No	
otu1147	*Porcellionides pruinosus* (Brandt, 1833)	No	
otu13	*Porcellionides sexfasciatus* (Budde-Lund, 1885)	No	
otu6875	*Psammotettix confinis* (Dahlbom, 1850)	No	
otu9840	*Steatoda triangulosa* (Walckenaer, 1802)	No	
otu11	*Anatrachyntis badia* (Hodges, 1962)	Yes	Introduced
otu26	*Xystrologa grenadella* (Walsingham, 1897)	Yes	Introduced
otu858	*Amegilla maderae* (Sichel, 1868)	Yes	Endemic
otu5394	*Brachyunguis tamaricis* (Lichtenstein, 1885)	Yes	Introduced
otu3948	*Laodelphax striatellus* (Fallén, 1826)	Yes	Native
otu94	*Loboptera decipiens decipiens* (Germar, 1817)	Yes	Introduced
otu8	*Aiolopus thalassinus thalassinus* (Fabricius, 1781)	Yes	Native
otu422	*Apatema fasciata* (Stainton, 1859)	Yes	MAC
otu1196	*Aphis gossypii* (Glover, 1877)	Yes	Native
otu1397	*Apis mellifera* (Linnaeus, 1758)	Yes	Introduced
otu315	*Argiope trifasciata* (Forskål, 1775)	Yes	?
otu7941	*Beosus maritimus* (Scopoli, 1763)	Yes	Native
otu30	*Blastobasis desertarum* (Wollaston, 1858)	Yes	MAC
otu46	*Blastobasis maroccanella* (Amsel, 1952)	Yes	Native
otu138	*Cadra figulilella* (Gregson, 1871)	Yes	Introduced

FCUP 23
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

OTUs	Scientific ID	Present in Madeira	Status
otu111	*Caracollina lenticula* (Michaud, 1831)	Yes	-
otu698	*Caradrina clavipalpis pinkeri* (Kobes, 1975)	Yes	Endemic
otu23	*Cardiocondyla emeryi* (Forel, 1891)	Yes	Introduced
otu20	*Chrysomya megacephala* (Fabricius, 1794)	Yes	Introduced
otu331	*Cinara cupressi* (Buckton, 1881)	Yes	Introduced
otu47	*Clogmia albipunctatus* (Williston, 1893)	Yes	Native
otu44602	*Condica capensis* (Walker, 1857)	Yes	Native
otu16104	*Cryptotermes brevis* (Walker, 1953)	Yes	Introduced
otu767	*Ctenolepisma longicaudata* (Escherich, 1905)	Yes	Introduced
otu4879	*Culex theileri* (Theobald, 1903)	Yes	Native
otu142	*Viteus vitifoliae* (Fitch, 1855)	Yes	Introduced
otu240	*Drosophila buzzatii* (Patterson & Wheeler, 1942)	Yes	Introduced
otu2267	*Duponchelia fovealis* (Zeller, 1847)	Yes	Introduced
otu102	*Dysdera crocata* (C.L. Koch, 1838)	Yes	?
otu2997	*Eluma caelatum* (Miers, 1877)	Yes	-
otu5590	*Empicoris rubromaculatus* (Blackburn, 1889)	Yes	Introduced
otu40494	*Gymnoscelis rufifasciata* (Haworth, 1909)	Yes	Native
otu33703	*Haploembia solieri* (Rambur, 1842)	Yes	Native
otu148	*Harpalus attenuatus* (Stephens, 1828)	Yes	Native
otu15808	*Harpalus tenebrosus* (Dejean, 1829)	Yes	Native
otu71	*Herpetogramma licarsisalis* (Walker, 1859)	Yes	Introduced
otu13239	*Idaea atlantica* (Stainton, 1851)	Yes	Endemic
otu1755	*Lepisma saccharina* (Linnaeus, 1758)	Yes	Introduced
otu2536	*Linepithema humile* (Mayr, 1868)	Yes	Introduced
otu521	*Liophrurillus flavitarsis* (Lucas, 1846)	Yes	?
otu2303	*Lithobius pilicornis* (Newport, 1844)	Yes	Introduced
otu126	*Macaroeris diligens* (Blackwall, 1867)	Yes	MAC
otu233	*Mangora acalypha* (Walckenaer, 1802)	Yes	?
otu88	*Nezara viridula* (Linnaeus, 1758)	Yes	Introduced
otu1070	*Oinophila v-flava* (Haworth, 1828)	Yes	-
otu7	*Ommatoiulus moreletii* (Lucas, 1860)	Yes	Introduced
otu250	*Opogona omoscopa* (Meyrick, 1893)	Yes	-
otu4641	*Opogona sacchari* (Bojer, 1856)	Yes	Introduced
otu105	*Paidiscura orotavensis* (Schmidt, 1968)	Yes	MAC
otu1026	*Paratrechina longicornis* (Latreille, 1802)	Yes	Introduced
otu10	*Periplaneta americana* (Linnaeus, 1758)	Yes	Introduced
otu145	*Pheidole megacephala* (Fabricius, 1793)	Yes	Introduced
otu497	*Phereoeca allutella* (Rebel, 1892)	Yes	-
otu2959	*Pholcus phalangioides* (Fuesslin, 1775)	Yes	?
otu248	*Pollenia pediculata* (Macquart, 1834)	Yes	Introduced
otu201	*Porcellio dilatatus* (Brandt, 1833)	Yes	-
otu1608	*Pselactus spadix sulcipennis* (Wollaston, 1854)	Yes	Endemic
otu174	*Pyralis farinalis* (Linnaeus, 1758)	Yes	Introduced
otu89	*Reticulitermes grassei* (Cléments, 1978)	Yes	Introduced
otu255	*Rodolia cardinalis* (Mulsant, 1850)	Yes	Introduced

FCUP 24
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

OTUs	Scientific ID	Present in Madeira	Status
otu235	*Sarcophaga africa* (Wiedemann, 1824)	Yes	Introduced
otu19819	*Scotophaeus blackwalli* (Thorell, 1871)	Yes	?
otu17996	*Scutigera coleoptrata* (Linnaeus, 1758)	Yes	Introduced
otu2930	*Sophonia orientalis* (Matsumura, 1912)	Yes	Introduced
otu1195	*Spilostethus pandurus* (Scopoli, 1763)	Yes	Native
otu118	*Spodoptera cilium* (Gueneé, 1852)	Yes	Introduced
otu1952	*Steatoda grossa* (C.L. Koch, 1838)	Yes	?
otu579	*Stomoxys calcitrans* (Linnaeus, 1758)	Yes	Introduced
otu224	*Technomyrmex pallipes* (F. Smith, 1876)	Yes	Introduced
otu260	*Tegenaria pagana* (C.L. Koch, 1840)	Yes	?
otu150	*Tetramorium caldarium* (Roger, 1857)	Yes	Introduced
otu12667	*Thanatus vulgaris* (Simon, 1870)	Yes	?
otu970	*Typhaea stercorea* (Linnaeus, 1758)	Yes	-
otu878	*Uloborus walckenaerius* (Latreille, 1806)	Yes	?

2. Moorish gecko diet

From a total of 250 prey items detected, 7 classes were identified: Arachnida, Chilopoda, Collembola, Diplopoda, Gastropoda, Insecta and Malacostraca. (Supplementary Table 2).

When comparing the obtained species-level list (Table 1) with the insect reference database collected in the six sampling localities, only three species from *T. mauritanica*'s diet match with the 56 prey taxa available: *Apis melífera*, *Beosus maritimus*, *Gymnoscelis rufifasciata* (Supplementary Table 3).

The results obtained from the relative read abundance (RRA) and frequency of occurrence (FOO) approaches are presented below in figures 7 and 8, respectively. As observed, the different treatments revealed slightly different results. Considering the RRA approach (Figure 7), there is a higher percentage of the classes Insecta and Diplopoda, compared to the FOO method. On the other hand, the frequency of occurrence method (Figure 8) presents a greater proportion of the remaining classes of arthropods found in the diet. Additionally, a more detailed view of the species present in the diet plus their corresponding abundances can be seen in Supplementary Figures 1 and 2.

FCUP 25
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island
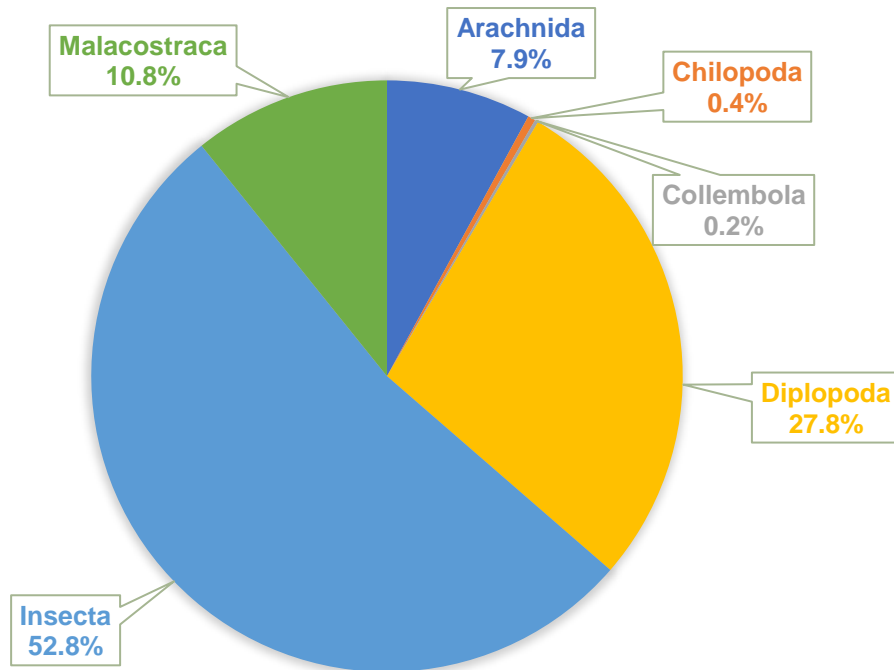
Figure 7: Pie chart showing the proportion of total reads (RRA) retained in the refined dataset with respect to their Arthropoda prey and the proportion of prey reads that belonged to different Arthropoda classes.
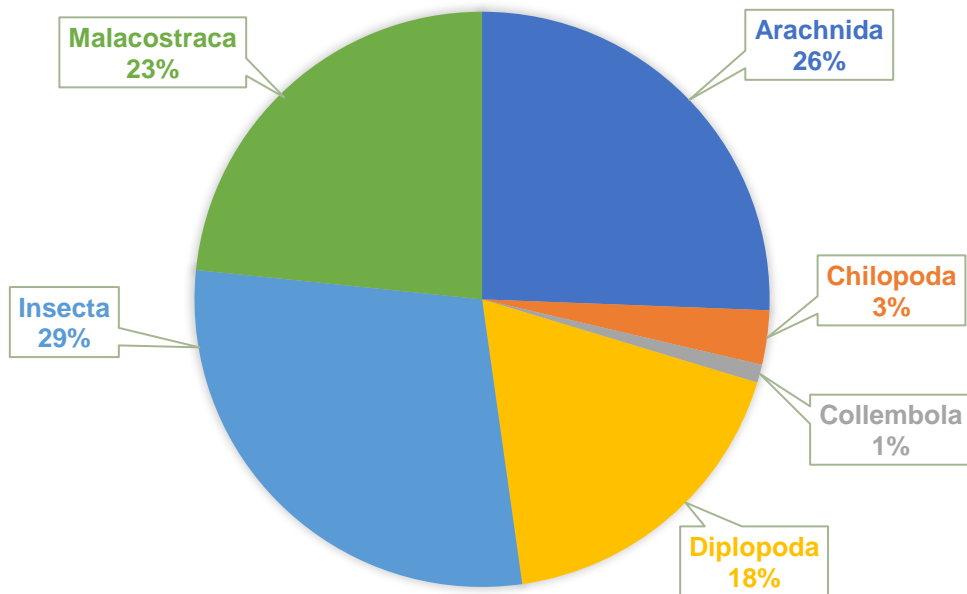
Figure 8: Pie chart showing the proportion of sample counts (FOO) retained in the refined dataset with respect to their Arthropoda prey and the proportion of sample counts that belonged to the different classes..

