In the blood and gut of lizards: the drivers of gut bacteria diversity and the impacts of blood parasites

Diana Silva e Vasconcelos
Master in Biodiversity, Genetics and Evolution
FCUP/ Cibio-InBio
2021

Orientador

David James Harris, Principal Researcher, CIBIO/InBIO-UP, BIOPOLIS

Coorientadores

Raquel Xavier, Auxiliary Researcher, CIBIO/InBIO-UP, BIOPOLIS

Isabel Damas-Moreira, Post-doc Researcher, Bielefeld University (Germany)

Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ______/______/_________

Acknowledgements

Since I started this Masters, I felt myself growing daily with all the challenges that came my way. They were two years of many emotions and achievements. But above all, I never imagined I could learn so much and grow as a scientist in just one year. Having this opportunity to work with incredible people who helped me and supported me made this journey more unique.

My biggest thank you is to my supervisors who taught me so much and were always available to help me with everything. Thank you James, in addition to everything I have learned from you, thank you for accepting to be my supervisor and for supporting me and giving me the opportunity to have worked on a project that gave me so much pleasure. Thank you Raquel for teaching me so much, for pushing and encouraging me, always with a smile, and being patience with me. Thank you Isabel, for all the advices, all the brainstorming, all the support, and funny stories that came at just the right moment.

To all the people that during this journey helped me in fundamental moments. A special thanks to Miguel and Ana Perera for all the advices and support. A Thank you to Frederico for all the assist and support, and to Ana Pereira, for all the help in many of my adventures with R.

A giant thank you to "Team *Podarcis*", my companions in the field, in lunches and stories. Thanks Isabel, for all the energy, and talks that made all the field days unique. To Joaquim, for being a good co-pilot and for being so patient with us. A thank you to our most recent acquisition, Carolina, may this have been the first of many adventures. Thanks to you for all your help, FFF!

I would like to thank the ICNF (Instituto da Conservação da Natureza e das Florestas) for giving us permission whenever necessary for the catches that were needed throughout the study.

A big thank you to my fellow Masters students who made all our meetings at the pool remarkable!

To my friends and family from the heart who have always been a fundamental support to me and have always been there at every moment.

To Orfeão Universitário do Porto, for making me grow so much at various levels, for giving me so much joy and courage in the last years and for always being present in my life, even before I was born.

Last but not least,

To Diogo, for all the patience, all the talks, all the confidence and all the strength you have given me over the last few years. A heartfelt thank you for everything.

Thank you to my aunt for all the support throughout my life and for all the understanding especially in this last stage. To my grandparents for being my inspiration at all levels, an example of kindness and achievement to follow!

To my brothers for all the patience and understanding. To my parents for being everything and my biggest motivation. To my father for making me fall in love with biology since I was a little girl and to my mother for always being there to support me and for always believing in me.

Thank you all.

Abstract

There is an increasing amount of evidence pointing towards the importance of bacteria and parasites in the ecology and dynamics of host populations. Complex microorganism communities living in intestinal tracts of the animals are known as the gut microbiome. These bacterial communities can impact the host's fitness, immune system and behavior, whereas microbiome composition and diversity can be influenced by countless factors such as the host's taxonomy, habitat, diet and social interactions with other individuals. In turn, blood parasites have a fundamental role in the ecosystem, weakening host fitness and reproductive ability, and thereby influencing population dynamics.

Five lacertid lizard species were used for the present work, three endemic and two introduced: in Parque das Nações, Lisbon, the invasive *Podarcis siculus* (n=20) and the native *Podarcis virescens* (n=22); another introduced species, *Teira dugesii* (n=20), was captured from a distinct area of Lisbon (9 kilometres from the Parque das Naçoes); and in Moledo, Northern Portugal, two endemic and syntopic species were studied - *Podarcis bocagei* (n=33) and *Podarcis lusitanicus* (n=8). This thesis comprises two investigations. A first study was conducted in order to better understand the role and influence of environmental factors and the host in the gut microbiome composition and diversity of these five species. The second study had the objective of evaluating the effect of blood parasites on host behavior (using a mirror for the behavioral test), with the two syntopic species found in Moledo as models.

This project allowed me to characterize the gut microbiome of these five species for the first time. Habitat/locality were the main factors driving gut microbiome differences between these species, and there was a greater microbiome diversity in species captured in Lisbon, the most urbanized studied site. I was able to verify a positive correlation between bacterial diversity and size in *P. siculus*, which indicates that it accumulates more bacteria, likely because bigger lizards explore more diverse environments. Additionally, I estimated bacteria transmission between sympatric lizards at Moledo and Parque das Nações. High transmission was verified for both populations. Results from the Lisbon population indicate that the invasive species, *P. siculus* might have acquired a higher proportion of local microbiota through the native species, *P. virescens* than vice versa.

The second study highlighted the infection prevalence differences between *P. bocagei* and *P. lusitanicus*, with a larger number of infected individuals in the former. However, *P. lusitanicus* registered a higher average parasite intensity. The species that interacted with the mirror the most, and was therefore considered bolder, was *P. lusitanicus*. Contrary to what was expected, the individuals that interacted with the mirror the most, were more parasitized.

This thesis aids in understanding the behavior and broadening the sphere of knowledge of microorganisms (bacteria and microparasites) and their interactions with the host. Such work is fundamentally important, and increased investment in these areas of knowledge is essential for species conservation.

Keywords: Gut Microbiome, Host, Environment, *Podarcis virescens, Podarcis siculus, Podarcis bocagei, Podarcis lusitanicus*, *Teira dugesii,* Apicomplexa, Behaviour.

Resumo

Existe um número crescente de evidências que apontam para uma grande importância das bactérias e dos parasitas na ecologia e nas dinâmicas dos hospedeiros e suas populações. Os animais têm complexas comunidades de microrganismos a viver no seu tracto intestinal, a que denominamos microbioma intestinal. Estas comunidades de bactérias podem influenciar e afetar a condição física do seu hospedeiro como a fisiologia, o sistema imunitário e também o seu comportamento. Por outro lado, a composição e a diversidade do microbioma intestinal pode ser influenciada por inúmeros factores como a taxonomia do hospedeiro, o habitat, a dieta e também as interações sociais entre indivíduos. Por sua vez, os parasitas que se encontram no sangue desempenham um papel fundamental no ecossistema, influenciando as aptidões físicas do animal, enfraquecendo a sua condição e possibilidade de reprodução e sobrevivência numa população.

Nesta tese foram utilizadas cinco espécies de lagartos, três endémicas e duas introduzidas, como objectos de estudo. No Parque das Nações, a *Podarcis siculus* (n=20) (invasora) e a nativa *Podarcis virescens* (n=22). E numa área isolada a 9 quilómetros do Parque das Nações, foi capturada a espécie introduzida, *Teira dugesii* (n=20). Em Moledo foram estudadas as espécies endémicas e sintópicas, *Podarcis bocagei* (n=33) e *Podarcis lusitanicus* (n=8).

Para compreender melhor o papel e a influência dos fatores ambientais e do hospedeiro na composição e diversidade do microbioma intestinal, foi realizado um primeiro estudo das cinco espécies mencionadas acima. Para um segundo estudo tínhamos como objectivo avaliar o possível efeito dos parasitas sanguíneos no comportamento de dois hospedeiros (utilizando um espelho no teste comportamental), e para tal utilizámos as espécies sintópicas que se encontravam em Moledo.

Neste projecto foi-nos possível caracterizar pela primeira vez o microbioma intestinal destas 5 espécies de lagartos. Os factores localidade e habitat, demonstraram ser os principais condutores de diferenças do microbioma intestinal entre estas espécies, havendo uma maior diversidade de microbioma nas espécies estudadas em Lisboa. Também observamos correlações positivas entre o tamanho e a diversidade bacteriana em *P. siculus*, indicando que esta pode obter mais bactérias dado que explora ambientes mais diversificados. Tendo em conta as duas populações com duas espécies coexistentes (Moledo e Parque das Nações) estimou-se a transmissão da diversidade bacteriana entre lagartos coexistentes. Deste modo, verificou-se transmissão elevada em ambas as populações, e em Lisboa os resultados indicam que a espécie invasiva, *P. siculus* pode ter adquirido uma maior proporção de microbiota local através da espécie nativa, *P. virescens*.

Os resultados do segundo estudo evidenciaram diferenças entre as duas espécies em termos de prevalência de infectados, com um número mais elevado de indivíduos infectados em *P. bocagei*. No entanto, a espécie *P. lusitanicus* foi a que registou maior intensidade de parasitas. A espécie que mais interagiu com o espelho, considerada por isso mais ousada, foi *P. lusitanicus*. Contrariamente ao esperado, os indivíduos que mais interagiram com o espelho foram os mais parasitados.

Os estudos elaborados ao longo deste trabalho constituem um auxiliar na compreensão do comportamento e no alargamento da esfera de conhecimento dos organismos e das suas interações com o hospedeiro. Assim, o aumento do investimento nestas áreas de conhecimento pode vir a revelar-se essencial para a conservação de espécies.

Palavras-Chaves: Microbioma intestinal, Hospedeiro, Ambiente, *Podarcis virescens, Podarcis siculus, Podarcis bocagei, Podarcis lusitanicus*, *Teira dugesii,* Apicomplexa, Comportamento.

Table of Contents

List of Tables

List of Figures

Abbreviations

ANOVA Analysis of Variance

PERMANOVA Permutational multivariate analysis of variance

ASV Amplicon sequence variant

DNA Deoxyribonucleic acid

PCR Polymerase Chain Reaction

rRNA Ribosomal ribonucleic acid

SVL Snout-vent length

PqN Parque das Nações

CHAPTER I

INTRODUCTION

1. Introduction

Lizards have been used as models in the field of parasitology since they have high levels and a great variety of parasites (Maia et al. 2012). Recently, they have also been used to study some aspects of host-microbiota interactions, such as the effects of gut microorganisms on thermoregulation (Moeller et al. 2020). In particular, lacertid lizards are easily observed and captured in nature and exist in large numbers, which can provide large sample sizes The wide distribution of these lacertid species, with many occurring in sympatry (Speybroeck et al. 2016), allow for comparative studies that indicate species-specific differences.

Parasites can have important deleterious effects on animal populations (Pedersen and Greives, 2008). Nonetheless, and despite their study has been ongoing for centuries, with knowledge consideringly expanding accordingly, many parasitic species remain undescribed (Carlson et al. 2020), which makes it even more fundamental to continue investing in this area. The importance of gut microbiome (i.e., the myriad of microorganisms and their genomes) on animal host's physiology and immune system is also undeniable (e.g. Broom and Kogut, 2018; Qian and Ho, 2020). Although the knowledge of the effects of microbiome on host behaviour still quite sparse among some animal groups, studies have shown that changes in the gut microbiome can lead to behavioural changes in insects, fish and rodents (Cryan and Dinan, 2012; Hsiao et al. 2013; Davis et al. 2016; De Palma et al. 2015; Sylvia et al. 2017). Still, this knowledge is largely lagging, especially for some host groups, and specifically for lizards.

Because of these factors described above, the studies described in this thesis can be very important in order to better understand the relationship between parasites and gut microbiome with their lizard hosts.

1.1 Reptiles

Reptiles (from the Latin Reptilia) belong to the phylum Chordata and are a class of tetrapods with more than 10,000 described species (Uetz et al. 2016). In the traditional sense it is a paraphyletic group, since birds are actually more closely related to crocodiles. The main groups of extant reptiles are turtles (order Testudines), tuatara (order Rhynchocephalia), crocodiles (order Crocodilia) and snakes and lizards (order Squamata). The reptile group is considered the largest vertebrate group after fish (~25,000 species) and birds (~11,000 species). Reptiles are found in all continents except Antarctica, although their main distributions comprise the tropics and subtropics (Pough et al. 1998). They occur in most habitats, from the open sea to mid elevations in mountainous habitats. Reptile activity is highly dependent on the temperature of the surrounding environment, as they are ectothermic and require an external heat source to raise their body temperature. Reptiles typically use exposure to sunlight to increase their body temperature and maximise physiological performance (Pough et al. 1998). Body temperature in reptiles is indeed an important factor for the optimal performance of several physiological functions, such as digestive efficiency, metabolic rate, locomotor performance and egg production (Angilletta, 2009; de la Cruz et al. 2014).

Podarcis (Reptilia, Lacertidae) wall lizards were only recognised as a distinct genus in the 1970s (Arnold, 1973) and are some of the most common reptiles across much of Europe, with 24 currently accepted species (Harris et al. 2005; Speybroeck et al. 2020). The phylogeny of the genus *Podarcis* (Lacertidae) was for a long time unresolved (Poulakakis et al. 2003) until recent studies including multiple nuclear genes (Salvi et al. 2021) and at the entire genomic level (Yang et al. 2021), resolved most phylogenetic relationships. Many of the known species of the genus *Podarcis* are believed to have had western European origins, their diversity being the result of several vicariance events mainly related to the fragmentation of western microplates during the Miocene (Oliverio et al. 2000).