FCUP | 26
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

3. Statistical Results

Overall, Porcellionidae, Julidae and Formicidae were the most frequent families, presenting higher percentages in all localities, with the exception of Funchal, where Julidae is replaced by Cosmopterigidae. Results on how the different Arthropod families are distributed among the six sampling locations (Figure 9), according to their frequency of occurrence in each faecal sample, demonstrate that Caniço and Praia Formosa appear to be the localities with higher alpha diversity. Similar results were obtained in the boxplot performed (Figure 10).
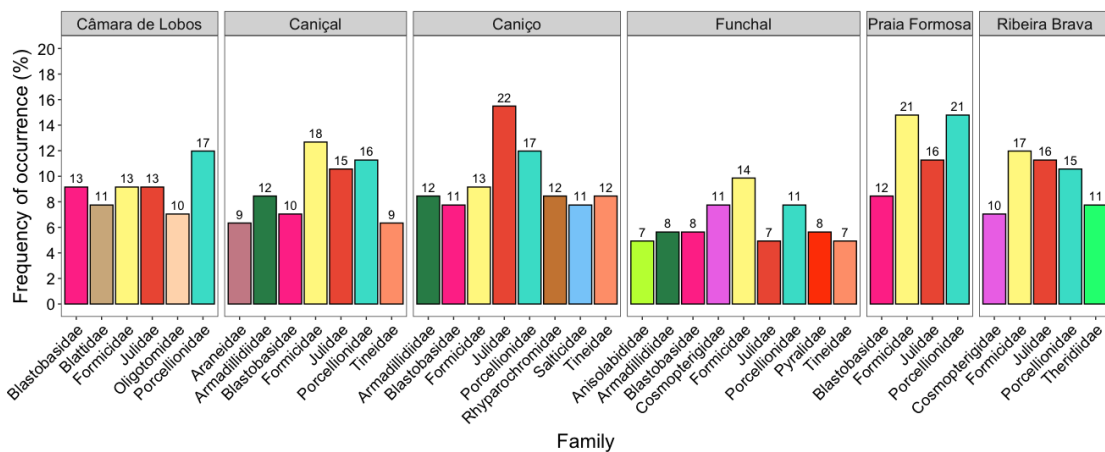
Figure 9: Family distribution across the six localities, according to their frequency of occurrence (%).
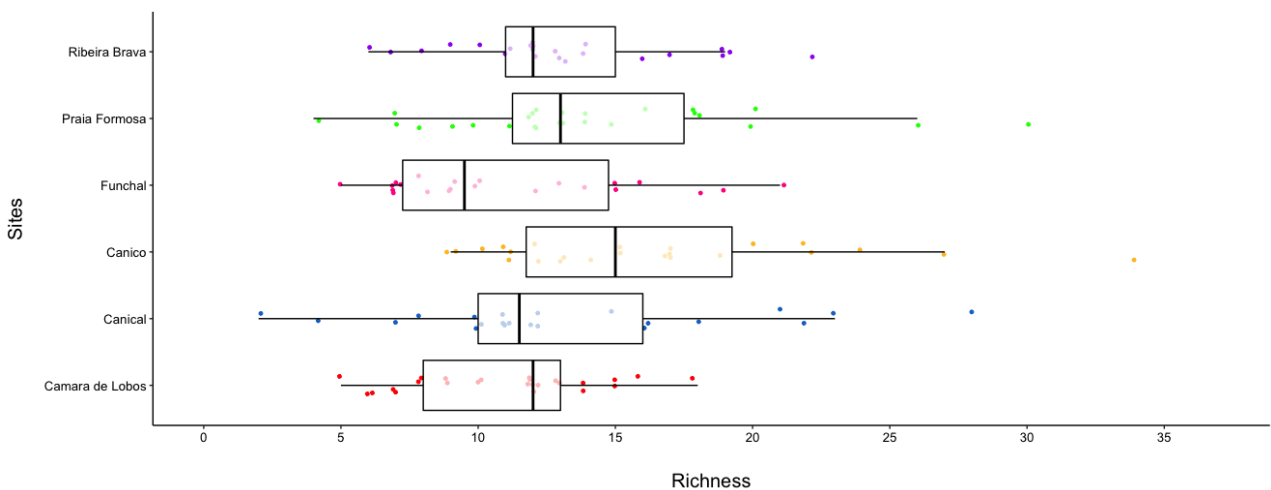
Figure 10: Boxplot showing the number of prey taxa detected in the six localities.

According to the GLM analysis, what seems to be affecting the richness is the locality, being the only variable presenting significant results (χ^2 = 34.865, *p* = 1.601e-

FCUP 27
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

06). On the contrary, sex ($p = 0.2746$) and Body Mass Index (BMI) ($p = 0.2598$) have no effect on the number of prey items consumed by the geckos (Table 2).

Table 2: ANOVA results of GLM for Sex, BMI and Locality variables. LR Chisq stands for Likelihood Ratio Chi-square, Df for degrees of freedom and Pr(>Chisq) for *p-value*.

Variable	LR Chisq	Df	Pr (>Chisq)
Sex	1.194	1	0.2746
BMI	1.270	1	0.2598
Locality	34.865	5	1.601e-06

According to the multiple comparison test (Supplementary Table 4), not all localities seem to be different from each other. The pairs that revealed significant differences were between Caniço and Câmara de Lobos ($p = 0.0012$), Caniço and Caniçal ($p = 0.0419$), Funchal and Caniço ($p < 0.001$) and lastly Funchal and Praia Formosa ($p = 0.0109$). Moreover, Funchal appears to be significantly less diverse than Praia Formosa and Caniço, and the latter seems to be significantly higher than Câmara de Lobos as well as Caniçal. Overall, through this analysis, Funchal appears to be the locality where prey detection was less diverse, and Caniço, on the opposite, presents a consistently higher richness average of prey items.

From the analysis of the rarefaction and extrapolation curves (Figure 11), it is possible to infer that Caniço is the richest sampling locality, with an observed richness (qD) of approximately 138 prey items detected, followed by Praia Formosa, with 112 OTUs. Those differences are not due to disparities in sample size between sites. Second, regarding SVL differences, the smallest geckos present more diverse diets than larger ones, with an observed richness of 177 prey items, in contrast with the 92 detected in larger animals, according to the sample coverage approach. The variable weight followed the same pattern, with lighter individuals presenting higher levels of diet richness, which was not a surprise since those two variables were correlated.

Moreover, as verified in the last curves, females present higher prey taxon richness when compared to males, but those differences are not significant, since there is overlap of the confidence intervals. In this analysis, an 80% sample coverage for all sites was achieved at least. Lastly, the results obtained based on the sex differences were similar to the ones mentioned for each variable independently (Supplementary Figure 3), so no further conclusions can be made.

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island
28
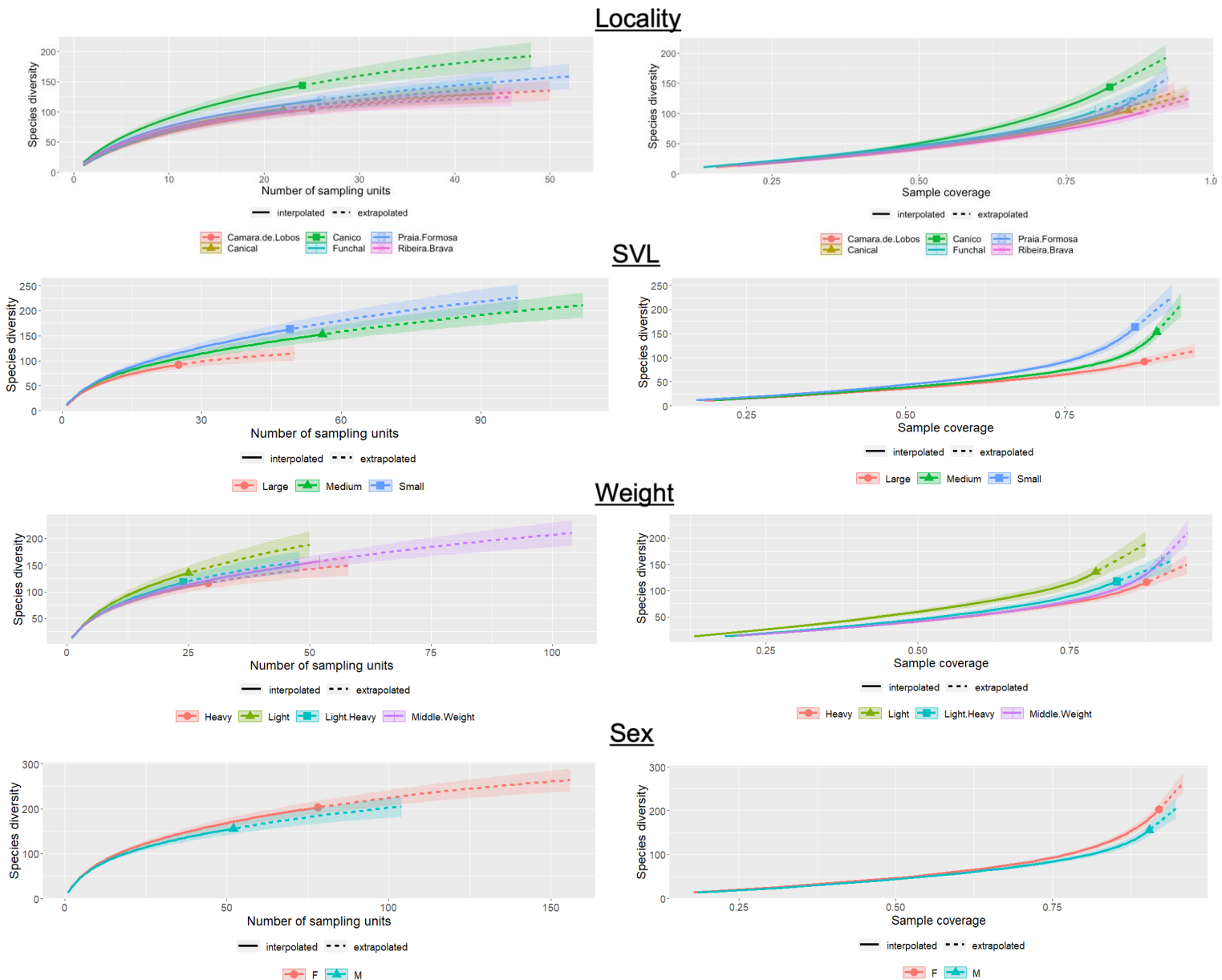
Figure 11: Rarefaction/Extrapolation (R/E) curves for Locality, SVL, Weight and Sex. Obtained according to the number of sampling units (left) and sample coverage (right) using iNEXT package from R.

Concerning composition analysis, significant differences in OTUs composition were observed among localities ($p = 0.0457$) (Supplementary Table 5), and there was no effect of data dispersion on the results (Supplementary Table 6).

From the PCoA analyses (Figure 12) we can verify that in each studied variable, samples were mixed and no groups were formed, which is common when using presence-absence data. This means that diet composition was very similar regarding localities, in either sex, weight or SVL.

FCUP 29
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island
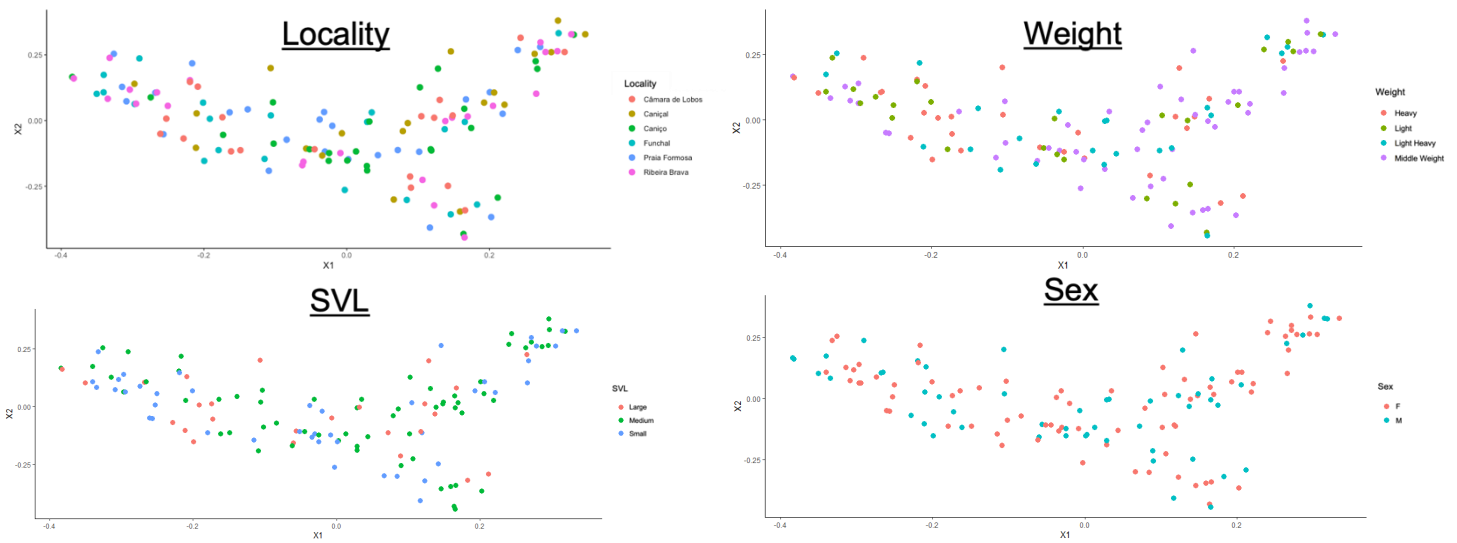
Figure 12: Principal Coordinates Analysis (PCoA) of the variables Locality, Weight, SVL and Sex.

The multilevel pairwise comparison (Supplementary Table 7) led to the conclusion that the pairs Funchal and Praia Formosa, Funchal and Caniçal, Funchal and Caniço as well as Caniço and Ribeira Brava exhibit significant differences among them.

Following the previous results, the similarity percentage analysis revealed that, between Funchal and Caniço as well as Funchal and Caniçal, Julidae is the family that contributed the most to the differences observed for these sites (Supplementary Figure 4). Besides, the differences among Funchal and Praia Formosa are mainly attributed to the Porcellionidae family. Finally, regarding Caniço and Ribeira Brava, the Rhyparochromidae family is the one contributing to the differences (Supplementary Figure 5).

Richness analysis between stages detected no significant differences ($p = 0.2453$) (Table 3). Also, the boxplot (Figure 13) revealed similar richness levels when comparing the number of prey taxa detected in juveniles and adult samples, despite of the sample size discrepancies.

Table 3: ANOVA results of GLM for stage variable. LR Chisq stands for Likelihood Ratio Chi-square, Df for degrees of freedom and Pr(>Chisq) for *p-value*.

Variable	LR Chisq	Df	Pr (>Chisq)
Stage	1.3499	1	0.2453

FCUP 30
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island
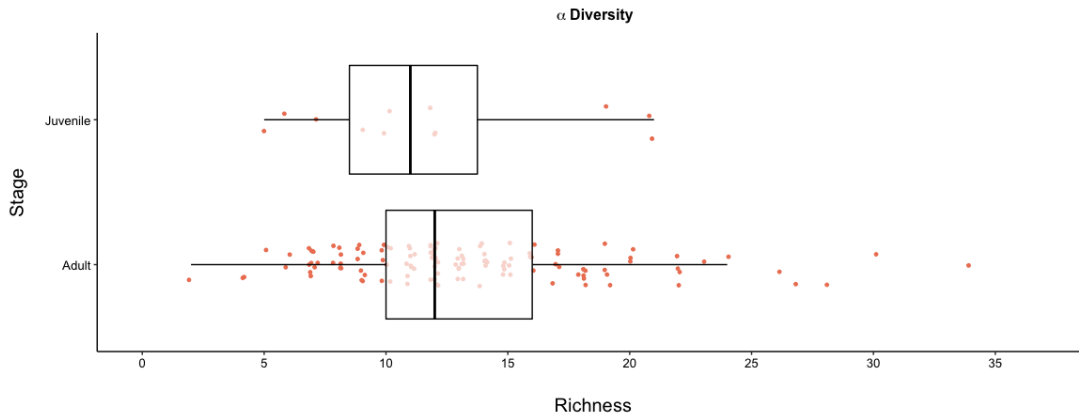
Figure 13: Boxplot showing the number of prey taxa detected in juvenile and adult samples.

Alike the previous results obtained for PerMANOVA, the composition analysis respecting the stage revealed significant results (Table 4) that do not seem to be associated with dispersion (Table 5).

Table 4: PerMANOVA results of stage effect in the diet of *T. mauritanica*. Df stands for degrees of freedom, SS for sum of squares, MS for means of squares, F Model for F-statistic, R2 for R-squared, and Pr (>F) for *p-value*.

Variable	Df	SS	MS	F Model	R2	Pr (>F)
Stage	1	0.620	0.62031	1.5174	0.01072	0.0388
Residuals	140	57.233	0.40881		0.98928	
Total	141	57.853			1.00000	

Table 5: PerMADISP results of stage variable. Df stands for degrees of freedom, SS for sum of squares, MS for means of squares, F value for F-statistic and Pr (>F) for *p-value*.

	Df	SS	MS	F value	Pr (>F)
Groups	1	0.001553	0.0015526	0.9885	0.3218
Residuals	140	0.219890	0.0015707		

Then, from the results of the rarefaction and extrapolation curves (Figure 14) it is possible to see that until a certain percentage of sample coverage (~0.63), juveniles revealed higher taxa richness compared to the adult stage. However, despite suggesting that juveniles present more diverse diets, the disparities in sample size between stages would require further analysis in order to make better conclusions.

FCUP | 31
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island
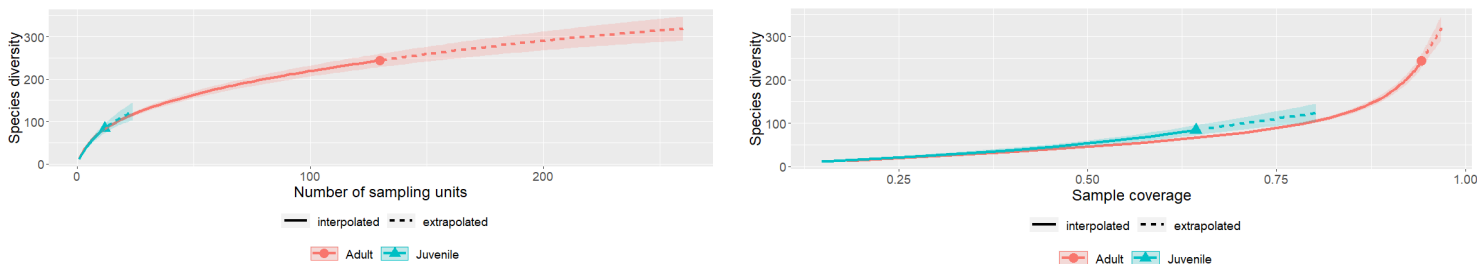
Figure 14: Rarefaction/Extrapolation (R/E) curves for stage. Obtained using iNEXT package from R.

Trogiidae, Oligotomidae, Julidae, Formicidae, Blastobasidae and Agelenidae were the groups contributing the most to the differences found in PerMANOVA between adults and juveniles (Figure 15), according to their average abundances in these stages (OTUs Frequency). In particular, the first two families as well as the latter occurred much more significantly in juveniles than in adults.
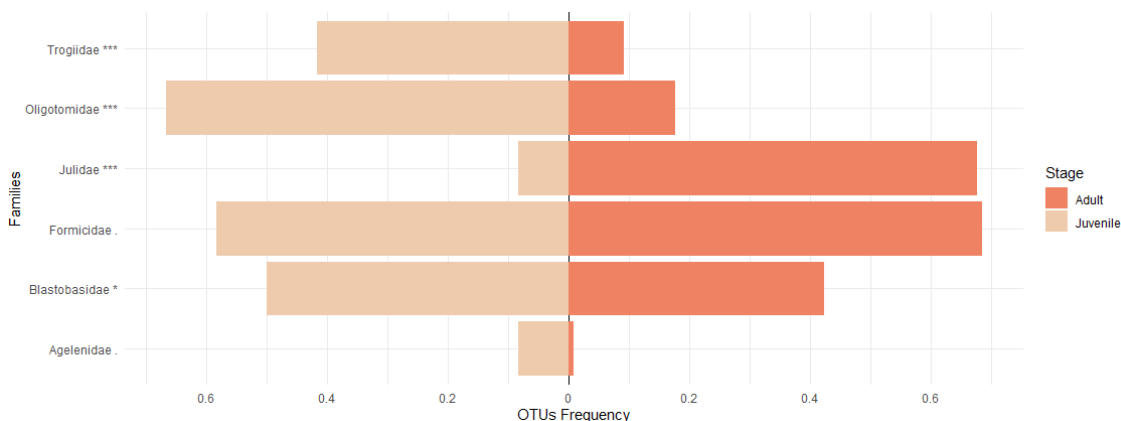
Figure 15: Results of similarity percentage analysis. Frequency of occurrence of Operational Taxonomic Units (OTUs) with the highest contribution to differences between the diets of *Tarentola mauritanica* in both stages. Magnitude of significance levels shown with asterisks: *** $p < 0.001$; * $p < 0.05$.

NDVI results (Table 6; Figure 16) revealed that Ribeira Brava, followed by Caniço, present the highest values for primary productivity. The results suggest that these localities should have the highest insect biomass and richness available for higher trophic levels, following Bailey et al. (2004) and Fernández-Tizón et al. (2020). At least for Caniço, this premise is true, since this is one of the localities with higher richness apart from Praia Formosa (Figure 10). Nevertheless, Praia Formosa has the lowest NDVI value, suggesting an opposite pattern in terms of insect richness. Not surprisingly, no correlation was found between the NDVI and richness per locality (~8% correlation;

FCUP 32
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Table 7). Thus, at least in this study, the NDVI cannot be used as a proxy for arthropod biomass or richness.

Table 6: NDVI values per locality.

Locality	NDVI
Câmara de Lobos	0.186599
Caniçal	0.0532623
Caniço	0.277893
Praia Formosa	0.045388
Funchal	0.1088005
Ribeira Brava	0.291147
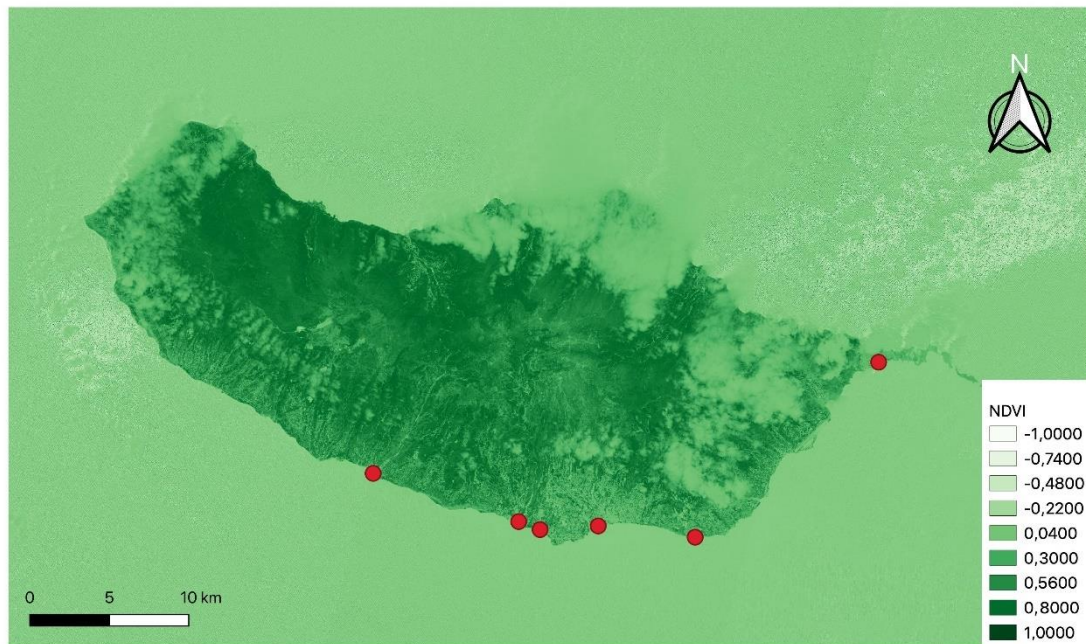
Figure 16: Gradient map of Madeira Island according to NDVI.

Table 7: Table of the estimated regression results.

Variable	Estimate	Std. Error	t value	Pr (>|t|)
(Intercept)	-0.051935	0.363762	-0.143	0.893
Richness	0.001875	0.003182	0.589	0.587

Residual standard error: 0.1165 on 4 degrees of freedom
Multiple R-squared: 0.07983, Adjusted R-squared: -0.1502
F-statistic: 0.347 on 1 and 4 DF, *p-value*: 0.5875

FCUP 33
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

4. Endemic Arthopods

Out of the 72 species composing the Moorish gecko's diet (Table 1), four of those – *Amegilla maderae* (Sichel, 1868); *Caradrina clavipalpis pinkeri* (Kobes, 1975); *Idaea atlantica* (Stainton, 1851); *Pselactus spadix sulcipennis* (Wollaston, 1854) – correspond to 6% of the species' diet and are classified as endemic (Figure 17).

All endemic and native species found belong to the class Insecta. *Amegilla maderae* is an endemic bee from Madeira, from the Hymenoptera family, that is usually seen visiting a large number of different plants, including the two endemic *Echium candicans* and *Echium nervosum* (Fellendorf et al., 1999).

Both *C. clavipalpis pinkeri* and *I. atlantica* belong to the Lepidoptera family, and *P. spadix sulcipennis* to the Curculionidae family. Unfortunately, not all species groups are well represented in the IUCN Red List, and Insecta appears to be one of them. Consequently, only two species, one native and one endemic, *Aiolopus thalassinus thalassinus* (Fabricius, 1781) and *Amegilla maderae* (Sichel, 1868), respectively, have a conservation status by IUCN, both considered as Least Concern (LC).
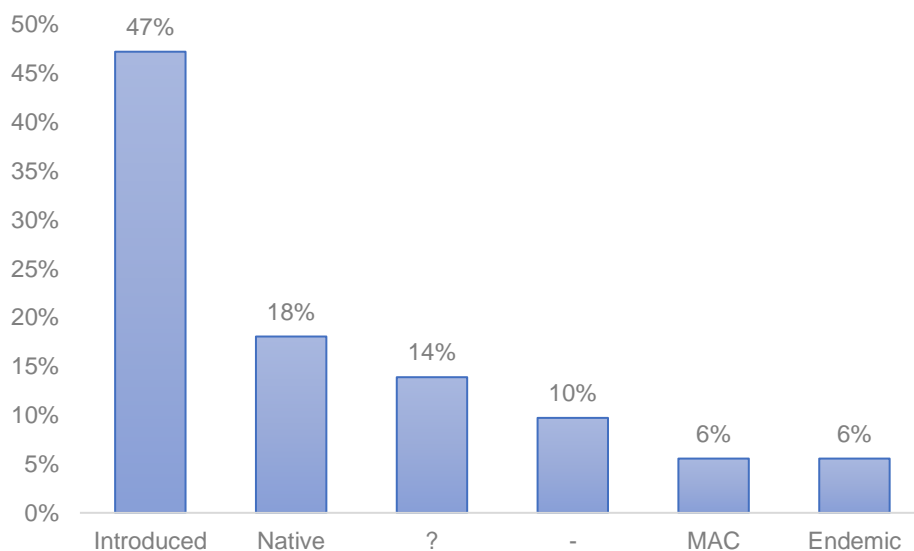
Figure 17: Bar plot representing species colonization status in Madeira Island. Classification following Borges et al. (2008). "MAC" represents Macaronesia endemisms and "-" stands for species whose colonization status appear in blank.