Besides the various *Podarcis* species that are native to Portugal (e.g. *Podarcis lusitanicus, Podarcis bocagei, Podarcis virescens or Podarcis carbonelli*) (Pinho et al. 2011; Dias et al. 2016; Caeiro-Dias et al. 2021), there are also two accidentally introduced species in Lisbon, *P. siculus* and *Teira dugesii* (Silva-Rocha et al. 2016; Ribeiro and Sá-Sousa, 2018).

1.1.1 Invasive reptile species

Various lizard species can become invasive after arriving to a new area. Invasive species cause significant negative impacts on ecosystems, either ecologically, economically or even socially (Mooney, 2001). Awareness regarding the impacts of invasive species increased rapidly in the last decades, as globalization increased the pathways and speed of invasions (Seebens et al. 2018). For example, *Anolis carolinensis*, a lizard that was introduced from North America via Guam was responsible for a severe decline in native endemic insect species through predation (Tamate et al. 2017; Yoshimura and Okochi, 2005). Since biological invasions endanger the native biodiversity, it is important to understand the traits of successful invaders to in order to manage and mitigate their spread (Van Kleunen et al. 2010; Blackburn et al. 2011). The ability of invasive species to learn from conspecifics (social learning) can also give individuals an advantage in novel environments (Mennen and Laskowski, 2018). This was made evident in several studies, for example in the case of invasive crayfish species which were shown to have a greater capacity for behavioral plasticity, where individuals of the invasive species showed evidence of retaining learned associations for longer than individuals of the native species (Hazlett et al. 2002).

A large proportion of invasive species have an effective response and adapt well to varied habitats, particularly those species that are used to more anthropogenic places (Pettit et al. 2021). A number of factors contribute to such anthropogenic adaptation. Invasive species may be more exploratory in urban environments due to their long history of co-inhabiting with humans in such disturbed habitats (Hufbauer et al. 2012). For example, the invasive *Hemidactylus* geckos in urban spaces, are more willing to explore the empty spaces around artificial lights compared to native geckos, which enhances their predatory ability (Zozaya et al. 2015). Several studies also showed that invasive species are bolder (i.e. more willing to take more risks or expose themselves to potential predators, Réale et al. 2007) in new habitats compared to native species (Short and Petren 2008; Carthey and Banks 2018). Furthermore, the constant contact with presence of people in urban environments can lead to animal habituation, over time leading to a situation where humans are no longer seen as a risk, which results in the reduction of anti-predatory responses (Geffroy et al. 2015; Blumstein, 2016). Ultimately bolder species can have an advantage in urban environments and there are already studies confirming that there is an increase in boldness in animals living in urban areas compared to populations living in non-urban areas (Myers and Hyman, 2016; Biondi et al. 2020). The success of these invasions can also be studied and predicted through the presence or absence of parasites in the species in question, which may condition their establishment (Poulin, 2017).

1.2 Parasites

Parasites can affect a range of ecological parameters and behaviours in animal populations, having significant impacts on population dynamics (Ebert, 2005). Parasitism is known to be one of the most effective symbiotic relation between organisms, involving a high dependency relationship but conferring parasites a greater reproductive success and chance of survival (Hudson et al. 2006). These interactions derive from natural selection and give rise to parasitic organisms that are better adapted to their environment and its variations (Poulin, 2007).

Many studies have shown that parasites perform an important role in the ecosystem (Torchin et al. 2003; Prenter et al. 2004) and can influence their hosts' behaviour, metabolism and reproductive success (Schmid-Hempel, 2011). While some parasites can significantly reduce host fitness (Hudson et al. 2002, 2006; Pedersen and Fenton, 2007), others can contribute to the host performance, with beneficial parasite functions benefiting the host (Combes, 2001; Hatcher et al. 2012). Macro and microparasites can exist throughout an animal's body, but a large proportion is found in the blood, developing large communities that can also be transmitted between hosts either through direct social contact or by an intermediate vector (Godfrey et al. 2009; Godfrey et al. 2006).

Hosts and respective parasites can interact and influence each other in various ways. A single parasite can have a variety of hosts (Wang et al. 2006), or be extremely host specific (Routtu and Ebert, 2015). In some cases, there may be parasitological differences between sexes of the host, as for example in the case of some birds, where a lower immune response and smaller resistance to infection in females is observed relatively to their male counterparts (Schmid-Hempel, 2003). Animals infected with parasites may respond with behavioural and physiological defensive mechanisms. For example, a change in body temperature is one of the most common (Kluger 1991), with consequences for the metabolism of the host as well as to the growth and transmission of the parasite (Schmid-Hempel, 2011). Specifically, ectothermic animals such as reptiles may respond by moving frequently to warmer locations, to increasing their body temperature (Richards-Zawacki, 2010), which may in turn help reduce, or even eliminate, infection. Such change in body temperature as a response to infection can have more complex outcomes and, in some cases, benefit the host (Muller and Schmid-Hempel, 1993). However, in other cases, it may end up benefiting the parasite and result in a successful manipulation of the host (Schmid-Hempel, 2011). Several studies in lizards show that parasitism can lead to a decrease in body condition (Dunlap and Mathies, 1993), lower reproductive production (Sorci et al. 1996), reduced social status (Dunlap and Schall, 1995) and reduction in speed (Oppliger et al. 1996).

Generally, studies on the impact of parasites on a host population include a comparison of the physiology, fitness, or behaviour of hosts that are naturally infected in the population with those that are free from parasites. Nevertheless, many species of parasites remain undescribed, and this may greatly confound assessments in the field. This is particularly true for the obligate parasitic species of the phylum Apicomplexa.

1.2.1 Apicomplexa

The phylum Apicomplexa, Levine 1970, forms a large and diverse group of unicellular protists with a wide environmental distribution, and which likely evolved from free-living photosynthetic organisms (Gubbels and Duraisingh 2012). They are obligate intracellular parasites, and their name derives from their apical end, which is determinant during the process of invasion of host cells. The Apicomplexa contain four clearly defined groups (Adl et al. 2005): the coccidians, the gregarines, the hemosporidians and the pyroplasmids. The Apicomplexa is a taxonomic group with a great interest to researchers as it is the only one that is solely made up of parasites (Morrison, 2009) and because it includes species of high medical and veterinary importance (e.g. Shivaramaiah et al, 2014; Orosz, 2021). Despite being a group of interest, it is one of the groups with the smallest knowledge regarding its biology, with only an estimated 0.1% of species having been described (Morrison 2009). Indeed, it is estimated that all vertebrate and invertebrate animals are host to at least one species of Apicomplexa (Morrison, 2009) and this parasitism often results in harmful diseases to their hosts (White, 2008; Wichmann et al. 2009; Voorhis et al. 2021). As such, their study is very important to understand more about their biology and their consequences for the health of the host.

Haemogregarins (Apicomplexa: Adeleorina) are heteroxous parasites that infect a wide variety of hosts, including many species of reptiles. This group consists of three families, the Hepatozoidae (Wenyon, 1926), and Karyolysidae (Wenyon, 1926), Haemogregarinidae (Léger, 1911). Within these families there are six genera of blood parasites, differentiated in sporogonic development in their invertebrate hosts. In reptiles, the most commonly identified Apicomplexa are *Hepatozoon* (Telford, 2009) coccidians, but their taxonomy is complicated. Coccidian species of *Karyolysus* (Labbé, 1894) are also common but identification is difficult, and these have an additional layer of complexity as they are hyperparasites of ticks and mites, which are their final hosts (Haklová-Kočíková et al. 2014) and in turn (especially mites) are also poorly studied. *Karyolysus* is a well-defined group, which differs from the closely related genera *Hepatozoon* (fig 1) in several features of its biology, such as morphology and life cycle. The life cycle of *Karyolysus* includes the motile sporokinetes that are formed in oocyst by a single germinal centre, and are subsequently released into the host organism, where they encyst as sporocysts. In contrast, the life cycle of *Hepatozoon* is characterised by a large polysporocyst oocyst (Haklová-Kočíková et al. 2014).

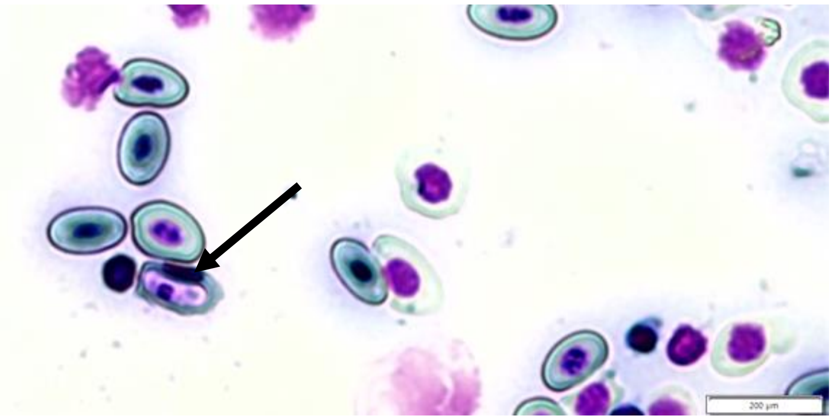

Fig. 1 – Picture of microscopic observation of a blood smear where a *Hepatozoon* spp. is indicated by the arrow. The photo was taken using OLYMPUS EP50 camera (40x objective) and the EPview software®. Picture by Diana Vasconcelos.

1.3 Gut Microbiome

Many microorganisms can be found living in the gastrointestinal tract of all animals, in particular bacteria. These microorganisms have a significant impact on host biology and can influence a variety of processes that can affect host fitness, including physiology, immune development and behaviour (Cryan and Dinan, 2012; Thaiss et al. 2016; Sylvia et al. 2017). Recent studies demonstrate that certain variations and changes in the composition of gut microbial communities can influence host physiology and also be a driver for disease (Martin et al. 2014; Boursier et al. 2016). Gut microbiomes can help with resistance to pathogens, nutrient uptake, energy acquisition, and digestion of certain substances (Rowland et al. 2018; Vavre and Kremer, 2014) and may also help their host adapt enabling a response to new metabolic challenges that may exist. An example of these challenges, is exploitation of new food sources (Delsuc et al. 2014; Hammer and Bowers, 2015), which in turn may have effects at the community level (Barrios-O'Neill et al. 2017). In other cases, specific bacterial taxa, such as *Bifidobacterium* and *Lactobacillus* in humans, have been identified with beneficial effects on host health (Walter, 2008)

Gut microbiota often have a symbiotic relationship with their host, mostly different levels of mutualism, where both host and symbiont benefit from the interaction. While these microbes use the host for shelter and nutrition and perform a variety of other important metabolic functions and assist their hosts with ecological interactions (Coyte et al. 2015; Douglas and Werren, 2016), thus being quite beneficial (Foster et al. 2017a); there are also many instances where only the microorganisms are benefited and the host suffers damage, for example in the case of some pathogenic bacteria (e.g. bateria

Escherichia coli or same *Clostridium* species; Khiav and Zahmatkesh, 2021 Mathipa and Thantsha, 2017).

Host taxonomy, habitat and diet have been considered central to gut microbiome composition and structure, but other factors such as social interactions or hosts' proximity have been recently proposed as important drivers of microbiome diversity (Moeller et al. 2013). Several studies have already been conducted on the gut microbiota in a range of vertebrates, such as mammals (e.g. Thaiss et al. 2016), fish (e.g. Xavier et al. 2019), birds (e.g. Hird et al. 2015) and amphibians (e.g. Bletz et al. 2016). However, microbiota of reptiles are relatively understudied, compared to those of other vertebrates. Most microbiome studies on lizards have been conducted in economically important or emblematic species, such as turtles (Arizza et al. 2019), snakes (Colston et al. 2015) and crocodiles (Willson et al. 2019). Available studies indicate that the gut microbial communities of reptiles are more similar to those of fish and birds when compared to mammals (Colston and Jackson, 2016), thus partially reflecting hosts' phylogenetic relations. There are also studies showing strong evidences that the microbiota of squamate reptiles derive from their environment and from horizontal transmission (via the environment or other host organisms) through interaction with other organisms, such as predatory encounters (Colston, 2017). However, the influence of individual host traits, such as age and sex are less studied.

Below, some key factors and their influence on this microbiome diversity and composition will be presented in more detail.

1.3.1 Diet

Diet is considered one of the main factors that can influence gut microbial community diversity and composition in species that have a resident microbiome (Muegge et al. 2011). Different dietary substrates can lead to the growth of microbial taxa with specialized metabolic functions which in turn can lead to variations in taxonomic abundance (De Filippo et al. 2010; Scott et al. 2013). Therefore, any drastic dietary changes that can arise are likely to induce changes in the gut microbiome (Ilmberger et al. 2014; Kohl et al. 2013). Even in humans, dietary differences between African and European populations have resulted in a divergent evolution of microbiomes in terms of species' composition (De Filippo et al. 2010).