FCUP 34
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Regarding the species classified as introduced by the previous analysis, another graphic was produced (Figure 18) representing the introduced species and corresponding families that appear to be more frequent, according to the FOO approach. Here, *Ommatoiulus moreleti*, *Periplaneta americana* and *Xystrologa grenadella* were the most frequent introduced species in the diet of the Moorish gecko.
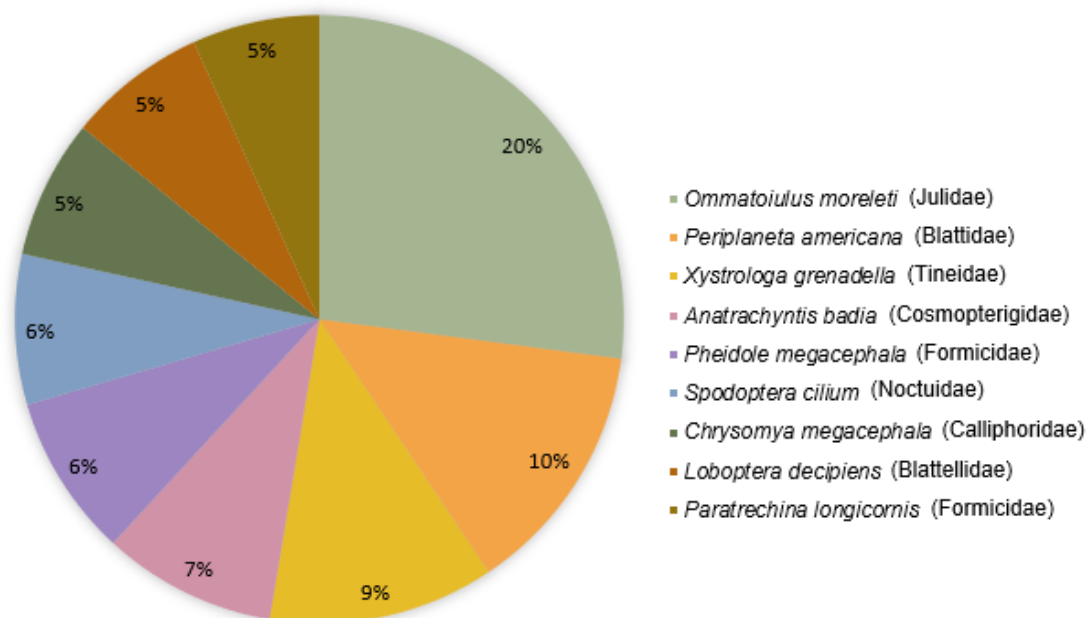
Figure 28: Pie chart representing the percentages of introduced species by frequency of occurrence and corresponding families present in *Tarentola mauritanica*'s diet.

5. Pests and Vector-borne diseases

Paratrechina longicornis, Nezara viridula, Aphis gossypii, Viteus vitifoliae, Opogona sacchari, Sophonia orientalis, Cameraria ohridella and *Linepithema humile* are the taxa classified in Madeira as agricultural pests according to the available bibliography, which are consumed by *Tarentola mauritanica* (Aguiar, 2009; Aguin-Pombo et al., 2007; Ribeiro et al., 2009). According to the FOO approach, the first species mentioned, ant *Paratrechina longicornis,* is undoubtedly the most consumed pest by the Moorish gecko (Figure 19). This indicates that many agricultural insect pests are breeding in areas frequented by the focal gecko species.

In addition, *Culex theileri* and *Typhaea stercorea* are the identified vectors of diseases such as the dog heartworm and salmonella. Unexpectedly, *Aedes aegypti*, known vector of several viruses, including dengue, was not detected in *T. mauritanica*'s diet.

FCUP 35
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island
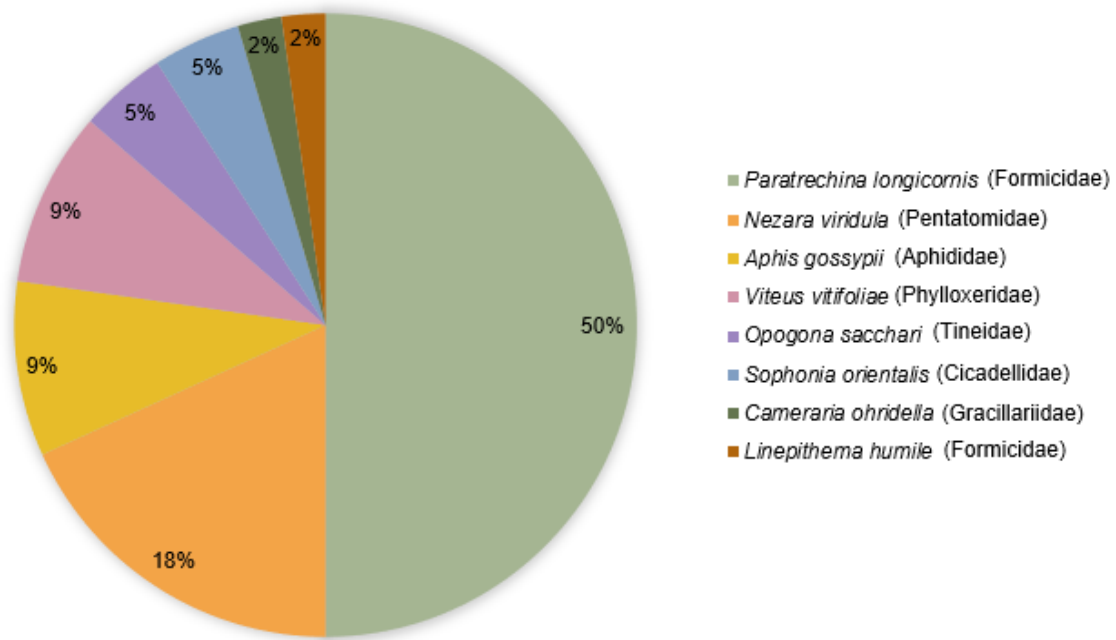
Figure 19: Pie chart representing the percentages of the identified arthropod pests by frequency of occurrence and corresponding families.

6. Detection of new species

In the list of all OTUs identified as comprising the Moorish gecko' diet (Table 1), aside from the species classified as present in the island, 19 of them consist most likely to new records of arthropod taxa to Madeira. According to the already mentioned table, these species are: fourteen Insecta (*Cameraria ohridella, Cerobasis guestfalica, Euborellia arcanum, Frankliniella tritici, Gryllodes supplicans, Lasius flavus, Liposcelis entomophila, Liposcelis paeta, Megaselia striolata, Oligotoma saundersii, Pheidole moerens, Plagiolepis pallescens, Plecia nearctica, Psammotettix confinis*); three Malacostraca (*Armadillidium vulgare, Porcellionides pruinosus* and *Porcellionides sexfasciatus);* and two Arachnida (*Holocnemus pluchei, Steatoda triangulosa*). It is important to mention that the species present in Malacostraca class were the ones that appear more abundantly in the diet, when comparing with the remaining ones (Supplementary Table 2).

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island
36

Discussion

This study, apart from being the first dietary study of *T. mauritanica* from Madeira, it is also the first metabarcoding assessment of any reptile species (introduced or endemic) from the island. Here, 250 prey items were detected encompassing seven different classes of invertebrates.

In this research, both relative read abundance (RRA) and frequency of occurrence (FOO) approaches were considered to evaluate differences in diet composition between them. The most noticeable difference is the bigger presence of the class Insecta, while the classes Malacostraca and Arachnida are significantly less present, in the RRA approach. These differences might be related with each family's digestibility or primer affinity to the different arthropod species, as it affects the amount of reads to be analyzed. According to Deagle et al. (2018), when it comes to the decision on how to interpret sequence counts, one should keep in mind that all methods have biases, meaning that this choice must be taken considering the kind of study. For instance, in dietary metabarcoding studies, taxon occurrences are typically the choice, since they are appropriate when food is clearly differentially digested; the sequence recovery bias is identified as being high (what usually occurs in animals with an insectivorous diet), or this bias is unknown and results cannot be cross-validated. The main problem with this approach is that rare food taxa are given the same importance as others, flattening the rank-abundance species curves frequently presented in dietary datasets (Deagle et al., 2018). However, it offers the advantage of moderating the impact of taxa-specific bias in marker signal, which simultaneously represents a big drawback of the RRA approach. Ideally, to correctly assess the quantitative ability of any study a mock community protocol should be carried out (Lamb et al., 2019). Since that was impossible to perform in this study, the ensuing diet composition discussion is based on the FOO approach.

The composition of the Moorish gecko diet in this study is, at some extent, in line with other dietary studies based mainly on morphological examinations (Gil et al., 1994; Hódar et al., 2006), as well as with other recent studies carried out with *Tarentola* genus from Cabo Verde (Pinho et al., 2018). Morphological examination has a tendency to underestimate prey incidence due to the fact that only items partially digested are detected (Ingerson-Mahar, 2002), while Metabarcoding is able to detect small or even invisible digested products. So, although the results cannot be compared in a straightforward way, the diet composition in all of them was almost exclusively made up of arthropods, with Insecta representing the predominant class in both studies. In fact,

FCUP 37
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

when comparing to Pinho et al. (2018), the number of Arthropoda prey taxa obtained more than doubled in this study, and two additional classes were detected, which is most likely related with the lower faunistic richness of Cabo Verde in terms of Arthropod taxa (Arechavaleta et al., 2005). Additionally, here it was possible to reach higher taxonomic resolution, with many prey items identified to species-level (Supplementary Table 2).

The predominance of the Porcellionidae, Julidae and Formicidae families in almost all the sampled sites show that the dietary composition does not vary widely among localities, which was also attested by the PCoA analysis. This could simply indicate that those taxa are more abundant, and therefore more accessible in the southern coast of Madeira, or maybe there is actually a preference for these families by the Moorish gecko. However, more studies regarding the incidence and abundance of arthropods per region would be beneficial for a better conclusion.

Interestingly, this dietary analysis apart from evidencing that this gecko captures preys belonging to diverse taxonomic groups, most of them are ground-dwelling arthropods (e.g. Julidae, Porcellionidae and Formicidae). These results might be indicative that foraging took place on the ground, in many different microhabitats, going in line with previous research (Hódar & Pleguezuelos, 1999; Sánchez-Piñero, 1994). Nevertheless, this strategy has only been observed in Mediterranean arid areas, where the availability of prey is lower, forcing them to forage actively on the ground instead of the usual sit-and-wait behaviour (Ananjeva & Tsellarius, 1986; Perry & Werner, 1981). Another possible explanation, albeit unlikely, could be that flying invertebrates feed on the ground-dwelling arthropods, and both prey levels were simultaneously amplified and identified as *T. mauritanica*'s prey items. Nevertheless, the strong presence of some Lepidoptera families such as Cosmopterigidae, Tinidae and Blastobasidae also indicates that these geckos are not just ground feeders, foraging on the walls and cliffs as well, possibly attracted to human lights at night-time. A study by Martin et al. (2018) concluded that artificial light influences the activity of wall geckos, not only their visual detection is improved, but also increasing the availability of invertebrate prey attracted by light.

The diversity in topography, microclimate and habitats that characterize Madeira Island, as well as the intercalated spatial distribution of land use (Santos, 2018), lead to abrupt changes in the landscape in just a few kilometers. Hence, and although the studied localities are very close to each other in a straight line, they are actually quite isolated from each other and comprised by distinct habitat combinations. Therefore, and not surprisingly, this is translated by significant differences in both richness and composition of OTUs among localities. The fact that Funchal appears consistently among the lowest prey taxon richness in all the analysis performed, could be associated

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

38

with the fact that this is the most urbanized and humanized area in Madeira, as it is demonstrated in the map showing the occupation typologies of the island (Figure 4). On the opposite, Caniço was consistently the locality presenting higher number and diversity of prey taxa, and coincidently it is also the place where this introduced species was first reported by Báez and Biscoito (1993).

Some studies, such as Bailey et al. (2004), have evidenced the existence of a correlation between richness and the amount of energy available, often measured by primary productivity. However, in this study, no correlation was found between richness and NDVI, suggesting that arthropod diversity in Madeira is not solely driven by primary productivity, but by some other unknown environmental factors.

Another interesting aspect revealed in this study was the fact that juveniles present higher interpolated OTU richness than adults, as well as differences found in the composition analysis. Regarding the first, it is known that juvenile head dimensions of *Tarentola mauritanica* are proportionally larger than in adults, and that prey size is an important factor in the feeding ecology of nocturnal geckos, as an energy-maximizing strategy (Ananjeva & Tsellarius, 1986). The differences found between juveniles and adults may solely be related to the different exploration of the territory. These geckos are extremely territorial and philopatric, with adults exploring the best places in terms of refuge and resources compared to juveniles (Campo & García-Roa, 2014; Martin et al., 2018). This means that even within the same population, there is microhabitat segregation between the two states, in which juveniles are obliged to explore a much larger territory than adults as they do not have their own refuge. Therefore, it is expected for juveniles to have a more varied diet as they cannot afford to be so selective.

The analysis of the Moorish gecko's diet revealed that it is composed by a small portion of endemic arthropods to Madeira (6%). Given the larger amount of endemic Arthropoda on the Island, it would be reasonable to expect slightly different results from the ones obtained, for this matter. From the 72 species found in the diet, just two species, one native and one endemic, have conservation status on IUCN both considered as Least Concern (LC). Therefore, if on one hand the results referring to the consumption of endemic species and species presenting least concern in conservation status seem encouraging, on the other hand, it highlights the lack of information regarding the conservation status of arthropods in the island, which limits, at some extent, the possible conclusions to be drawn in this study. On the opposite, the large proportion of the species found were classified as introduced. Thus, taking into consideration that island ecosystems are more susceptible to foreign competitors, pathogens and predators than continental ecosystems, this research highlights for the need to raise awareness

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

39

regarding introduced species, particularly in European islands such as Madeira as they are important natural heritage (Silva et al., 2008).

Regarding arthropods pests, the mostly consumed species by *T. mauritanica* (the milliped *Ommatoiulus moreleti*) is responsible for multiple damage in a small number of crop types, including canola, lucerne and lupin seedlings, which are most susceptible to be attacked (Douglas et al., 2017). However, in a recent study (Douglas et al., 2019) evaluating the damage caused by this specimen on specially cultivated and wild-type *Lupinus angustinus* seedlings, also present in Madeira Island, concluded that the damage to all crop seedlings was related to life-stage, sex and size, being the large females, the ones causing more damage. However, millipedes and terrestrial isopods are known to play a beneficial role on decomposing plant matter and recycling nutrients back into the soil (Vos et al., 2011). Likewise, the isopod *Armadillidium vulgare* (Table 1), not described to Madeira yet, is likely to cause damage to a variety of crops as well, such as pulses, cereals and oilseeds (Douglas et al., 2017).

In Madeira, the Banana moth, *Opogona sacchari* (Lepidoptera: Tineidae) (Table 1) is a common pest spread across banana plantations, especially where there are high densities of these plants. This organism usually prefers decomposed tissues to develop, however the larvae can easily attack nearby healthier tissues as well, attacking directly the fruit during the months with higher humidity and lower temperatures (November to April) (Ribeiro et al., 2009). Other frequently attacked hosts are plants of commercial interest such as sugar cane (*Cana sacarina*), and ornamental plants commonly growing in gardens like *Strelitzia regiae*, especially those bordering banana plantations.