1.3.2. Habitat

Another factor that can have an influence on the gut microbiome is the habitat (Amato et al. 2013). However, probably its potential to affect the gut microbiome can be increased with the association between the habitat and food resources available at a particular location (Barelli et al. 2015).

Urban areas are rapidly expanding, affecting natural landscapes, and causing dramatic changes in terrestrial ecosystems and with associated impact on wildlife ecology, biodiversity, and function (Grimm, 2008; Seto et al. 2012). For animals living in urban environments, interactions with urban landscapes can influence animal movement, predation risk, and reproductive success and also available food resources (Weiser and Powell, 2010). For many species, the selective pressures created by the expanding urban landscape (Foley, 2005) may lead to local extinctions and consequent decreases in local biodiversity (Concepcion, 2015), although still there are generalist species that can thrive and adapt to urban environments (Ducatez et al. 2018). The success of these species is mostly enabled by behavioral adaptations, such as broadening their diet by exploiting anthropogenic food sources, which can often be variable (Lowry, 2013). Dietary changes resulting from habitat alteration can in turn affect and modify the gut microbiome and its diversity. While this may have positive effects, when gut microbiota remains healthy and assisting the organism in necessary functions, it can also have negative effects (Rocca et al. 2019). For example, a recent study in urban coyote hosts showed that the consumption of anthropogenic carbohydrate-rich foods altered their microbiome, negatively affecting their body condition, with potential consequences to parasite susceptibility and conflict-prone behavior (Sugden et al. 2020).

Habitat sharing may also have effects on the composition and diversity of the gut microbiome. For example, sympatric members of different species that share the same habitat, such as gorillas and chimpanzees, have a more similar gut microbiome than allopatric members of the same species (Moeller et al. 2013).

Despite the rapid increase in urbanization and its impact on ecosystems, most microbiome studies are conducted on animals in captivity or in natural environments. However, humans and wild animals coexist in such environments, and given the recent awareness of the role transmission may have on gut microbiome of co-existing species (Ducatez et al. 2018; Alm et al. 2018), more studies are needed to improve our understanding of how urban pressure can influence gut microbiome transmission and health between co-existing hosts.

1.3.3 Behaviour

Associations of the gut microbiome with diet as well as with social interactions have been documented in wild animals, both to understand the effect of host behaviour on the microbiome, as well as effects in the opposite direction, i.e. the influence of the microbiome on host behaviour. These links can ultimately play a key role in host fitness and influence community-scale dynamics.

Recent research has been important in demonstrating and understanding how the vertebrate gut microbiome can have diverse and powerful effects on the brain and behaviour (Cryan and Dinan, 2012; Bharwani, 2017; Sylvia et al. 2017). Gut microorganisms perform a variety of vital functions for their hosts, including communicating with the immune system, brain and endocrine axes to mediate physiological processes and affect individual behaviour. There is also evidence that gut bacteria can have an impact on neurological outcomes, potentially altering behaviour and also potentiating or aggravating disorders of the nervous system (Sampson and Mazmanian, 2015; De Palma et al. 2015). It is presently well established that this occurs because the microbiome extends its influence to the brain through various pathways that link the gut to the central nervous system.

Furthermore, an important part of the immune system resides in the gut (Vighi et al. 2008) and increasing numbers of studies are continuing to demonstrate that gut microbes may be intrinsically linked to stress-induced changes, which may have a compound effect and alter the animal's behaviour (Neufeald et al. 2011; Foster et al. 2017b). Importantly, many other recent observations indicate that the gut microbiota by altering aspects of their hosts' neurological function, can lead to alterations on mood and behaviour, including anxiety, social behaviour, depression, and even mate choice (Desbonnet et al. 2010; Bravo et al. 2011; Hsiao et al. 2013; Sharon et al. 2010).

1.4 Experimental approached of behaviour studies focusing on reptile agonistic behaviour

Lizards are territorial animals that may have agonistic encounters with another individual for a limited resource, such as food, shelter, partners and territory (Hack, 1997). These encounters entail risks for the parties involved, such as injury, predation or even mortality (Lange and Leimar, 2001) and may involve various types of stimuli and responses from the lizard. Territorial communication in lizards overall relies on visual and chemical stimulus. The success of communication depends on the signal transmitted between a transmitter and a receiver, which must be clear to be able to perceive them in the face of other potential stimuli (Fuller and Endler, 2018). Lizards often use chemical stimuli for their communication, but they also use plenty of visual stimuli which can be based on more aggressive behaviours.

Research on lizards' visual stimuli mainly focusses on four methodological approaches: staged direct encounters with between lizards, silicone models that mimic the shape and colours of the animal, video playbacks shown to the lizards, and the use of a mirror to reflect the lizards in test. The later can have many advantages: mirrors are the most commonly used visual stimuli in experimental designs because they are easy to obtain, inexpensive, and can generally fulfil the objective of stimulating agonistic behaviours against the reflected image, related to physiological and morphological aspects (Balzarini et al. 2014). Furthermore, the signal is enhanced by positive feedback (Scali et al. 2019), because the postures or behaviours made by the lizard are immediately replicated by the mirrored lizard. This methodology has been successfully applied to several lizard families, such as Agamidae, Phrynosomatidae, and Dactyloidae, which have very different physical characteristics, and results are more noticeable when the lizards are being particularly territorial, for example during the breeding season (Farrell and Wilczynski, 2006; Osborne et al. 2012).

1.5 Objectives

This thesis is focused on five species of lizards in Portugal, three native *Podarcis virescens* (Geniez et al. 2014), *Podarcis bocagei* (Seoane, 1884)*, Podarcis lusitanicus* (Caeiro-Dias, 2021) and two introduced, *Podarcis siculus* (Rafinesque-Schmaltz, 1810), and *Teira dugesii* (Milne-Edwards, 1829). Because some populations of these species live in sympatry (sharing habitat type), and some are native and others are invasive, they were considered good models to carry out an evaluation and comparison of parasitological and gut microbiome aspects.

There is already a high degree of knowledge regarding the parasites infecting these lizards in Portugal. For example, in the Parque das Nações (Lisbon, central Portugal), invasive *P. siculus* was found to be less parasitised by haemogregarines, than native congeneric *P. virescens*, both in terms of prevalence and intensity of infection (Tomé et al. 2021). In the sympatric populations of *P. lusitanicus* and *P. bocagei* residing in Moledo (Northern Portugal), previous studies indicate that the former had higher levels of parasite intensity compared to the latter (Maia et al. 2015). As such they become populations of interest to perform further studies concerning parasites and their influences and consequences.

The first objective of this thesis was to characterise for the first time the gut microbiome of these five species, and specifically to understand which factors might influence and predict the diversity and composition of bacteria present in the gut of these species. My working hypothesis was that host taxonomy (i.e. factor species), habitat, size and sex could be used as predictors of microbiome diversity and composition. Additionally, I also wanted to investigate potential transmission of microbiota between sympatric hosts.

The second objective of this thesis was to use an experimental approach to investigate the relation between the parasites present in lizards and the boldness they could show in behavioral studies. According to the studies already mentioned, my working hypothesis was that a low rate of parasites in the blood favors a bolder behaviour on the part of the individual, demonstrated by a greater interaction with the visual stimulus (the mirror).

The results of this thesis are presented in two different chapters, in the form of manuscripts. Below, I give a detailed overview of studies species and sites, and a general overview of the methods used in both main chapters.

2. Methods

In this thesis, various methodologies were used in order to study the parasites, gut microbiome and also the behaviour of lizard species.

2.1 Study areas

In this thesis, individuals studied were captured in two different areas: Parque das Nações (PqN, Lisbon, central Portugal) and Moledo (North of Portugal).

The PqN is an urban area that was built very recently (about 24 years ago), with extensive human influence, including buildings such as the Gare do Oriente, the Altice Arena, Lisbon Oceanarium, International Lisbon Fair, Casino de Lisboa and the Vasco da Gama Tower among others. There are also many supermarkets, cafes, hairdressers, and restaurants which makes this a highly frequented place. In PqN, the green areas were developed linearly, and occur throughout the area, defining a network of corridors that follow the pedestrian circuits. The plant species are varied, ranging from those native to Portugal and those originating in different regions of the world. The entire studied area is characterized by a Mediterranean climate, i.e. hot and dry summer and a wet and rainy winter, most precipitation occurring in the period between October and April (Santos et al. 2012).

The species captured in PqN were *Podarcis siculus,* a recent invasive species, and *Podarcis virescens,* a native species, that are living in sympatry in the gardens (38°76'22.4 "N, 9°09'44.3 "W). Another species was studied in Lisbon, *Teira dugesii* introduced from Madeira Island, that lives in a totally artificial environment and in a more isolated but equally urbanised site, 9.2 km west from the PqN (see figures 2-5).

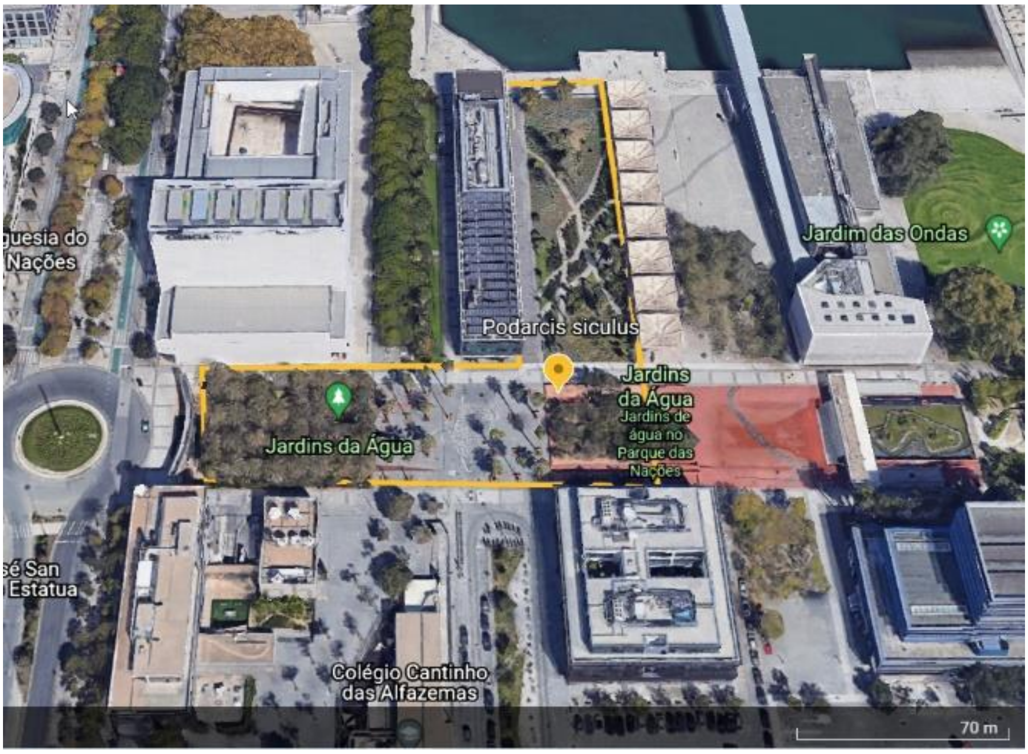

Fig. 2 - Capture sites of the invasive species *Podarcis siculus*, in the gardens in front of the Lisbon Oceanarium (Parque das Nações).

Podarcis siculus, was captured in an area with lawn gardens with a more exotic appearance, in front of the Oceanário de Lisboa (fig 2). The species is still present at this site after its introduction about two decades ago (González de la Vega et al. 2001).

The garden area of Parque das Nações where the individuals of the native species *P. virescens* were captured is located between the Vasco da Gama Tower and the Altice Arena (fig 3), about 900 meters away from the exact place where *P. siculus* was captured. This a large area of gardens all along the street, parallel to the building of the Lisbon International Fair, and where there are various substrates, such as walls and artificial structures on the ground, taller vegetation, trees and also more undergrowth vegetation such as shrubs and grasses. Although the collections were made in this location, this species, *P. virescens*, is spread in many more places all over the Parque das Nações.

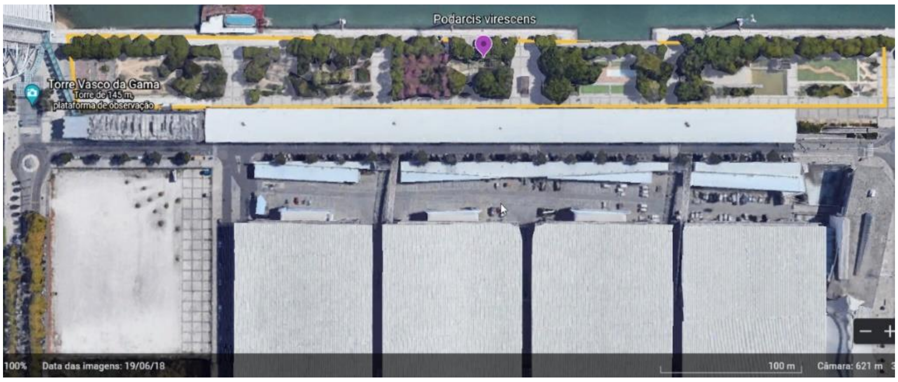

Fig. 3 - Capture sites of the native species *Podarcis virescens*, in the gardens near Torre Vasco da Gama (Parque das Nações).