The leafhopper, *Sophonia orientalis* (Homoptera: Cicadellidae) (Table 1) is a very recent presence in Madeira, found for the first time in Funchal in the year 2000 (Aguin-Pombo et al., 2007). Like many other introduced species, it became widely spread all over the island in just a few years. This species is a serious pest to many economically important plants in the Hawaiian archipelago, where it was observed attacking banana plantations (Aguin-Pombo et al., 2007). However, the greatest damage produced by this species was registered for the common guava tree, *Psidium guajava* (also present in Madeira) causing reductions of 23% (Aguin-Pombo et al., 2007). Nevertheless, there are also reports of nymphs feeding on *Vitis vinifera*, which produces the well-known Madeira wine, another important crop of the island (Aguin-Pombo et al., 2007; Ribeiro et al., 2009). The fact that this introduced species is known to affect two of the main cultures present in Madeira, requires special care through constant monitoring of its potential detrimental effects.

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

40

Inside vineyards many groups of insects can be observed, namely species belonging to Cicadellidae family, also known as leafhoppers. In 1882, the insect *Viteus vitifolli* was introduced to Madeira Island, and in a decade, it was able to reduce the wine cultivated area from 2500 ha to 500 ha (Aguiar, A., personal communication). Nowadays, the main threats to this culture are the leafhoppers *Sophonia orientalis* and *Scaphoideus titatus*. The latter was first reported in 2010 and is the vector of the golden flavescence in vineyards, a very important disease caused by a phytoplasma, transmitted during the feeding process; however, it was not found in *T. mauritanica*'s diet. Fortunately, the disease was not yet detected on the island, despite the insect being widely dispersed in the vineyards of the north coast of Madeira. When the phytoplasma is absent, this insect can cause damage to the plants when in high numbers, while feeding on it (Cravo, 2015).

Across Europe, horse-chestnut trees (*Aesculus hippocastanum*) are severely damaged by the leaf miner *Cameraria ohridella* (Table 1). To evaluate these effects, a study was conducted over the course of ten years. The investigators came to the conclusion that 75% of the leaf area of horse-chestnut trees was damaged by *Cameraria ohridella*, while red chestnut trees (*Aesculus carnea*) were only affected when growing close to highly infested horse-chestnut trees (Koskella et al., 2017; Straw & Williams, 2013).

Regarding Formicidae, a very relevant family present in *T. mauritanica* diet, there are records of two known pests, the big-headed ant, *Pheidole megacephala*, and the Argentine ant, *Linepithema humile*. First records of these species date back to the nineteenth century, when both populations exploded in the 1850s and 1890s, respectively (Wetterer et al., 2006). According to Wetterer et al., (2006), even after 150 or more years of residence, *P. megacephala* and *L. humile* did not became dominant and maintained restricted to a small part of the island, causing little impact over native ants already present.

In summary, 10 of the total 93 species found in the diet are classified as pests by EPPO (European and Mediterranean Plant Protection Organization), an organization that aims to protect plants, strategize against the introduction and spread of dangerous pests, and to promote safe and effective control methods. *Anatrachyntis badia, Laodelphax striatella, Daktulosphaira vitifoliae* and *Duponchelia fovealis* are the identified pests in this database apart from the already mentioned.

Concerning vector-borne human diseases, *Aedes aegypti* does not seem to be present in the diet of the Moorish gecko, neither was it detected in the field traps. *Tarentola mauritanica* is mostly crepuscular/nocturnal, frequently foraging around artificial lights (Tkaczenko et al., 2014), but they can also be active during the day

FCUP 41
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

(Fulgione et al., 2019) A possible explanation for the absence of *Aedes* mosquitoes in the diet composition could be due to its non-phototactic and diurnal activity, not regularly occurring around artificial lights (Christophers, 1960). However, an *Aedes* mosquito was detected in the diet of the also nocturnal *Tarentola gigas gigas* from Cabo Verde (Pinho et al., 2018). These results suggest that in Madeira the activity of the Moorish gecko is mainly crepuscular/nocturnal, not overlapping with the one characterizing *Aedes aegypti*, and hence, there are little opportunities for the gecko to prey on these mosquitos. Moreover, the non-detection of *A. aegypti* as prey can also mean that its representation in the gecko's diet is minimal and unable to be exposed in a metabarcoding approach. Surely, in the future the insect traps should be placed during the day, increasing the chances of detecting and collecting *Aedes* mosquitos.

Instead, another mosquito from the Culicidae family, *Culex theileri,* was detected in a female gecko from Funchal. This is a mammophilic mosquito that serves as a vector for several zoonotic diseases, and it is widely distributed across Europe, North Africa, and southwest Asia (Demirci et al., 2012). This species has been proven to be a competent vector of human and domestic animal pathogens, caused by *Wuchereria bancrofti* (main cause of lymphatic filariasis) and *Dirofilaria immitis* (the dog heartworm), respectively (da Silva et al., 2014; Demirci et al., 2014; Demirci et al., 2012). The latter is known to be endemic to continental Portugal, and as of 2015 it was the only known vector of the dog heartworm disease. Since then, other species have also been identified as being potential vectors of this disease (Ferreira et al., 2015). Nonetheless, there is evidence that *Culex theileri* likely is the main vector of the dog heartworm disease in Madeira, where it is a major issue, as it is estimated to prevail in 30% of dogs (Clemente, 1996; Santa-Ana et al., 2006).

Typhaea stercorea (Table 1) is a beetle who carries *Salmonella enterica*, being able to enter broiler houses due to its capability to fly. Since it also inhabits cars, crates and catching machinery, this infection can be widely spread. The infection occurs when chickens eat contaminated beetles or are in contact with contaminated litter and fomites. *Salmonella enterica serovar infantis* (*S. infantis*) was proven to be potentially carried by this beetle between successive broiler cycles (Hald et al., 1998). However, results from another study reveal that salmonella transmission from contaminated beetles to chickens may not always happen. Despite this result, the same study concluded that the beetles were the reservoir of *Salmonella enterica serovar indiana* (*S. indiana*) between two consecutive broiler flocks (Skov et al., 2004), going in line with the previous study.

Given the huge biodiversity threat caused by either accidental or intentional introduction of species, and their potential ecological effects, biocontrol is presented as

an important alternative management option between pest prey species and its introduced antagonists (De Clercq et al., 2011). However, several criteria need to be carefully investigated prior to the introduction, as well as an intense follow-up is required for the assessment and improvement on the predictability of the biological control (Louda et al., 1997).

In the case of *T. mauritanica*, it is true that it seems to have little impact on Madeira's endemic arthropods, while feeding mainly on different pests and species which can potentially spread vector-borne diseases. This indicates that it could be a potential good candidate for biocontrol of these problematic species (Monagan Jr. et al., 2017). However, due to the lack of knowledge regarding the conservation status of several arthropod taxa, in particular the endemic ones, it is hard to be sure if the impact is indeed not significant. Furthermore, the used approach (metabarcoding) allows only to have a qualitative perspective on the taxa *T. mauritanica* is feeding on, and not about the quantity consumed by this gecko (Pompanon et al., 2012). Therefore, even if only 6% of the diet is composed by endemic species, it is not possible to know the real impact of *T. mauritanica* on the population of these species. In conclusion, further studies are needed to understand if *T. mauritanica* can really be a formal biocontrol agent of pests and species that spread diseases, without having negative impacts upon endemic species. In an informal and natural way, the study suggests that yes, *T. mauritanica* is serving as predator of problematic species, hence acting as a biocontrol agent.

Finally, through the results obtained during the assignment of each prey taxa to their respective taxonomic identification, it was possible to detect 19 species in Madeira that are still unknown to occur in the island until this date. These new reports might contribute to the update of Madeira's arthropods fauna checklist that is dated to 2008, highlighting also the importance of metabarcoding diet analyses as an indirect way to detect and update lists of cryptic species such as invertebrates.

FCUP 43
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Conclusion

The obtained results provide supporting evidence of the positive impact of generalist predators, such as *Tarentola mauritanica*, on the control of crop pests in agricultural landscapes, and the role of diversified agroecosystems in sustaining both functionally diverse communities and crop production in tropical agroecosystems.

The richness and composition of the sampled sites seem to be affected by the sampling locality, with Funchal presenting the lowest values, and on the contrary, Caniço the highest. Nevertheless, this richness differential is unrelated to local primary productivity. The more abundant families in the diet of the Moorish gecko are ground-dwelling Julidae, Porcellionidae and Formicidae, evidencing that this predator is capable of active foraging apart from the typical "sit-and-wait".

Moreover, the smallest percentage of endemic species found in the diet, followed by their limited conservation status information, indicates that if on one hand *T. mauritanica* does not seem to preferable prey on these protected species, it also evidences that more conservation research is needed regarding endemic arthropods in island ecosystems.

The results from this study also demonstrate that despite the possibility of false inferences due to contaminations, along with DNA reference sequences deficiency, as well as the fact that DNA metabarcoding only provides taxa occurrences in the samples, instead of their relative abundances, this is a reliable method with great potential to improve biomonitoring terrestrial insular ecosystems, highly vulnerable to global change and introduced species.

Overall, this research suggests that this species of gecko might indeed work as a natural agent of biological control of some of the agricultural pests that affect the two most popular and profitable crops for the island (wine and bananas), feeding on eight Arthropoda species already known as pests. This number is most likely underestimated due to the lack of information on this topic. The benefits of lizards to agricultural ecosystems have been studied over the years, however the correlation of feeding habits, in particular lizard species as biological factors to environmental resistance, are not properly studied yet, which constitutes a barrier to the implementation of more adequate measures regarding agricultural systems.

FCUP 44
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

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FCUP 45
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

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FCUP

Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

46

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47

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50

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FCUP 52
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FCUP 53
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Appendix

Supplementary Table 1: Insect primer list of Fragments LC and B2 used for each sample and negative control (NC) amplified for COI mitochondrial gene. Identification based on highest taxonomic resolution according to the colleague entomologist Ysabel Gonçalves.

Fragment / Specimen	LC		B2	
	Fw	Rv	Fw	Rv
Uresiphita gilvata	fwhF1-NGS	Ind_C_R	BF2	BR2
Udea numeralis	fwhF1-NGS	Ind_C_R	BF2	BR2
Udea ferrugalis	fwhF1-NGS	Ind_C_R	BF2	BR2
Psara bipunctalis	fwhF1-NGS	Ind_C_R	BF2	BR2
Palpita vitrealis	fwhF1-NGS	Ind_C_R	BF2	BR2
Pyrausta sanguinalis	fwhF1-NGS	Ind_C_R	BF2	BR2
Chrysodeixis chalcites	fwhF1-NGS	Ind_C_R	BF2	BR2
Euplesia dubiosa	fwhF1-NGS	Ind_C_R	BF2	BR2
Autographa gamma	fwhF1-NGS	Ind_C_R	BF2	BR2
Leucania loreyi	fwhF1-NGS	Ind_C_R	BF2	BR2
Notuidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Gymnoscelis rufifasciata	fwhF1-NGS	Ind_C_R	BF2	BR2
Rhodometra sacraria	fwhF1-NGS	Ind_C_R	BF2	BR2
Ascotix fortunata wollastoni	fwhF1-NGS	Ind_C_R	BF2	BR2
Geometridae	fwhF1-NGS	Ind_C_R	BF2	BR2
Pyralidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Microlepidoptera	fwhF1-NGS	Ind_C_R	BF2	BR2
Sciaridae	fwhF1-NGS	Ind_C_R	BF2	BR2
Simulium sp.	fwhF1-NGS	Ind_C_R	BF2	BR2
Tachinidae	Ind_LCO1490	Ind_C_R	BF2	BR2
Calliphoridae	fwhF1-NGS	Ind_C_R	BF2	BR2
Sphaeroceridae	fwhF1-NGS	Ind_C_R	BF2	BR2
Agromyza sp.	fwhF1-NGS	mlCOIintR-NGS	BF2	BR2
Limoniidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Hippoboscidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Lonchoptera lutea	fwhF1-NGS	Ind_C_R	BF2	BR2
Drosophilidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Sepsidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Muscidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Lauxanidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Syrphidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Tipula paludosa	fwhF1-NGS	Ind_C_R	BF2	BR2
Tipula sp.	fwhF1-NGS	Ind_C_R	BF2	BR2
Sarcophaga sp.	fwhF1-NGS	Ind_C_R	BF2	BR2
Chyronomidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Phoridae	fwhF1-NGS	Ind_C_R	BF2	BR2
Phaneroptera sp.	fwhF1-NGS	Ind_C_R	BF2	BR2
Homoptera	fwhF1-NGS	Ind_C_R	BF2	BR2
Beosus maritimus	fwhF1-NGS	Ind_C_R	BF2	BR2
Dieuches schmitzi	fwhF1-NGS	Ind_C_R	BF2	BR2
Nysius sp.	fwhF1-NGS	Ind_C_R	BF2	BR2
Lygaeidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Pinalitus conspurcatus	fwhF1-NGS	Ind_C_R	BF2	BR2
Miridae	fwhF1-NGS	Ind_C_R	BF2	BR2
Cydnidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Apis mellifera	fwhF1-NGS	Ind_C_R	BF2	BR2

FCUP 54
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Fragment	LC		B2	
Specimen	Fw	Rv	Fw	Rv
Vespidae	jgLCO1490-NGS	mlCOlintR-NGS	BF2	BR2
Formicidae	fwhF1-NGS	Ind_C_R	ind_ArF5	INDs_HCO2198
Hymenoptera	fwhF1-NGS	Ind_C_R	BF2	BR2
Cloeon peregrinator	fwhF1-NGS	Ind_C_R	BF2	BR2
Psocoptera	fwhF1-NGS	Ind_C_R	BF2	BR2
Scobicia barbata	fwhF1-NGS	Ind_C_R	BF2	BR2
Carabidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Staphilinidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Chrysomelidae	fwhF1-NGS	Ind_C_R	BF2	BR2
Chrysopidae	jgLCO1490-NGS	Ind_C_R	BF2	BR2

Supplementary Table 2: List of the identified OTUs to the maximum resolution obtained, according to their occurrence in the faecal samples.

Class	Order	Family	Final ID	Sample Counts
Arachnida	**Araneae**	Agelenidae	Tegenaria pagana	2
		Araneidae	Argiope trifasciata	3
			Mangora acalypha	28
		Cheiracanthiidae	Cheiracanthium sp.otu371	3
		Clubionidae	Porrhoclubiona sp.otu742	1
			Porrhoclubiona sp.otu76	31
			Porrhoclubiona sp.otu5603	2
		Corinnidae	Liophrurillus flavitarsis	2
		Dysderidae	Dysdera crocata	18
		Gnaphosidae	Gnaphosidae otu160	6
			Scotophaeus blackwalli	1
		Oecobiidae	Oecobiidae otu5965	5
		Philodromidae	Thanatus vulgaris	2
		Pholcidae	Holocnemus pluchei	1
			Pholcus phalangioides	1
		Salticidae	Macaroeris diligens	27
			Salticus sp.otu57	14
			Salticidae otu21431	1
			Salticidae otu2154	2
		Theridiidae	Paidiscura orotavensis	31
			Steatoda grossa	2
			Steatoda triangulosa	1
		Uloboridae	Uloborus walckenaerius	3
		Unknown	Araneae otu103	15
			Araneae otu114	12
			Araneae otu1383	1
			Araneae otu1684	3
			Araneae otu177	7
			Araneae otu191	11
			Araneae otu229	2

Class	Order	Family	Final ID	Sample Counts
			Araneae otu2322	4
			Araneae otu241	4
			Araneae otu257	10
			Araneae otu268	10
			Araneae otu306	3
			Araneae otu335	6
			Araneae otu582	1
			Araneae otu14330	1
			Araneae otu553	1
			Araneae otu13026	1
			Araneae otu4886	1
Chilopoda	**Geophilomorpha**	Geophilidae	Geophilidae otu973	2
	Lithobiomorpha	Lithobiidae	Lithobius pilicornis	1
			Lithobius sp.otu115	7
			Lithobius sp.otu357	3
	Scolopendromorpha	Cryptopidae	Cryptopidae otu516	3
			Cryptopidae otu22210	2
			Cryptopidae otu12337	1
			Cryptopidae otu29794	1
	Scutigeromorpha	Scutigeridae	Scutigera coleoptrata	2
	Unknown	Unknown	Chilopoda otu13019	1
Collembola	**Entomobryomorpha**	Entomobryidae	Entomobryidae otu358	1
			Entomobryidae otu9176	1
	Unknown	Unknown	Collembola otu232	3
Diplopoda	**Julida**	Julidae	Julida otu634	2
			Ommatoiulus moreleti	88
			Julidae otu6277	6
Gastropoda	**Stylommatophora**	Geomitridae	Actinella sp.otu11340	1
		Hygromiidae	Ashfordia sp.otu256	9
			Caracollina lenticula	4
Insecta	**Blattodea**	Blattidae	Periplaneta americana	44
		Ectobiidae	Loboptera decipiens	24
			Loboptera sp.otu159	15
		Kalotermitidae	Cryptotermes brevis	1
		Rhinotermitidae	Reticulitermes grassei	19
		Unknown	Blattodea otu1160	7
	Coleoptera	Carabidae	Harpalus attenuatus	3
			Harpalus tenebrosus	1
		Chrysomelidae	Chrysomelidae otu11977	1
		Coccinellidae	Rodolia cardinalis	7
		Curculionidae	Curculionidae otu217	3
			Pselactus spadix	3
		Elateridae	Elateridae otu293	7
		Mycetophagidae	Typhaea stercorea	1

FCUP | 56
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Class	Order	Family	Final ID	Sample Counts
		Tenebrionidae	Tenebrionidae otu261	7
			Tenebrionidae otu463	2
		Unknown	Coleoptera otu18	36
			Coleoptera otu9361	1
			Coleoptera otu15534	1
	Dermaptera	Anisolabididae	Euborellia sp.otu50	25
			Euborellia arcanum	2
	Diptera	Bibionidae	Plecia nearctica	1
		Calliphoridae	Chrysomya megacephala	24
		Ceratopogonidae	Ceratopogonidae otu139	1
		Culicidae	Culex theileri	1
		Drosophilidae	Drosophila buzzatii	5
		Ephydridae	Psilopa sp.otu296	8
		Limoniidae	Diptera otu36	15
		Muscidae	Limnophora sp.otu2086	1
			Stomoxys calcitrans	1
		Phoridae	Megaselia striolata	3
		Polleniidae	Pollenia pediculata	2
		Psychodidae	Clogmia albipunctatus	26
		Sarcophagidae	Sarcophaga africa	4
		Sciaridae	Bradysia sp.otu3192	1
		Syrphidae	Eumerus sp.otu342	1
		Unknown	Diptera otu1213	2
			Diptera otu13832	1
	Embioptera	Oligotomidae	Haploembia solieri	30
			Oligotoma saundersii	1
	Hemiptera	Aphididae	Aphis gossypii	4
			Brachyunguis tamaricis	1
			Cinara cupressi	1
		Cicadellidae	Cicadellidae otu130	18
			Cicadellidae otu79	22
			Edwardsiana sp.otu21255	1
			Psammotettix confinis	1
			Sophonia orientalis	2
			Cicadellidae otu8157	2
			Cicadellidae otu7733	1
		Delphacidae	Hemiptera otu87	17
			Tagosodes sp.otu2178	1
			Laodelphax striatella	1
		Lygaeidae	Spilostethus pandurus	1
		Miridae	Miridae otu323	4
		Pentatomidae	Nezara viridula	8
		Phylloxeridae	Daktulosphaira vitifoliae	4
		Reduviidae	Empicoris rubromaculatus	1

FCUP 57
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Class	Order	Family	Final ID	Sample Counts
		Rhyparochromidae	Rhyparochromidae otu74	28
			Scolopostethus sp.otu340	9
			Rhyparochromidae otu40343	1
			Rhyparochromidae otu38566	1
			Beosus maritimus	1
		Unknown	Hemiptera otu134	10
			Hemiptera otu2088	1
	Hymenoptera	Apidae	Amegilla maderae	1
			Apis mellifera	3
		Formicidae	Cardiocondyla emeryi	15
			Lasius flavus	1
			Nylanderia sp.otu16	39
			Nylanderia sp.otu27	24
			Paratrechina longicornis	22
			Pheidole megacephala	28
			Pheidole moerens/navigans	7
			Plagiolepis pallescens	1
			Technomyrmex pallipes	6
			Tetramorium caldarium	11
			Linepithema humile	1
		Unknown	Hymenoptera otu534	1
	Lepidoptera	Autostichidae	Apatema fasciata	12
		Blastobasidae	Blastobasis desertarum	40
			Blastobasis maroccanella	25
			Blastobasis sp.otu137	14
			Blastobasis sp.otu584	1
		Cosmopterigidae	Anatrachyntis badia	30
			Coccidiphila sp.otu1091	1
			Pyroderces sp.otu73	18
		Crambidae	Duponchelia fovealis	1
			Herpetogramma licarsisalis	14
		Depressariidae	Depressariidae otu808	1
			Depressariidae otu8778	1
		Geometridae	Geometridae otu6245	1
			Gymnoscelis rufifasciata	1
			Idaea atlantica	1
		Gracillariidae	Cameraria ohridella	1
		Noctuidae	Caradrina clavipalpis	2
			Spodoptera cilium	26
			Condica capensis	1
		Pyralidae	Cadra figulilella	18
			Isauria sp.otu220	3
			Pyralidae otu1453	1
			Pyralidae otu19	21

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

58

Class	Order	Family	Final ID	Sample Counts
			Pyralis farinalis	3
		Stathmopodidae	Neomariania sp.otu1212	6
			Stathmopodidae otu366	2
		Tineidae	Oinophila v-flava	3
			Opogona omoscopa	3
			Phereoeca allutella	2
			Xystrologa grenadella	39
			Opogona sacchari	2
		Unknown	Lepidoptera otu166	3
			Lepidoptera otu2481	1
	Orthoptera	Acrididae	Aiolopus thalassinus	43
		Gryllidae	Gryllidae otu550	3
			Gryllodes supplicans	16
		Mogoplistidae	Mogoplistidae otu205	4
		Tettigoniidae	Tettigoniidae otu274	3
	Psocodea	Liposcelididae	Liposcelis entomophila	1
			Liposcelis paeta	14
			Liposcelis sp.otu759	4
		Myopsocidae	Myopsocus sp.otu600	1
		Trogiidae	Cerobasis guestfalica	17
	Thysanoptera	Thripidae	Thripidae otu300	21
			Frankliniella tritici	1
	Zygentoma	Lepismatidae	Ctenolepisma longicaudata	2
			Lepisma saccharina	1
			Lepismatidae otu504	1
			Lepismatidae otu937	1
			Thermobia sp.otu13056	2
			Lepismatidae otu46687	1
			Lepismatidae otu2661	1
		Unknown	Zygentoma otu885	1
	Unknown	Unknown	Insecta otu1064	1
			Insecta otu109	3
			Insecta otu1337	1
			Insecta otu155	18
			Insecta otu168	24
			Insecta otu1695	1
			Insecta otu202	13
			Insecta otu20610	6
			Insecta otu213	8
			Insecta otu219	7
			Insecta otu24941	4
			Insecta otu258	8
			Insecta otu2632	2
			Insecta otu3286	1

FCUP 59
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Class	Order	Family	Final ID	Sample Counts
			Insecta otu3490	1
			Insecta otu3610	1
			Insecta otu387	1
			Insecta otu4020	1
			Insecta otu4410	1
			Insecta otu80	1
			Insecta otu862	1
			Insecta otu24097	3
			Insecta otu2859	1
			Insecta otu246	2
			Insecta otu3562	1
			Insecta otu1645	1
			Insecta otu1526	2
			Insecta otu8267	1
Malacostraca	**Isopoda**	Armadillidiidae	Armadillidium vulgare	52
			Eluma caelatum	4
		Porcellionidae	Porcellio dilatatus	3
			Porcellionidae otu1083	3
			Porcellionidae otu1490	2
			Porcellionidae otu51	24
			Porcellionidae otu6883	4
			Porcellionides pruinosus	51
			Porcellionides sexfasciatus	62
			Porcellionidae otu21377	1
			Porcellionidae otu32383	2
			Porcellionidae otu8282	2
			Porcellionidae otu35758	2
			Porcellionidae otu4291	2
			Porcellionidae otu10927	3
			Porcellionidae otu11269	3
			Isopoda otu11790	1
			Porcellionidae otu11231	1
			Porcellionidae otu14068	3
			Porcellionidae otu14356	1
			Porcellionidae otu17357	1
			Porcellionidae otu21357	2
			Porcellionidae otu2677	1
			Porcellionidae otu34584	2
			Porcellionidae otu7417	1
			Porcellionidae otu20239	1
		Unknown	Isopoda otu123	11
			Isopoda otu194	8
			Isopoda otu4822	5
			Isopoda otu7102	1

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island
60

Supplementary Table 3: Insect reference database collected in the six sampling sites.

Order	Family	Genus	Species
Lepidoptera	Crambidae	*Uresiphita*	*Uresiphita gilvata*
Lepidoptera	Crambidae	*Udea*	*Udea numeralis*
Lepidoptera	Crambidae	*Udea*	*Udea ferrugalis*
Lepidoptera	Crambidae	*Psara*	*Psara bipunctalis*
Lepidoptera	Crambidae	*Palpita*	*Palpita vitrealis*
Lepidoptera	Crambidae	*Pyrausta*	*Pyrausta sanguinalis*
Lepidoptera	Noctuidae	*Chrysodeixis*	*Chrysodeixis chalcites*
Lepidoptera	Notuidae	*Euplesia*	*Euplesia dubiosa*
Lepidoptera	Notuidae	*Autographa*	*Autographa gamma*
Lepidoptera	Noctuidae	*Leucania*	*Leucania loreyi*
Lepidoptera	Notuidae		
Lepidoptera	Geometridae	*Gymnoscelis*	*Gymnoscelis rufifasciata*
Lepidoptera	Geometridae	*Rhodometra*	*Rhodometra sacraria*
Lepidoptera	Geometridae	*Ascotix*	*Ascotix fortunata wollastoni*
Lepidoptera	Geometridae		
Lepidoptera	Pyralidae		
Lepidoptera	Microlepidoptera		
Diptera	Sciaridae		
Diptera	Simulidae	*Simulium*	*Simulium sp.*
Diptera	Tachinidae		
Diptera	Calliphoridae		
Diptera	Sphaeroceridae		
Diptera	Agromyzidae	*Agromyza*	*Agromyza sp.*
Diptera	Limoniidae		
Diptera	Hippoboscidae		
Diptera	Lonchopteridae	*Lonchoptera*	*Lonchoptera lutea*
Diptera	Drosophilidae		
Diptera	Sepsidae		
Diptera	Muscidae		
Diptera	Lauxanidae		
Diptera	Syrphidae		
Diptera	Tipulidae	*Tipula*	*Tipula paludosa*
Diptera	Tipulidae	*Tipula*	*Tipula sp.*
Diptera	Sarcophagidae	*Sarcophaga*	*Sarcophaga sp.*
Diptera	Chyronomidae		
Diptera	Phoridae		
Orthoptera	Tettigonidae	*Phaneroptera*	*Phaneroptera sp.*
Homoptera			
Heteroptera	Lygaeidae	*Beosus*	*Beosus maritimus*
Heteroptera	Lygaeidae	*Dieuches*	*Dieuches schmitzi*
Heteroptera	Lygaeidae	*Nysius*	*Nysius sp.*
Heteroptera	Lygaeidae		
Heteroptera	Miridae	*Pinalitus*	*Pinalitus conspurcatus*

FCUP 61
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Order	Family	Genus	Species
Heteroptera	Miridae		
Heteroptera	Cydnidae		
Hymenoptera	Apidae	*Apis*	*Apis mellifera*
Hymenoptera	Vespidae		
Hymenoptera	Formicidae		
Hymenoptera			
Ephemeroptera	Baetidae	*Cloeon*	*Cloeon peregrinator*
Psocoptera			
Coleoptera	Bostrichidae	*Scobicia*	*Scobicia barbata*
Coleoptera	Carabidae		
Coleoptera	Staphilinidae		
Coleoptera	Chrysomelidae		
Neuroptera	Chrysopidae		

Supplementary Table 4: Multiple comparison test results among localities.