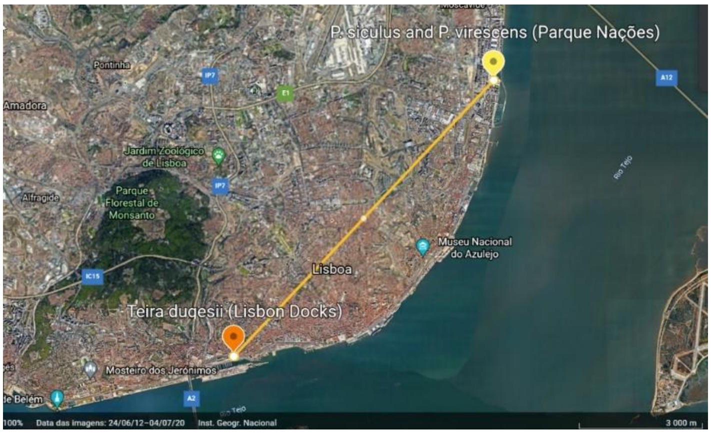

Fig. 4 - Distance between the capture site (around 9,22 quilometers) of the two sympatric species (*Podarcis virescens* and *Podarcis siculus*) represented with yellow marker and the introduced species *Teira dugesii*, with orange marker.

The site where the Maderian lizard, *Teira dugesii*, was captured is located near the Alcantara neighbourhood (38°70'33.8 ''N, 9°16'54.1 ''W), close to the port area of Lisbon (fig 4), living on the ground under bushes near the railway fence, along a belt area extending about 1.15 metres.

The first specimens of this species were detected for the first time in 1992 (Sá-Sousa 1995) in this area, which is a completely artificial and very restricted habitat (fig 5) where this species is established until today (pers. obs.). Although this area is more reserved than PqN, it has a lot of movement caused by the transport of products carried out there.

Fig. 5 - Site of capture of the introduced species, *Teira dugesii*, near the Lisbon Docks.

The second study area is a more isolated area near Moledo Beach (41°50'19.2 "N 8°52'24.5 "W) along an extension of around 0.5 kms (Fig 6). Being a coastal location, it is characterized by being windy and a little cooler during the cold season, but can reach very high temperatures in summer. There are different types of habitats in this area. On one side there is a sandy area with rocks and some undergrowth that is separated by a small concrete road from the area with more vegetation. The vegetated side has several places to shelter like walls, bigger rocks and also has bushes and small trees. It is an area used for agricultural purposes, although most of the fields seem to be abandoned. As far as disturbance is concerned, there is little human movement at this site in comparison with the Lisbon studied areas, so it can be considered as being in a non-urban area. The biggest human disturbances are mainly on the walkway which people use for leisure and sport (in the summer time the human presence increases a little). There are also some flocks of sheep and goats that go to the fields where the lizard species feed in the morning, which may disturb them during this short period of time. In this area, individuals of the species *P. bocagei* and *P. lusitanicus* living in syntopy (in the same habitat and observed in close proximity) were captured (Rivas, 1964).

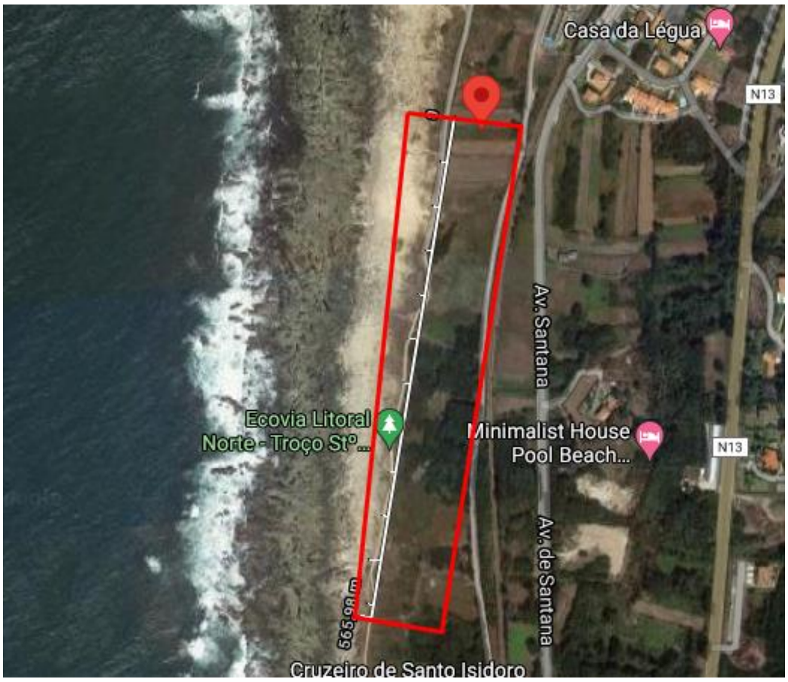

Fig. 6 - Place of capture of both species in the Moledo population (*Podarcis bocagei and Podarcis lusitanicus*), near Moledo Beach.

2.2 Study species

2.2.1 Podarcis siculus

Podarcis siculus, is considered the most abundant lizard species in southern Italy (Verderame and Scudiero, 2019), and occurs all over the country, with the exception of the Alps. This species has been introduced in several places in the Mediterranean region, including Portugal, Spain, France, Montenegro, Croatia, Turkey, Libya, Tunisia (Podnar et al. 2005) and also to the USA. A total of 52 subspecies of *P. siculus* have been described, based on minor morphological differentiations (Podnar et al. 2005).

It is a robust and medium-sized lizard (Freiría, 2005) that can reach 90 mm in snout-vent length, and there are adults with a body mass of 7- 14 grams (fig 7). This species can be found in various types of habitat, such as lawn areas, bushes, orchards, vineyards, urban areas, stone walls and buildings, also showing a greater preference for arid and sunny sites than other *Podarcis* species (Capula et al. 1993). It has a robust head and a green dorsum, brownish on the back, it has sexual dimorphism, with males having a more intense green dorsal colouration and a greater number of femoral pores than females (Tosini et al. 1992; Vogrin, 2005; Salvador, 2015). This is an oviparous species, characterised by an annual reproductive cycle, with a breeding season between March and July. They feed mainly on insect larvae and worms, but also occasionally on fruits and vegetables (Capula et al. 1993; Rugiero, 1994; Zuffi and Giannelli, 2013). It is a species with great plasticity in the spatial use of habitat, occupying natural and modified habitats, agricultural or urban, and with a high adaptability to climatic conditions, characteristics that will have favoured its passive transport with goods (Carretero and Silva-Rocha, 2015). In its sites of its introduction may be predated by wild cats (Pleguezuelos et al. 2002), and pet trade is also a threat to this species, since these lizards can be used as food for other reptiles. Because it is an introduced species and is in possible expansion, it was classified as of Low Concern on a global scale. This classification is mainly due to its tolerance to a wide variety of habitats, its wide distribution and its high number of individuals.

The region of Rome was pointed out as the most likely origin of the individuals introduced in Lisbon (Silva-Rocha et al. 2014), through phylogenetic analyses. The first citation of the Italian lizard *P. siculus* in Portugal, reports an observation of individuals in the Parque das Nações area (González de la Vega et al. 2001). The individuals introduced in Lisbon are believed to have been transported from their place of origin along with various materials and plants approximately two decades ago, during the preparations for Expo98 event (González de la Vega et al. 2001), where they would be taking refuge or thermoregulating (Silva-Rocha et al. 2012). This contrasts to the introductions in the United States of America, which were related to the pet trade (Kolbe et al. 2013). More studies report evidence of anthropogenic introduction of *P. siculus* through trade of olive trees imported to Spain from Calabria (Italy) (Rivera et al. 2011; Valdeón et al. 2010).

Previous studies on lizard populations from PqN suggest that *P. siculus* and *P. virescens* use the available microhabitats differently. This is commonly reported for sympatric species, when they enter into direct competition end up spatially separated many times, probably due to differences in structural niche, or differences in environment in the same habitat or even result from the displacements of individuals by competition, or by anti-predatory behaviour (Downes and Bauwens, 2002). In this area *P. siculus* avoids totally artificial substrates and preferentially uses habitats with a preferentially terrestrial substrate texture, which are poor in tree and shrub cover, and they use available vegetation cover as shelter. However, despite being bolder, it currently has a more limited and restricted spatial distribution compared to the native *P. virescens* and there are still no reports of marked spatial invasion by *P. siculus* into the preferred areas of *P. virescens* (Vignoli et al. 2012). *P. siculus* in PqN may face some challenges, with recorded events of predation of an adult specimen of this species by the gecko *Tarentola mauritanica*, which is also known to consume juveniles of other species of the genus *Podarcis* (Pellitteri-Rosa et al. 2015).

Fig. 7 - Ventral and dorsal pictures from a male *Podarcis siculus*. Pictures taken by Diana Vasconcelos.

2.2.2 Podarcis virescens

This species was previously included in the paraphyletic species complex *Podarcis hispanicus*, as morphotype II (Harris and Sá-Sousa, 2001, 2002; Pinho et al. 2006), and was later raised to full species as *Podarcis virescens* by Geniez et al. (2014), given that the epithet virescens is a derivative of the Latin verb "viresque" meaning "to turn green, become green". It is an Iberian endemism (Dias et al. 2016) and has been suggested to belong to the Least Concern category due to its extensive range and acceptable tolerance to habitat modification (Carretero and Salvador, 2016).

At the national level, this lizard predominates in typically Mediterranean conditions and in regions below 400 metres altitude, in the centre and south of the country, not being found north of the Douro River (Harris and Sá-Sousa, 2002). Its habitat is dominated by plains and lowlands and it is also found in human habitats, like other Iberian *Podarcis* species, preferring especially hard substrates, such as walls or outcrops, in relatively humid situations (Geniez et al. 2014).

It is a species with a moderately robust and flattened head and body with dorsal patterns often greenish or light brown in colour, and a yellowish or orange belly (Fig 8) (Harris and Sá-Sousa, 2001). The back of the males is often greenish in spring, especially on the posterior part. This colouration disappears during the summer. The average head and body length is 54.3 mm with adult males between 40 and 62 mm (Geniez et al. 2014). Sexual dimorphism is evident, with both sexes differing in size, shape and intensity of the colouration pattern, with males being larger than females and males having well-developed femoral pores (Kaliontzopoulou et al. 2011; Carretero and Salvador 2016). *Podarcis virescens* is an active and generalist predator, feeding mainly on individuals of the class Arachnida and the orders Coleoptera, Hemiptera, Hymenoptera and Diptera (Juan, 1997). The greatest threats to this species are probably the same as for the other lizard species, such as domestic cats (Loureiro et al. 2008).

The population of *P. virescens* in PqN may have its distribution influenced by this introduction of *P. siculus*, although negative impacts have not yet been specifically identified. Several observations in Parque das Nações point to a scenario of no expansion by the invasive *P. siculus* (Loureiro et al. 2008; Ribeiro e Sá-Sousa, 2018). However, the data obtained suggest that this site division was most likely due to competitive exclusion, and as *P. siculus* has a larger body than *P. virescens*, it may be an advantage in competitive encounters as larger species tend to be dominant over smaller ones (Downes and Bauwens, 2002). This feature seems to have favored *P.siculus* over the native species, since in Lisbon the species are shown to be sympatric but not syntropic.

Fig. 8 - Ventral and dorsal pictures from a male *Podarcis virescens*. Pictures taken by Diana Vasconcelos.

2.2.3 Teira dugesii

Teira dugesii (Milne-Edwards, 1829), the Madeiran wall lizard, is a species of small lizard belonging to the family Lacertidae that is endemic to the Madeiran archipelago including the Selvagens islands. This species was placed in the genus *Lacerta* but was later transferred to the subgenus *Teira* based on morphology (Richter, 1980). This lizard can reach up to 20 cm in length, although adults are usually between 10 to 15 cm. Its colour can range from light brown to dark grey with some marbling, with some specimens, usually the males, also being able to display iridescent colours such as green, blue and violet (Fig 9). Males can be easily distinguished from females by the presence of a yellow nuptial fold on the underside of their hind legs. In its native habitat, although it is essentially an insectivorous species, it also feeds on ripe fruit and berries. It occurs across the Madeira Archipelago, where it is abundant and present in practically all types of terrestrial habitats of the archipelago, from the coast to the highest mountains, through urban areas, gardens, rural areas, pastures and beaches. There are also some populations in the Azores Islands, which were also introduced from Madeira, probably accidentally during the 19th century by ships travelling between the two archipelagos (Silva-Rocha et al. 2016).

In mainland Portugal, there is a small population in the port area of Lisbon, probably accidentally carried on transport ships, which is originated from Madeira Island (Silva-Rocha et al. 2016) having been detected for the first time in 1992 (Sá-Sousa 1995). A later study (Sá-Sousa pers. obs) found that the population has remained stable in size since its discovery.

Fig. 9 - Ventral and dorsal pictures from a male *Teira dugesii*. Pictures taken by Diana Vasconcelos.

2.2.4 Podarcis bocagei

Podarcis bocagei (Seoane, 1884) displays a clear phylogeographic pattern, evidently different from the other Iberian *Podarcis* belonging to the *P. hispanicus* complex (Pinho et al. 2008; Kaliontzopoulou et al. 2012). Males exhibit greenish tones on the dorsal area with the pattern generally spotted on the greenish background, which during the mating season their ventral area also displays reddish or yellowish tones. Females are brownish on the back, displaying green dorsal stripes (fig 10).

It is an endemic species of the northwest Iberian Peninsula that occurs north from the Douro River and with some relatively isolated populations in Trás-os-montes, requiring relatively humid climates (Sá-Sousa, 2001). Its distribution is abundant and it is capable of occupying different types of habitats, such as scrubland, forests, meadows or rocky areas, and it can be found in stony settlements, hedges and walls, preferably in sunny areas. It is also well adapted to humanised landscapes such as farmland, settlements and industrial areas.

This species lives in sympatry with *P. lusitanicus* in some populations in Portugal, and in particular there is a sympatric population in Moledo. The differentiation between *P. lusitanicus* and *P. bocagei* took place about three million years ago and they are considered sister lineages (Caeiro-Dias et al. 2021). Their adaptation to different types of habitat may have favoured the isolation between the two and while *P. bocagei* is found more on the ground, *P. lusitanicus* lives more on rocks (Carretero et al. 2015). Furthermore, males of *P. bocagei* and *P. lusitanicus* are able to chemically discriminate between females of their species and the other, although females do not have the same ability to discriminate males of both species (Carretero et al. 2015). These differences in chemical signals and discrimination between females likely contributed to an isolated reproduction (Barbosa et al. 2006). Head morphology is also different in the two species, being more robust in *P. bocagei* and flattened in *P. lusitanicus*, which is related to the type of habitat in which each one lives (Kaliontzopoulou et al. 2012).

Fig. 10 - Ventral and dorsal pictures from a male *Podarcis bocagei.* Pictures taken by Diana Vasconcelos.

2.2.5 *Podarcis lusitanicus*

The species *Podarcis guadarramae*, was formally considered as a species by Geniez et al. (2014), where the subspecies *Podarcis guadarramae guadarramae* was assigned to the eastern populations of the called Type 1A lineage (following Pinho et al. 2008) and the western populations of Type 1B were named as a new subspecies, *Podarcis guadarramae lusitanicus* (Geniez et al. 2014), which was recently considered as a full species, *P. lusitanicus* and not actually the sister taxon of *P. guadarramae* (Caeiro-Dias et al. 2021).

Podarcis lusitanicus (Geniez et al. 2014), the recently accepted species is found mainly in northern Portugal and north-western Spain. It is an insectivorous, diurnal, small-sized lizard with an average snout-vent length in males between 41.5 and 62.5 mm and the females having a range between 40 and 60 mm (Carretero et al. 2015). The average weight is 2.8 g in adult males and 2.1 g in adult females (Galán, 1986). It has a flatter head than *P. lusitanicus* and the male Iberian wall lizards have mostly reticulate dorsal pattern, while females have a pattern with longitudinal lines with straight edges (fig 11). There is also territoriality between males, where larger males dominate smaller males (Carretero et al. 2015). The distribution of *P. lusitanicus* in northern Portugal, is somewhat irregular, with populations located on open natural rocky outcrops and artificial stone walls around agricultural fields, using areas with rocks and less vegetation for thermoregulation and shelter (Diego-Rasilla and Pérez Mellado, 2003). Studies in northern Portugal (Gerês) indicate that its abundance increases in repeatedly burned areas possibly due to the burned areas being favourable habitats, such as bare rocks (Mateus, 2014).

In Portugal, in this population, *P. lusitanicus* lives in sympatry with *P. bocagei* on the Portuguese coast where there is a large overlap of microhabitats, although *P. lusitanicus* tends more to use open rocky habitats than *P. bocagei* (Sillero and Gonçalves-Seco, 2014). However, it is not the difference in locomotor ability of the two species that explains such use, but this difference may be attributed more to shelter use due to a greater flattening of the head in *P. lusitanicus* (Kaliontzopoulou et al. 2012).

Fig. 11 - Ventral and dorsal pictures from a male *Podarcis lusitanicus*. Pictures taken by Diana Vasconcelos.

2.3 Field work

For this study we sampled 103 adult lizards, three species (*P. siculus, P. virescens* and *T. dugesii*) were captured in Lisbon on the 15th and 16th of September 2020 and two species (*P. bocagei* and *P. lusitanicus*) in Moledo on the 28th and 29th of September 2020. The lizards were caught with a noose, consisting of a slipknot at the end of a fishing rod. This method is used by most herpetologists because it is a more effective method and does not harm the animal during capture. After capture, the lizards were removed from the noose as quickly as possible to cause them the least possible stress.

2.3.1 Microbiome sampling

Cloacal swabs were collected immediately after capture with a sterile swab (MedicalWire), inserting it into the entrance of the cloaca (taking care not to touch the cloacal area of the lizard with the hand to avoid contamination). After the animal was identified with a number written on the belly with a permanent ink, and swab sample was identified accordingly. The tip of the swab was cut, and swabs were immediately stored in eppendorfs at −20 °C, until transportation on dry ice to the CIBIO-InBIO laboratory (University of Porto), where they were kept at −80 °C until further processing. Still in the field, data was taken from each lizard, such as species, sex, tail condition (regenerated or original) and also the Snout-vent length (SVL) using a digital calliper. Dorsal and ventral photographs of the lizard were also taken. Before releasing, a small part of the tail was removed and kept in a microtube capped with 96% ethanol to preserve the material. With the blood released from this cut, blood smears were made, and spots on Whatman filter paper. This collection of blood samples was not always be possible due to the lack of fluidity and coagulation of the blood. All these samples were identified and stored in a small ice box, for better conservation. At the end of the sampling, the lizards were released at the location where they were caught.

2.4 Laboratory work

2.4.1 Parasite analysis: Blood smears

To extract DNA from blood parasites of a lizard we can use either tissue or blood samples, since both work well for this group of parasites (Maia et al. 2014). In this study both analyses were performed using the samples collected during fieldwork after the capture of the lizard. In this study we obtained from the captured lizards muscle tissue samples (lizard’s tail), blood smears (obtained from the blood released in the tail cutting) and blood spots on Whatman filter paper (stored and kept at -20°C). The blood smears were used for microscopy analysis which may allow us to detect parasitic gamont stages, and in this way enable morphological identification. The blood spots were used for genetic analysis.

Blood smears were obtained by smearing a drop of blood on a glass slide, which was air-dried and then fixed with absolute methanol, as soon as possible. On the following days, the slides were stained with Giemsa standard stain (ratio of 1:9) for 45 minutes and left to dry at room temperature. During the preparation of blood smears, some care is needed to obtain the best quality blood smears possible. When performing them in the field it’s important to avoid dust, excessive heat, humidity or exposure to the sun. Furthermore, at the time of fixing and staining, care must be taken with timing, as this may compromise the quality of the sample (Valkiunas et al. 2008).

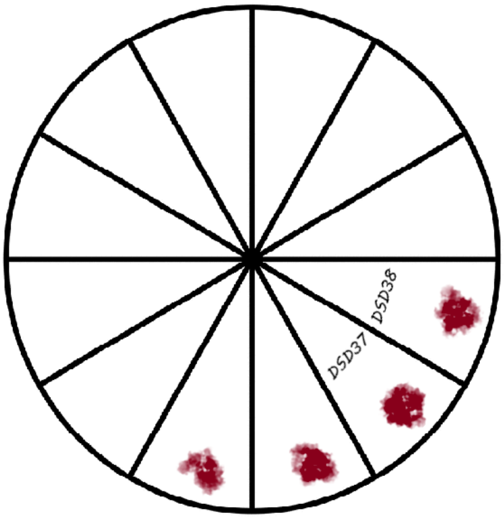

Fig. 12 – Illustration of the blood spots in Whatman filter paper, with the identification of each sample in each section. Image by Diana Vasconcelos.

2.4.2 Microscope analysis

Microscopy was developed in the 15th century by Antonie van Leeuwenhoek and is a method that has been used for centuries. It is used to help detect, identify and quantify blood parasites (Moody, 2002) and is the main source for current knowledge about parasites and is widely used in the study of Apicomplexan parasites, including haemogregarins (Amo Fargallo et al. 2005; Roca and Galdón 2010). It allows the detection of parasites in their different stages of development, quantify parasite intensity (percentage of infected cells per individual) and obtain the parasite prevalence (the percentage of infected individuals in a population). It is also possible to observe the morphology of the parasite and the type of blood cell being parasitised (Moody, 2002). One of the points in favour of its use is also that it is a comprehensive technique, well studied, easily used and understood, and above all is economical. And despite all the technological advances and innovation in recent years, it continues to be widely used to study various groups of parasites

In this study, to identify the parasites in these lizards, we used microscopy. Microscopy may be unable to correctly identify at the genus level some haemogregarines, because in the gamont stage the cells have a similar appearance (Telford, 2009) and because different morphologies are observed in different developmental stages, which can lead to misidentifications (Jakes et al. 2003). As such, the use of molecular tools to complement microscopy is highly desirable (Sloboda et al. 2007).

Microscopy may not be the fastest method, but with the help of available software built into the microscope interface, such as the most recent EPview software® coupled with the OLYMPUS EP50 built-in camera with the 40x objective, it can easily capture images for later observation and analysis. There is also ImageJ® software with a cell counter plug-in, which makes the work easier and reduces time on the microscope. With this software the parasite intensity can be assessed by counting the number of infected cells within a previously defined total number of cells (Margolis et al. 1982; Bush et al. 1997). However, the total number of erythrocytes counted varies according to studies and researcher preferences, ranging from 2,000 (Amo Fargallo, et al. 2005; Amo López et al. 2005) to 10,000 cells (Valkiunas et al. 2008).

For this thesis, the value assumed for counts was a minimum of 2000 total erythrocytes, which is considered enough to give accurate results (Godfrey et al. 1987).

Fig. 13 – Picture of microscopic observation of a blood smear with a Hepatozoon (red circle), among erythrocytes (yellow circle), using EPview software® with the OLYMPUS EP50 camera (40x objective). Picture by Diana Vasconcelos.

2.4.3 Parasite DNA extraction, amplification and sequencing

Due to the difficulties and possible uncertainties in identifying parasites with microscopic examination, molecular determination of positive samples can be used in conjunction with microscopic assessment, giving further information that is invaluable for species identification and for phylogenetic reconstruction. In this thesis, the method used for DNA extraction of the tissue samples (lizard tails) was the high-salt method (Sambrook et al. 1989). Following DNA extraction, amplification was performed via polymerase chain reaction (PCR) (de Waal, 2012), performed with the temperatures shown in the table below (table 1), and using Bioline's MyTaq™ HS Mix, 2x and incorporating primers that are designed to amplify the parasite of interest (eg Ujvari et al. 2004). In this case, primers were originally designed to amplify a 600 bp long region of the 18S rRNA gene of haemogregarines, HepF300 and HepR900 (Ujvari et al. 2004), following the conditions of Maia et al. (2012) and which are known to amplify the DNA of several different Apicomplexa infecting lizards. The 18s rRNA gene is considered a good marker and has been almost universally used for phylogenetic reconstructions of protists, including Apicomplexa organisms (Perkins et al. 2011). These rRNA genes are mostly used because they have some characteristics that facilitate their amplification, such as a large abundance of their transcripts in the cell, and also allow the easy design of primers as they have highly conserved regions, but which provide phylogenetic information necessary (Perkins et al. 2011). While these primers were designed to amplify haemogregarines, they also amplify other related parasites (Maia et al. 2012), so a positive PCR reaction alone cannot be used to confirm the presence of a particular parasite – examination of the resulting sequences is needed.

Table 1 – PCR termoclycler conditions used in this study with HepF300 and HepR900 primers.