Locality Differences	Estimate	Std. Error	z value	Pr (>|z|)
Caniçal – Câmara de Lobos = 0	0.09287	0.09214	1.008	0.9148
Caniço – Câmara de Lobos = 0	0.33099	0.08454	3.915	0.0012
Praia Formosa – Câmara de Lobos = 0	0.16081	0.08598	1.870	0.4185
Funchal – Câmara de Lobos = 0	-0.13663	0.09653	-1.415	0.7159
Ribeira Brava – Câmara de lobos = 0	0.11342	0.08994	1.261	0.8049
Caniço – Caniçal = 0	0.23812	0.08186	2.909	0.0419
Praia Formosa – Caniçal = 0	0.06794	0.08383	0.810	0.9654
Funchal – Caniçal = 0	-0.22950	0.09446	-2.430	0.1448
Ribeira Brava – Caniçal = 0	0.02055	0.08708	0.236	0.9999
Praia Formosa – Caniço = 0	-0.17017	0.07636	-2.229	0.2229
Funchal – Caniço = 0	-0.46762	0.08638	-5.413	<0.001
Ribeira Brava – Caniço = 0	-0.21756	0.07760	-2.804	0.0563
Funchal - Praia Formosa = 0	-0.29745	0.08921	-3.334	0.0109
Ribeira Brava - Praia Formosa = 0	-0.04739	0.08095	-0.585	0.9919
Ribeira Brava – Funchal = 0	0.25006	0.09179	2.724	0.0700

Supplementary Table 5: PerMANOVA results of variables (Sex, Locality, SVL, Weight) effect in the diet of *T. mauritanica*. Df stands for degrees of freedom, SS for sum of squares, MS for means of squares, F Model for F-statistic, R2 for R-squared, and Pr (>F) for *p-value*.

Variable	Df	SS	MS	F Model	R2	Pr (>F)
Sex	2	0.9	0.44998	1.21227	0.01698	0.1365
Locality	5	2.265	0.45301	1.22044	0.04273	0.0457
Weight	3	1.19	0.39658	1.0684	0.02244	0.3085
SVL	2	0.815	0.40753	1.09792	0.01538	0.2722
Sex:Locality	8	3.254	0.40671	1.09571	0.06138	0.1449
Sex:Weight	3	1.04	0.34672	0.93408	0.01962	0.6521
Locality:Weight	15	5.33	0.35536	0.95736	0.10055	0.7293

FCUP 62
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Variable	Df	SS	MS	F Model	R2	Pr (>F)
Sex:SVL	2	0.784	0.39212	1.0564	0.01479	0.3515
locality:SVL	10	3.338	0.33377	0.8992	0.06296	0.8987
Weight:SVL	3	1.126	0.37519	1.01077	0.02123	0.4417
Sex:Locality:Weight	5	1.841	0.36824	0.99205	0.03473	0.5035
Sex:Locality:SVL	2	0.848	0.42416	1.14272	0.016	0.2029
Locality:Weight:SVL	2	0.955	0.47743	1.28623	0.01801	0.0573
Residuals	79	29.324	0.37119		0.55318	
Total	141	53.01			1.00000	

Supplementary Table 6: PerMADISP results of locality variable. Df stands for degrees of freedom, SS for sum of squares, MS for means of squares, F value for F-statistic and Pr (>F) for *p-value*.
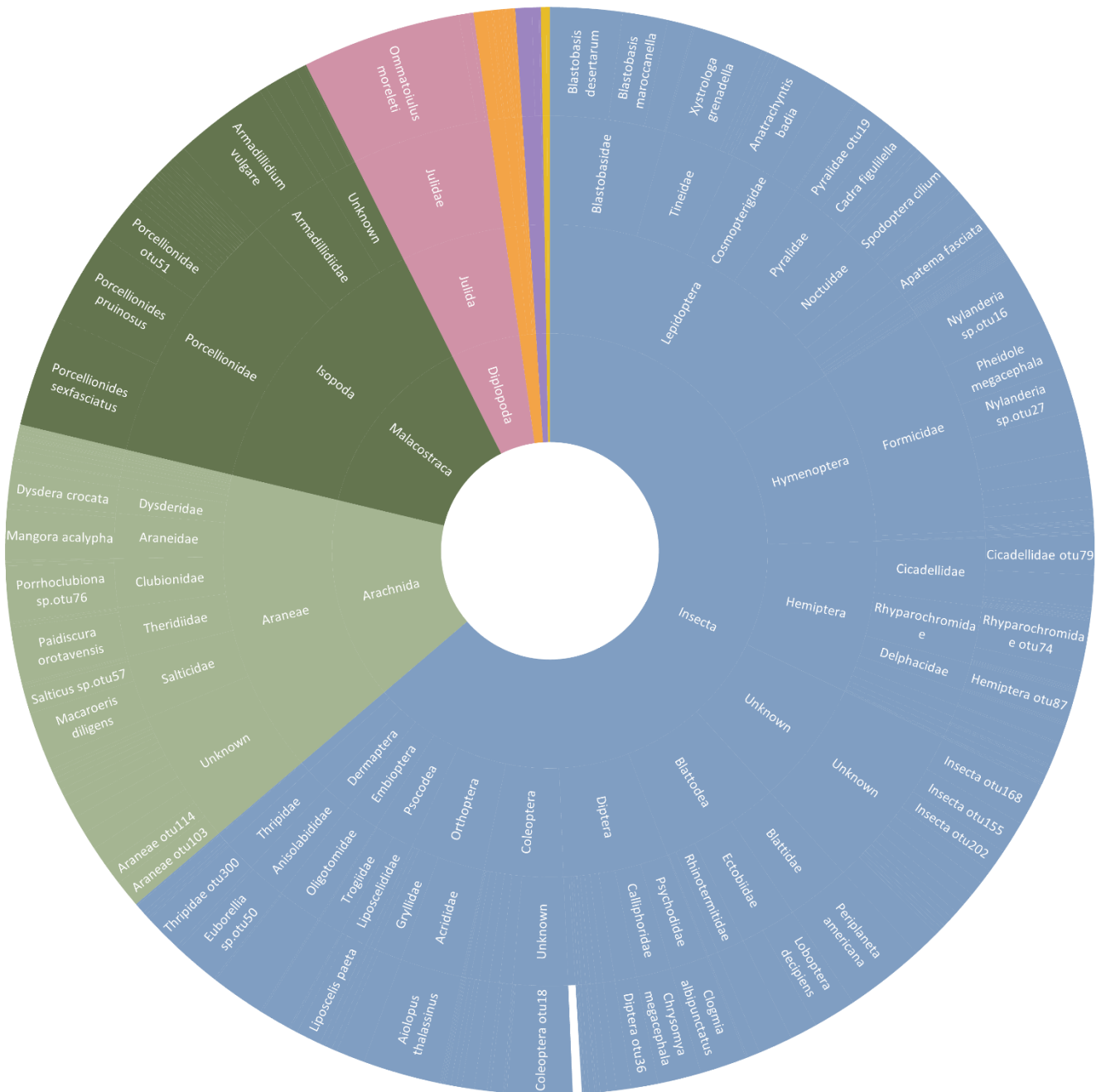
	Df	SS	MS	F value	Pr (>F)
Groups	5	0.009363	0.00187	1.1339	0.3455
Residuals	136	0.224602	0.00165		

Supplementary Table 7: Multilevel pairwise comparison among localities. Df stands for degrees of freedom, SS for sum of squares, F Model for F-statistic, R2 for R-squared, and p. adjusted sig for adjusted *p-value*.

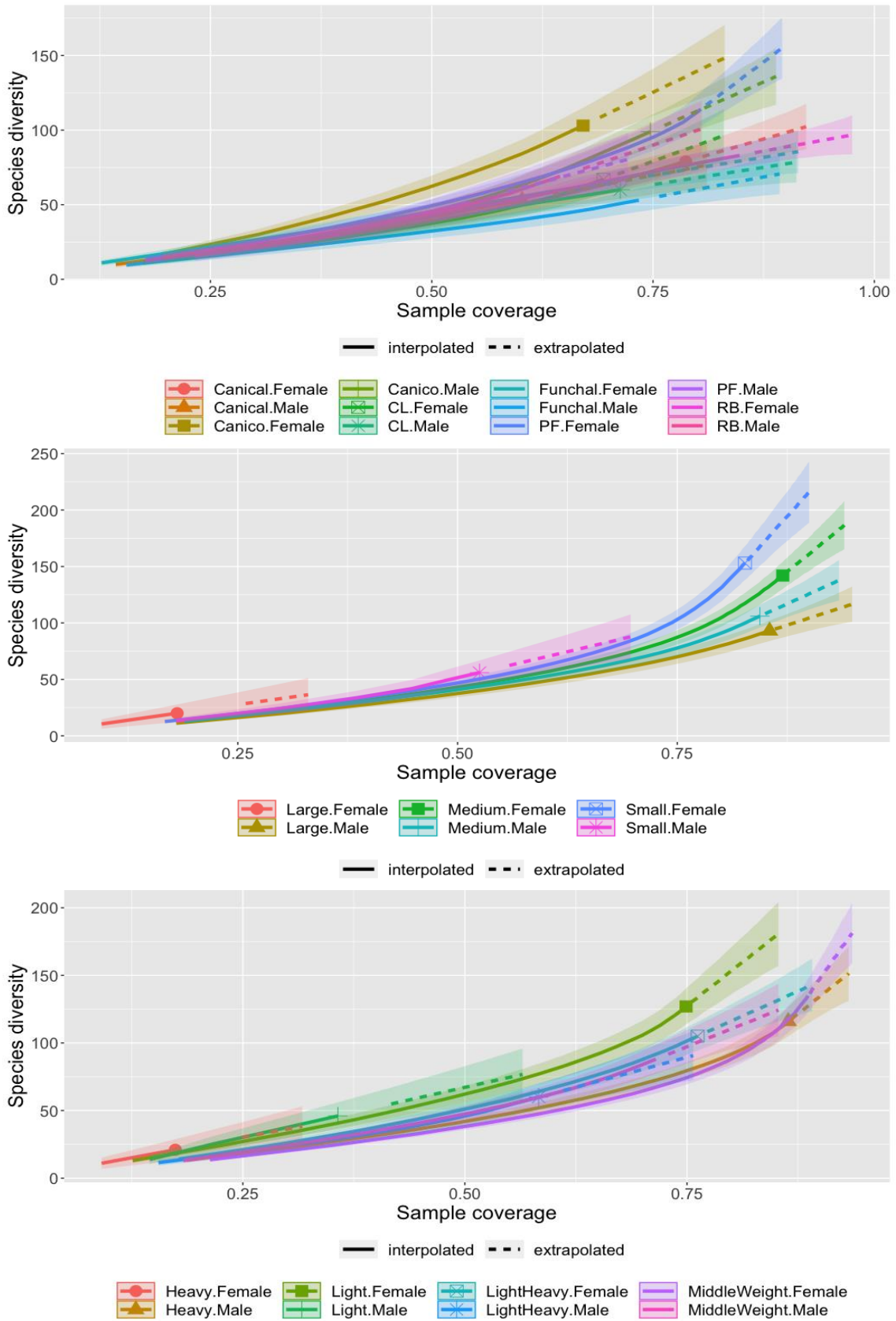| Pairs	Df	SS	F Model	R2	*p. value*	p. adjusted sig
Funchal vs Praia Formosa	1	0.7054591	1.9626133	0.04364989	0.016	0.240
Funchal vs Caniçal	1	0.7002644	1.9062509	0.04899601	0.028	0.420
Funchal vs Caniço	1	0.8110678	2.3591169	0.05318223	0.005	0.075
Funchal vs Ribeira Brava	1	0.5553142	1.5298367	0.03597090	0.117	1.000
Funchal vs Câmara de Lobos	1	0.3193137	0.8675811	0.02291092	0.576	1.000
Praia Formosa vs Caniçal	1	0.4187761	1.2084881	0.02796877	0.261	1.000
Praia Formosa vs Caniço	1	0.4487815	1.3694922	0.02831314	0.131	1.000
Praia Formosa vs Ribeira Brava	1	0.2818557	0.8182745	0.01747767	0.607	1.000
Praia Formosa vs Câmara de Lobos	1	0.5396272	1.5544738	0.03569033	0.083	1.000
Caniçal vs Caniço	1	0.4427562	1.3409349	0.03166994	0.138	1.000
Caniçal vs Ribeira Brava	1	0.3764902	1.0771889	0.02622353	0.400	1.000
Caniçal vs Câmara de Lobos	1	0.4326232	1.2248114	0.03290309	0.228	1.000
Caniço vs Ribeira Brava	1	0.6523710	1.9797986	0.04214149	0.021	0.315
Caniço vs Câmara de Lobos	1	0.4041207	1.2215896	0.02893282	0.251	1.000
Ribeira Brava vs Câmara de Lobos	1	0.3999644	1.1422389	0.02776317	0.304	1.000