	Temperature (ºC)	**Time (min)**	**Number of cycles**
Initial denaturation	95	10:00	1x
Denaturation	95	00:30	40x
Annealing	61	00:45	
Extension	72	1:00	
Final extension	72	10:00	1x
Cooling	12	∞	-

2.4.4 Microbiota DNA extraction

A major challenge for microbiological studies is to maintain uniform and accurate DNA extraction in the presence of samples with a wide range of bacterial content. For bacterial sampling studies, various collection methods have already been used, but we chose to use an extraction kit by the non-invasive method, as used in some previous studies (e.g. Xavier et al, 2020). To obtain the best results and avoid contamination, it is important to perform DNA extraction using a dedicated laboratory suitable for this purpose. For this study, the samples used were cloaca swabs from the captured lizards and the kit used was the DNeasy® PowerSoil® Kit (QIAGEN, Hilden, Germany). The tips of the swabs were placed in PowerBead tubes containing 60 μL of C1 solution and shaken horizontally for 10 minutes. The remaining steps were performed according to the kit manufacturer's instructions. At the time of each extraction batch a negative control was also used for quality control.

The V4 region of the 16S rRNA gene (~ 250 bp) was amplified from each sample using the Kozich et al. (2013) protocol and sequenced in a single MiSeq run. This service was outsourced to Michigan University (https://microbe.med.umich.edu/). The 16S rRNA V4 region has been widely used to characterise bacterial communities from various vertebrates (Chiarello et al. 2018; Colston and Jackson, 2016; Wong and Rawls, 2012).

2.4.5 Microbiome sequence quality filtering and statistical analysis

The raw FASTQ sequences were processed using the QIIME2 (Quantitative Insights into Microbial Ecology 2) (Bolyen et al. 2019) analysis package, version 2020.11 (available at https://qiime2.org) to perform quality filtering, definition of amplicon Sequence Variants (ASVs), and taxonomic classification. Raw sequence data were imported using Casava 1.8 demultiplexed (paired-end) command and quality filtered using the q2-demux plugin of the DADA2 pipeline (Callahan et al. 2016). We removed all ASVs present in extraction and PCR blanks and also the ASVs with less than 36 reads, which corresponded to 0.001% of total read count (3586752 (number total reads) x 0.001 =36 reads). The SILVA 138 database (Pruesse et al. 2007; Quast et al. 2013) was chosen for taxonomic assignment. The ASV counts table was converted to the format *biom* for use in R Software v.4.1.1 (R Core Team 2020). Read counts were normalized using negative binomial distribution (McMurdie and Holmes, 2014).

In a large proportion of gut microbiome studies, diversity indices (alpha-diversity and beta-diversity) are widely used as tools to characterise the microbiome (Scepanovic et al. 2019). Alpha-diversity describes the variety of the microbial community in a single sample (intra-samples), taking into account the number of different taxa and their relative abundances. While Beta-diversity measures the diversity of microbial community composition between different samples. Using the R package *phyloseq* (McMurdie and Holmes, 2013) the bacterial alpha-diversity (intra-sample) was calculated using Observed, Shannon, Faith's phylogenetic diversity (PD), Fisher, ACE and Chao richness, InvSimpson and Simpson indices. The beta-diversity (inter-sample) was also estimated using Bray–Curtis, Jaccard, phylogenetic Unifrac unweighted and phylogenetic Unifrac weighted distances. The Principal Coordinates Analysis (PCoA) function that is a method to explore and to visualize similarities or dissimilarities of data, was used to visually assess dissimilarity between samples. Differences in alpha diversity between locality, species and sex were analyzed using a linear model analysis (lm(alpha div~species * sex) with ANOVA. Differences in beta diversity between locality, species and sex (adonis(div~ local + species + sex, permutations = 10000)) were assessed using permutational analysis of variance (permanova) with 10000 permutations as implemented in adonis function of the R vegan package (Oksanen, 2008). Correlations between individual size and bacterial alpha diversity were also tested using the Pierce correlation test. Bacterial transmission between species was also estimated using the FEAST software (Shenhav et al. 2019), between each pair of species from sympatric populations (Moledo and Parque das Nações).

2.5 Behavioural experiments

2.5.1 Mirror experiment

The species chosen for the mirror test, were the two resident species in Moledo *(P. bocagei* and *P. lusitanicus*). These particular species, *P. bocagei* and *P. lusitanicus*, have not yet been studied for this purpose and with this method, but it is believed that they should show territorial behaviour in the presence of other individuals, similar to the previously studied species *Podarcis muralis* (Scali, 2019). These species were chosen because they are two species with sexual dimorphism, with males larger in size and having larger heads than females. The location is also a point in favour in this choice, since it is a quieter area with less movement, which facilitates the performance of these tests. Furthermore, one of our aims in this study is to see if the parasite levels of individuals with a bolder behavioural pattern are lower. This is based on the results of a study where the parasite level between a native and an invasive species, it was observed that the invasive species would have a lower number of parasites in the blood and presented a bolder behaviour, which facilitated in its invasion. Although it is not an invasive/native relationship, my working hypothesis was that a low rate of parasites in the blood favours a bolder behaviour on the part of the individual, demonstrated by a greater interaction with the visual stimulus (the mirror). This population became the ideal model system as it was characterised by high infection rates with *Hepatozoon*.

Adult males of both species were captured between May and August 2021, to try to maximize territorial response according to proximity to the reproductive season (Carretero et al. 2015). The first trial of experiments was actually performed during the autumn of 2020, but the reaction was low (presumably since this was outside the reproductive season), and as such the data for this work was the one collected between May-August 2021. Before starting the captures, the whole set up was prepared with the terrariums ready to receive the individuals (fig 14). The individual boxes were all the same dimensions (40 × 30 × 30 cm) with a refuge positioned in the middle of the box, and a small container with water *ad libitum*. A mirror (15 × 15 cm) was previously placed on the side of the box, but properly covered with cardboard so as to be only exposed at the beginning of the test. A cardboard box was also used around all terrariums in order to reduce external stimuli and without interfering with the interior of the terrarium. The terrariums were cleaned with alcohol between individuals to maintain the same conditions for all. After capture, the lizard was placed in the respective terrarium and acclimated for 30 minutes in the sun to increase body temperature and at the end of this period the test was initiated with ae video recorded for 15 minutes, after taking the covering from the mirror. This test was performed in the shade, with the covering by a parasol, without moving the terrarium to avoid any disturbance. The conditions were the same for all individuals. For recording, a GoPro was used, placed on a tripod previously set up. At the end of the tests and sampling, the lizards were released at the location where they were caught.

Fig. 14 - Images showing the set up used and previously prepared for the behavioural experiment.

CHAPTER II

MANUSCRIPTS

MANUSCRIPT I

Host and enviromental factors influencing gut microbiota in sympatric species of lizards in portugal

Abstract

Host-gut microbiota interactions are quite complex and can substantially affect the ecology and evolution of both counterparts. There are several factors that are known to influence the composition and diversity of the gut microbiota, such as host taxonomy, habitat, diet and more recently social interactions or host proximity. In this study we aimed to investigate the influence of taxonomy, sex, host size and habitat/locality on gut microbiota diversity and understand if they could be used as predictors of gut bacterial diversity in five lizard species collected from two different sites in Portugal. Additionally, microbial transmission was tested in cases where species lived in sympatry. We collected individuals of *Podarcis bocagei (n=33)* and *Podarcis lusitanicus (n=8)*, which live in sympatry, from an area near the beach of Moledo, northern Portugal, and *Podarcis siculus (n=20)*, an invasive species and *Podarcis virescens (n=22),* which were collected in Lisbon (Parque das Nações), where they also live in sympatry. Additionally, *Teira dugesii* (n=20) an introduced species, also collected from Lisbon (Docas de Alcantara). We collected cloacal swabs (n= 103) from captured lizards and sequenced the V4 region of the 16s rRNA gene of the bacterial communities for each sample using high-throughput sequencing. Habitat/locality was found to be the main driver of differences in bacterial diversity, with species effects more evident at finer taxonomic scales. We also observed a significant and positive correlation between size and bacterial alpha diversity in the invasive species, *P. siculus*, which we suggest may due to their higher exploratory behaviour or boldness. Finally, although estimates of bacterial transmission between lizards were high in both sympatric populations, the invasive *P. siculus* seems to have acquired a higher proportion of local microbiota from the sympatric native *P. virescens*.

Keywords: Microbiome, *Podarcis*, *Teira*, Invasive species, Metabarcoding, Sympatry, Transmission.

Introduction

The gut microbiome can have major impacts on host development, behaviour and fitness, with subsequent impacts on the dynamics of ecosystems (Thaiss et al. 2016). However, in turn, the gut microbiome can also be influenced by several factors. Host taxonomy, sex and size, as well as the environment, such as the habitat or available prey items are known to affect gut microbiota (e.g. Xavier et al. 2019; Muegge et al. 2011). In addition, although less explored, social interactions between hosts can also be a major driver of the gut microbiome (Moeller et al. 2013). Ultimately, host and gut microbiota interactions are quite complex with evolutionary and ecological implications for both counterparts (McFall-Ngai et al. 2013).

Changes in the gut microbiome, and their consequences, have been studied in many mammals (e.g. Thaiss et al. 2016), birds (e.g. Hird et al. 2015), fishes (e.g. Xavier et al. 2019), and amphibians (e.g. Bletz et al. 2016). Contrastingly, there have been fewer studies conducted in reptiles and many of these connections remain to be further explored, especially in the case of lizards. Nevertheless, host taxonomy and ecology seem to be important drivers of reptile gut microbiota diversity. For example, diet was seen to influence gut microbiota of the crocodile lizard, *Shinisaurus crocodilurus,* where a diet with earthworm was healthier compared to lizards fed with loaches that resulted in higher abundances of pathogens or opportunistic pathogens (Jiang et al. 2017). Diet and habitat were found to have a compound effect on the microbiological gut diversity of water dragon, *Intellagama lesueurii*, with lizards that live in urban areas having higher diversity than populations living in their native habitat, likely due to differences in food resources (Littleford-Colquhoun et al. 2019). Host phylogeny and habitat were also seen to influence the gut microbiota of venomous snakes (Smith et al 2021) (see supplementary table A1 for an overview of microbiome studies in reptiles).

In an attempt to contribute to the existing knowledge on gut microbiota of reptiles, we analyzed and compared the diversity and composition of gut bacterial communities of five reptile species captured in Portugal: *P. siculus*, *P. virescens, P. guadamarrae, P. lusitanicus* and *Teira dugesi*. Our main goal was to understand whether habitat/locality and host factors (species, size, sex) modulate the gut bacterial diversity of these five lizards. To achieve this, we used non-invasive sampling (cloacal swabs) to obtain a proxy for gut bacterial communities and high throughput sequencing of a portion of 16S rRNA.

Methods

Studied species and habitat

We collected *P. bocagei* and *P. lusitanicus* from a semi-natural habitat in Moledo (fig 16) (41°50'19.2 "N 8°52'24.5 "W). This location has limited human disturbance and is a sparsely urbanized area with lots of vegetation and natural and artificial shelters (the walls of agricultural properties) for lizards. These two species live in syntopy, being both small-sized, diurnal, insectivorous and showing sexual dimorphism. Males of *P. bocagei* are usually bigger than females and have greenish tones on the dorsal area with the pattern generally spotted, while females are brownish on the back with stripes (Carretero et al. 2015). Males of the species *P. lusitanicus* are also usually larger than females, and have mostly reticulate dorsal pattern in dark tones, while females have a pattern with more longitudinal lines (Carretero et al. 2015). Ecological adaptation is seen as being a major factor favouring the isolation between these two species. Specifically, while *P. lusitanicus* lives more on rocks, *P. bocagei* is found more on the ground (Carretero et al. 2015).

Two other lizard species, *P. siculus* and *P. virescens* were both captured in Lisbon, at the Parque das Nações (38°76'22.4 "N, 9°09'44.3 "W). *Podarcis siculus* is an invasive species, introduced to Parque das nações about two decades ago (fig 15 B,C) (González de la Vega et al., 2001). It is a medium-sized lizard (Freiría, 2005) with sexual dimorphism, with males being larger than females (Vogrin, 2005; Salvador, 2015). It is a species with great plasticity in the spatial use of habitat and can be found in various types of habitat which may favor the invasion success of this species (Carretero and Silva-Rocha, 2015). On the other hand, *P. virescens* is a smaller size and with sexual dimorphism where males are also larger than females. These sympatric species live in an anthropogenic environment, in artificial gardens near to residential and business areas, close to one of Lisbon’s biggest malls where there is considerable human disturbance throughout the day. The feeding of these two species specifically in this population is not well studied, but it is thought that despite sharing the same habitat, the feeding of *P. siculus* is somewhat more varied than that of *P. virescens*. *P. virescens* is known to be insectivorous and to feed mainly on individuals of the class Arachnida and the orders Hymenoptera, Hemiptera, Coleoptera and Diptera (Juan, 1997), while *P. siculus* usually consumes various arthropods (Zuffi and Giannelli, 2013) but can also consume fruits and nectar (Mačát et al, 2015) and there is even a study (Vervust et al. 2010), where a morphological alteration of its digestive tract is found, as a way of adapting to a more herbivorous diet. This more versatile diet and adaptive behaviour and morphology, may explain the successful colonisation of *P. siculus* (Vervust et al. 2007; Vervust et al. 2010 Damas-Moreira et al. 2019, 2020). Finally, *T. dugesii, a* medium sized lizard was captured near the Alcantara docks, close to the port area of Lisbon (38°70'33.8 "N, 9°16'54.1 "W). This species is thought to have been accidentally introduced by transport ships originating from Madeira Island almost three decades ago, in 1992 (Sá-Sousa 1995). Like the *Podarcis spp* captured in Lisbon, it occupies an anthropogenic area, although much less visited, and is found close to railway tracks with limited vegetation cover (fig 15D). Relatively to its diet, *T. dugesii* has a more omnivorous and generalist diet, feeding preferentially on insects but also on small fruits (Sadek, 1981).