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island | 63
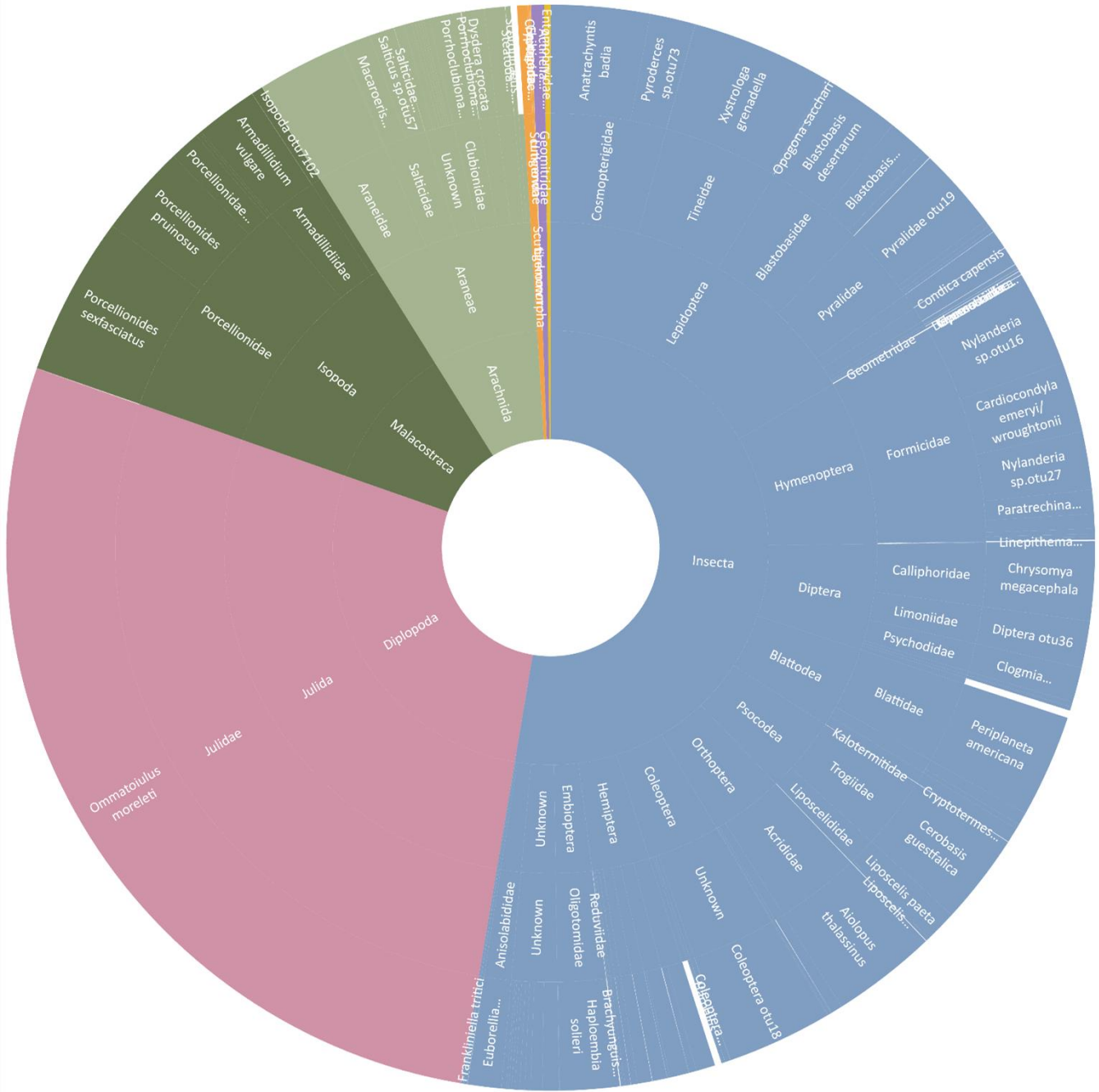
Supplementary Figure 1: Diet diversity, based on reads.

FCUP 64
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

Supplementary Figure 2: Diet diversity, based on sample counts.

FCUP
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island

65

Supplementary Figure 3: Rarefaction/Extrapolation (R/E) curves for Locality-Sex, SVL-Sex and Weight-Sex. Obtained using iNEXT package from R.

FCUP 66
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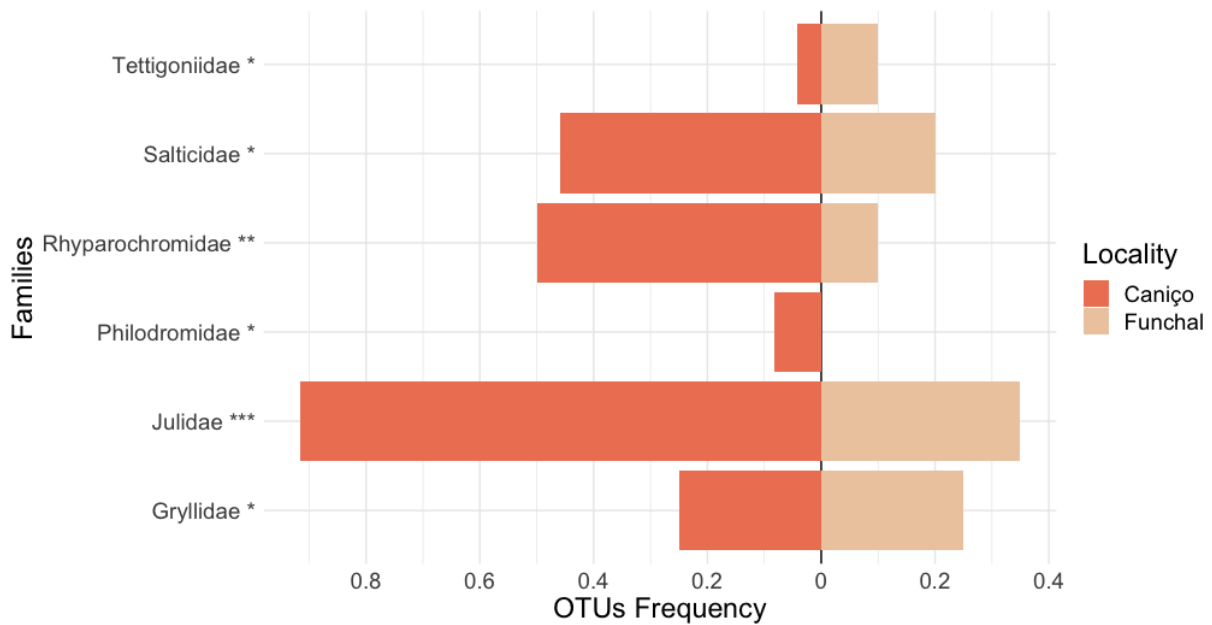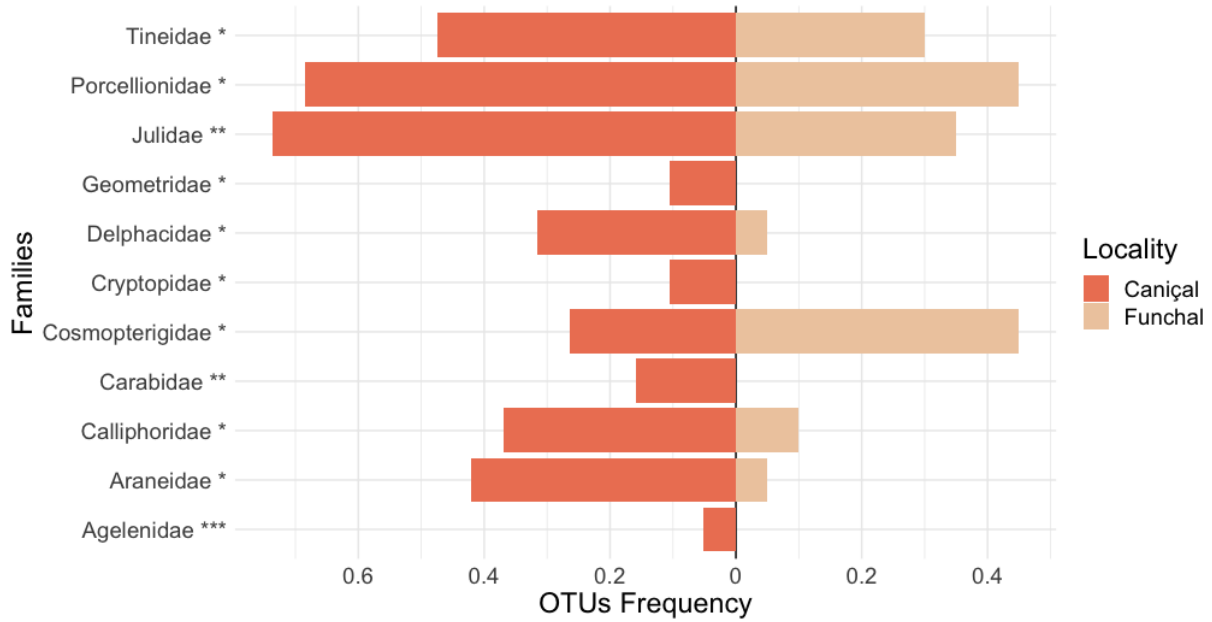
Supplementary Figure 4: Results of similarity percentage analysis for Caniçal vs Funchal and Caniço vs Funchal. Frequency of occurrence of Operational Taxonomic Units (OTUs) with the highest contribution to differences between the diets of *Tarentola mauritanica* in each locality. Magnitude of significance levels shown with asterisks: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

FCUP 67
Can an introduced gecko act as biological pest controller? Exploring the diet of Tarentola
mauritanica in Madeira Island
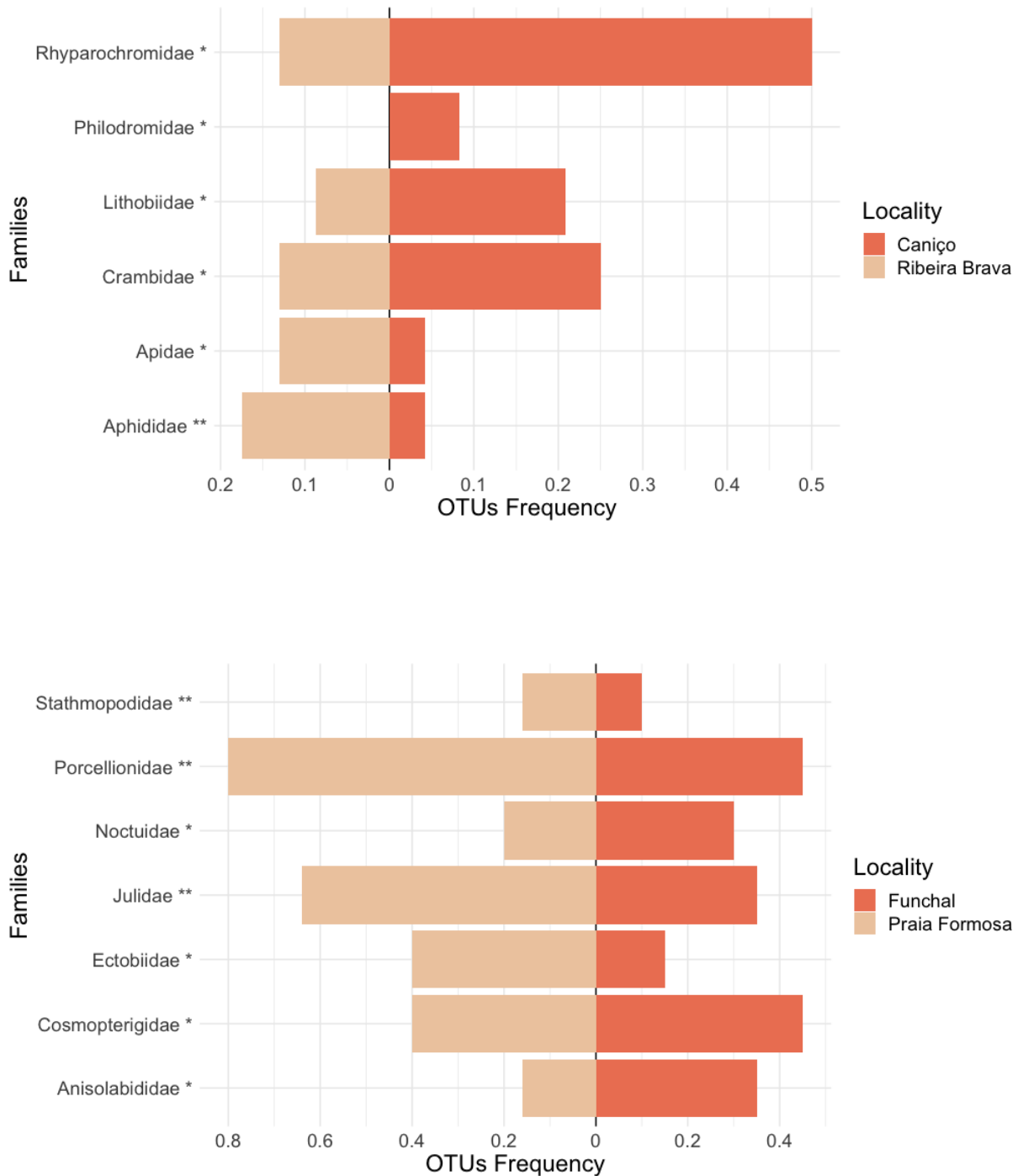
Supplementary Figure 5: Results of similarity percentage analysis for Caniço vs Ribeira Brava and Funchal vs. Praia Formosa. Frequency of occurrence of Operational Taxonomic Units (OTUs) with the highest contribution to differences between the diets of *Tarentola mauritanica* in each locality. Magnitude of significance levels shown with asterisks: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.