Fig. 15 – Images that represent the locations of the populations from Lisbon. The distance between the two populations from the Parque das Nações (*P. siculus and P. virescens*) represented with a yellow marker, and *Teira dugesii* at Lisbon Docks, represented with orange marker (A); Capture sites of the species from Parque das Nações, *P. siculus* (B) and *P. virescens* (C), surrounded by a yellow line. Site where specimens of the introduced species, *Teira dugesii* (D), were captured.

Fig. 16 - Capture site of the species from Moledo, *P. bocagei* and *P. lusitanicus* , surrounded by a yellow line.

Sampling

For this study, we sampled 103 adult lizards in September 2020: *Podarcis bocagei* (n=33), *Podarcis lusitanicus* (n=8), *Podarcis siculus* (n=20), *Podarcis virescens* (n=22) and *Teira dugesii* (n=20).

Immediately after collecting the lizards, we swabbed each individual's cloaca, using a sterile swab and ensuring that neither the swab nor the lizard's cloaca touched anything, to avoid any contamination. Swabbing was performed by inserting it lightly into the entrance of the cloaca. The swabs were then immediately placed in ice boxes during field work and were then kept at -80°C in the laboratory until further processing. After swabbing, still in the field, data was taken from each lizard, such as species, sex, and also the snout-vent length (SVL - from head to cloaca) using a digital caliper (+- 0.01mm error).

DNA from the swabs was extracted using the DNeasy® PowerSoil® Kit (QIAGEN, Hilden, Germany), with all steps performed according to the manufacturer's instructions. DNA concentration and quality was measured on the Epoch™ Microplate Spectrophotometer (BioTek Instruments, Inc.; United States of America). The V4 region of the rRNA gene (~ 250 bp) of the bacterial communities was amplified for each sample and the respective extraction blanks and PCR controls using the Kozich et al. (2013) protocol and the amplicons were sequenced in a single MiSeq run (Illumina). The V4 region of this gene is commonly used for the characterization of bacterial communities in various taxa (Colston and Jackson, 2016; Chiarello et al. 2018). Due to the lack of a dedicated laboratory for bacterial DNA amplification at CIBIO, this part of the protocol was outsourced to University of Michigan.

Statistical analysis

Raw FASTQ sequences were denoised using QIIME2 (Quantitative Insights into Microbial Ecology 2) (Bolyen et al. 2019) version 2020.11. Demultiplexed FASTQ sequences were imported using the "*Casava 1.8 format demultiplexed (paired-end)"* format. After an initial quality assessment, forward and reverse reads were trimmed to 220bp and 200bp, respectively. Further quality filtering was performed using the "*q2-demux*" plugin from the DADA2 pipeline (Callahan et al., 2016). The SILVA 138 database (Pruesse et al. 2007; Quast et al. 2013) was chosen for taxonomic assignment. The final ASV (amplicon sequence variants) table was imported in*.biom* format for use in R Software v.4.1.1 (R Core Team 2020). Bacterial taxonomic alpha-diversity (intra-sample) and beta-diversity (inter-sample) were estimated using the *phyloseq* package (McMurdie and Holmes, 2013). Specifically, as a measure of alpha-diversity the number of observed ASVs, and the Shannon, Faith's phylogenetic diversity (PD), Fisher, ACE, Chao richness, InvSimpson and Simpson indices were estimated. Beta-diversity was estimated using Bray-Curtis, Jaccard, Unifrac phylogenetic unweighted and Unifrac phylogenetic weighted distances. We used the Principal Coordinate Analysis (PCoA) to visually assess dissimilarity between samples.

Statistical differences in alpha diversity between locality/habitat, species and sex were also analyzed by performing a linear model (lm (alphadiv~ local + species + sex)). Differences in the proportion of the most abundant taxa at the genera level were also assessed using the linear model (lm (bacterial genus~ species*sex)). Differences in beta diversity between locality, species and sex were assessed using permutational analysis of variance (PERMANOVA) with 10000 permutations, implemented using the *adonis* function (Adonis (divbeta~ local + species + sex, permutations = 10000)) of the R vegan package (Oksanen, 2008). Correlations between individual size and bacterial alpha diversity were also tested using Pierce's correlation test for all species individually.

Finally, bacterial transmission between each pair of species from sympatric populations living in Moledo and Parque das Nações was estimated using FEAST software (Shenhav et al. 2019). Due to a discrepancy in the number of samples in the Moledo populations (*P. bocagei, P. lusitanicus*) we used only part of the individuals of *P. bocagei* (with individuals as similar as possible to the P. *lusitanicus* sample (sex and SVL)) to balance the number of samples and to avoid overestimating the transmission.

Results

After quality control and taxonomic assignment, we removed sequences from Archaea, Eukaryota, Mitochondria, Chloroplast and some unassigned ASVs from the final dataset. We also removed the sequences present in only one sample and with less than 36 reads. This step eliminated ASVs that had a minimal count of less than 0.001% of the total number of reads (3586752 (number total reads) x 0.001% =36) that were present in a single sample. After filtering steps, the final ASV table encompassed 3923 unique ASVs, in a total of 39 bacteria phyla. The most abundant phyla of bacteria detected, belonged to Firmicutes, Bacteroidota, Actinobacteroidota, Proteobacteroidota and Campylobacterota (fig 17)

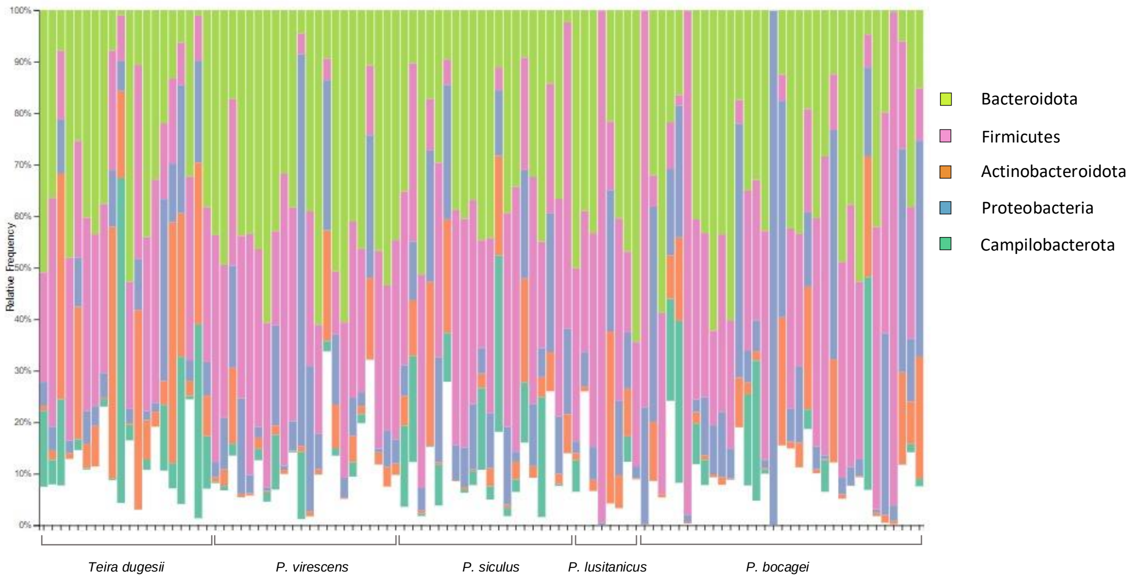

Fig. 17 - Relative frequency of the most abundant bacterial phyla in the gut microbiome of the species studied (*T. dugesii, P. virescens, P. siculus, P. lusitanicus, P. bocagei*).

Microbial alpha-diversity was significantly different between Moledo populations (*P. bocagei* and *P. lusitanicus*) and Lisbon populations (which includes the three species *P. siculus*, *P. virescens* and *T. dugesii*) (Table 2), with Lisbon populations showed consistently higher alpha-diversity indices (fig 18). By comparing the species within populations, it was possible to detect that in Parque das Nações (Lisbon) the level of microbiota diversity was higher in the invasive *P. siculus* than in the native *P. virescens* (fig 19).

Table 2 – Results of analysis of variance (ANOVA) performed with various alpha diversity indices.

Alpha-diversity indices **(factor “local” significative)**	**F-statisct**	**P-value**
Shannon	5.9115	<0.001 (0.0003053)
Observed	19.4695	<0.001 (2.122e-10)
PD	18.0354	<0.001 (1.154e-09)
InvSimpson*	8.7855	0.00032
ACE*	27.2169	<0.001 (5.185e-10)
Fisher*	19.8491	<0.001 (6.626 e-0.8)

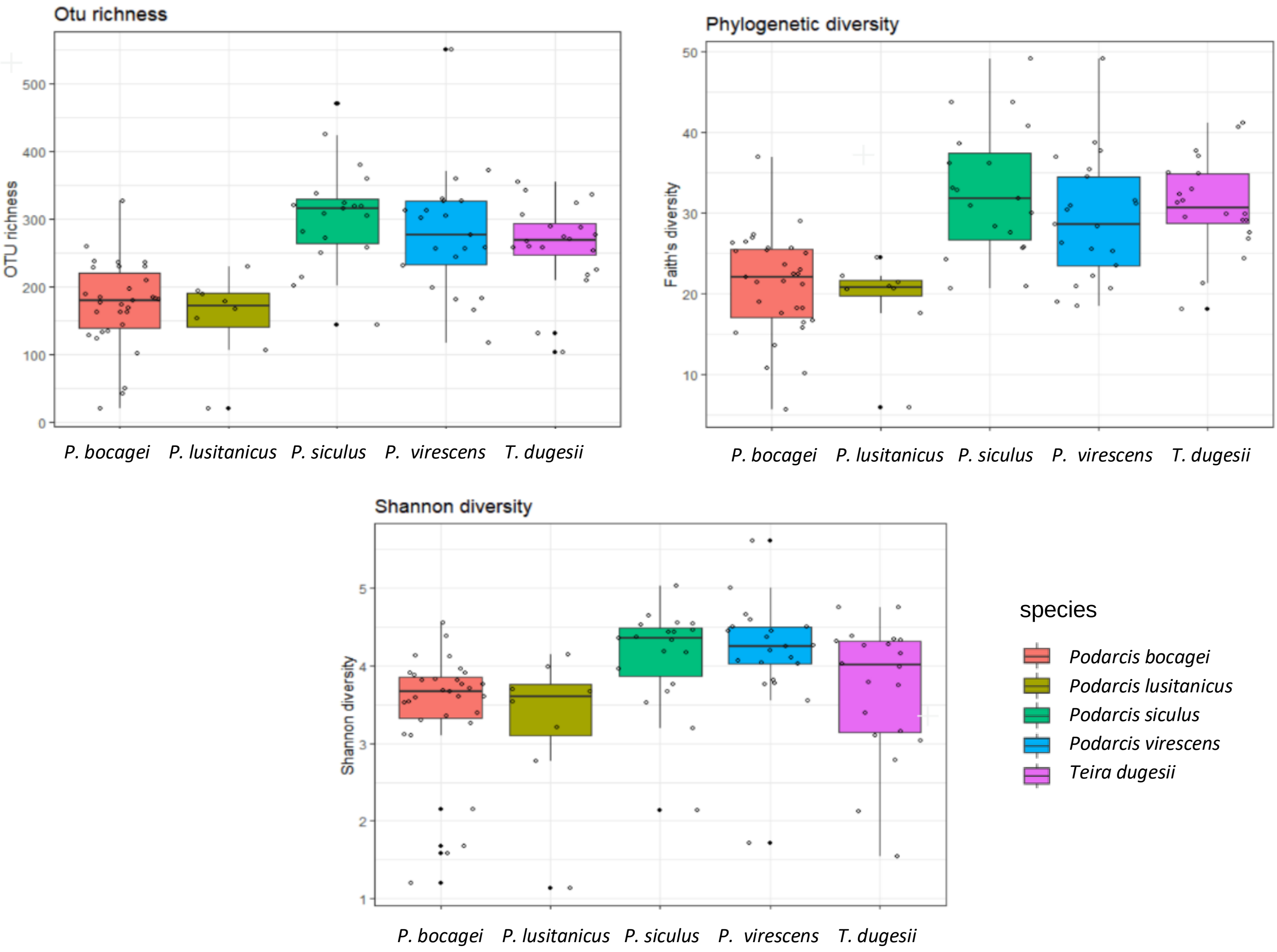

Fig. 18 - Boxplots showing the median values and interquartile ranges for the alpha diversity indices *(Phylogenetic diversity, Shannon diversity and number of observed ASVs)* for the gut microbiome of the five species under study. Samples are represented by open circles and outliers are shown as black circles *(*) The boxplots for the alpha-diversity indices not presented here can be found in the supplementary material.*

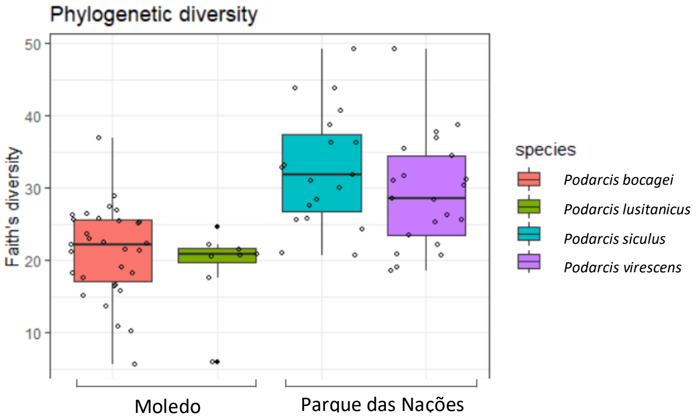

Fig. 19 - Boxplots showing the median values and interquartile ranges for the alpha diversity indice for the gut microbiome representing the diversity of microbiome of the sympatric species from Moledo and Parque das Nações. Samples are represented by open circles and outliers are shown as black circles

Sampling localities also showed significant differences in beta-diversity using Bray–Curtis ($R^2 = 0.186$ and $p<0.001$), and phylogenetic Unifrac unweighted ($R^2 = 0.180$ and $p<0.001$) distances (fig 20) (table 3).

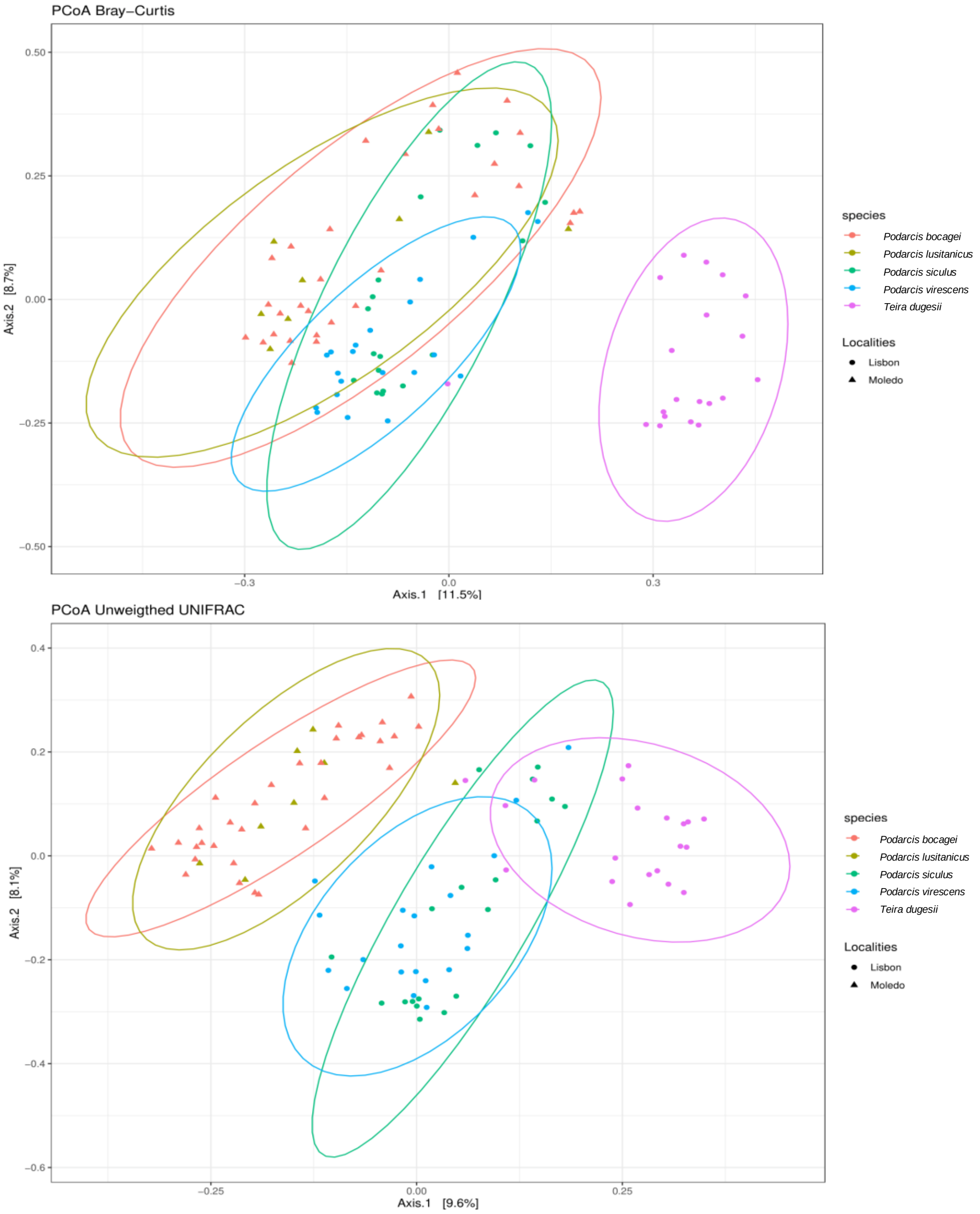

Fig. 20- PCoA plots representing Bray–Curtis and Unweighted Unifrac distances.

Table 3 - Permutational multivariate analysis of variance (PERMANOVA) analysis results for the significance of "Local" using diferente distances.

Beta-diversity distances **(factor "local")**	**R^2**	**P-value**
Bray–Curtis	0.18602	<0.001 (9.999e-05)
Unifrac phylogenetic unweighted	0.18007	<0.001 (9.999e-05)
Unifrac phylogenetic weighted	0.10178	0.005599

Significant differences in the proportion of the most abundant genera between *P. bocagei* and P. *lusitanicus* were found only for genus *Corynebacterium* (F-statistics= 6.823 p= 0.013) at Moledo (appendix: fig A3). Differences in the proportion of the most abundant genera between *P. siculus* and *P.virescens* were found for phyla *Gastranaerophilales* (F-statistics= 6.324, p= 0.003); *Corynebacterium* (F-statistics= 6.887, p= 0.002); *Kocuria* (F-statistics= 4.639, p= 0.0138); *Staphylococcus* (F-statistics= 6.767, p= 0.002); and *Odoribacter* (F-statistics= 11.609, p= 6.398e-05) (appendice: fig A1). Additionally both species and sex significantly affected the abundance of *Akkermansia* (sex: F-statistics= 5.191, p= 0.026; species: F-statistics= 3.467, p= 0.038) (appendix: fig A2). Finally, the interaction between species and sex (species*sex) significantly affected the proportion of *Romboutsia* (F-statistics= 3.475, p= 0.038) and *Pseudomonas* (F-statistics= 3.412, p= 0.040) (appendix: fig A2)

Pierce Correlation test showed significant and positive correlations between SVL and bacterial alpha diversity in the invasive species, *P. siculus* (fig 21). For the remaining species no significant results were found.

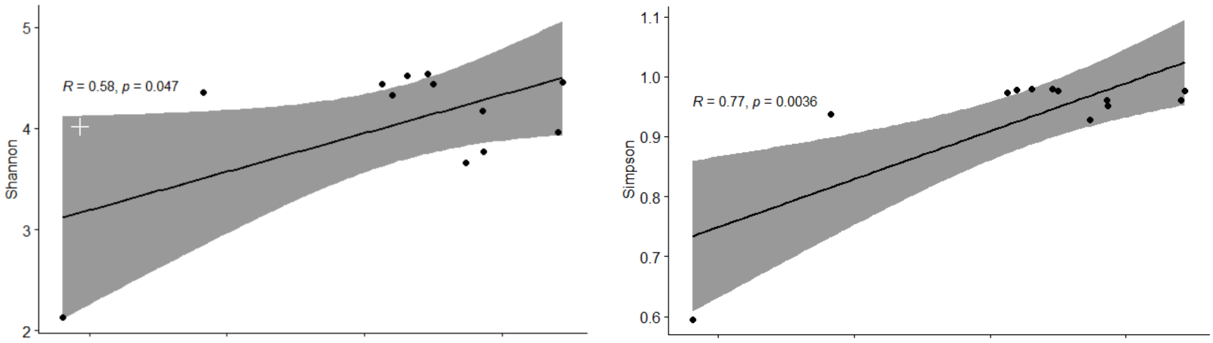

Fig. 21 - Correlation test between size (SVL) and bacterial alpha diversity in the *Podarcis siculus.*

The FEAST software indicated that the level of bacterial transmission between sympatric species was high. Nevertheless, while between the syntopic *P. lusitanicus* e and *P. bocagei* transmission was balanced in both directions (*P. bocagei* -> *P. lusitanicus* ~ 71% on average, and *P. lusitanicus* -> *P. bocagei* ~ 69% on average), between the sympatric *P. siculus* and *P. virescens* this did not seem to be the case with transmission from the native *P. virescens* to the invasive *P. siculus* estimated to be 72% on average, and from *P. siculus* to *P. virescens* about 55% on average.

Discussion and conclusion

In the present study, we characterized the gut bacterial microbiota of five lizard species captured in Portugal (*P. siculus*, *P. virescens*, *P. bocagei*, *P. lusitanicus* and *T. dugesii*) using a metabarcoding approach. Results showed habitat was the major diversity predictor which significantly influenced both alpha and beta-diversity. Nevertheless, sex and size also had an effect, albeit more discrete, in gut bacterial communities.

Habitat effects on lizard’s gut bacteria

Although these five species do not share the same habitat, they all showed the same most abundant bacteria phyla (Firmicutes, Bacteroidota, Actinobacteroidota, Proteobacteroidota and Campylobacterota) in their gut microbiome, although these phyla vary in value and order of abundance among these species. These bacteria phyla were already found in several studies of lizards and were also the most abundant in the gut microbiome of these lizard species (Holmes et al. 2019; Montoya-Ciriaco et al. 2020).

The habitat at the two sites was very different, with lizards from Lisbon living in a more urbanized and artificial habitat, with greater environmental disturbance, compared to Moledo, a semi-natural habitat. Differences in habitat may lead to differences in the composition or diversity of the gut microbiome (Amato et al. 2013), especially by influencing the available diet items (Littleford-Colquhoun et al. 2019). In our study, a higher microbiota diversity was observed in the more urbanized environment. These higher values can be justified by the higher probability of lizard hosts exploiting a higher variety of diet items, including human food waste and garbage. Additionally, environmental microbiota, which could be transferred to lizards, may also be more diverse in these urban habitat when compared with semi-natural habitats. Furthermore, the co-existence with other species, not only of reptiles but also of other coexisting animals (cats, dogs, rats), the constant movement of people, may leave their bacterial fingerprint in the environment, which can be acquired by these urban lizards. On the other hand, species inhabiting in more natural conditions, with less disturbance, may come into contact with somewhat more homogenous environmental microbiota. The present results are in agreement with a previous study where the gut microbiome of populations from urban and semi-natural habitats was different, with higher diversity in urban populations, in water dragons *Intellagama lesueurii* (Littleford-Colquhoun et al. 2019).

Host taxomony, sex, size and potential boldness effects on gut microbiota diversity

In this study, host taxonomy was seen to significantly influence the proportion of the most abundant genera. Additionally, an interaction between species and sex was also found to influence the gut microbiota of P. *virescens* and *P. siculus*. The influence of host taxonomy, which can be a proxy not only for host genetics but also it's general ecology, is commonly found in many animals (Moeller et al. 2013, 2014) including reptiles (Smith et al. 2021). The influence of sex found here in the abundance of major bacterial groups between the two sexes of *P. virescens* and *P. siculus*, has already been observed in a previous study with lizards (*Sceloporus virgatus)*, where difference in immune responses between sexes and also sex-specific host-microbe interactions, perhaps related to hormone levels were proposed to explain this (Martin et al. 2010). In the present study the influence of the sex factor was small in relation to the effect of habitat and host taxonomy. Nonetheless, the influence it exerts could be linked to differences in the ecology of the two sexes. It is important to note that differences in the size of females versus males may lead to differences in feeding behaviour, and consequently on the microbial diversity that comes from feeding.

Interestingly, we also found a positive correlation between individual's size and alpha bacterial diversity in *P. siculus*. This lizard is larger than the other studied species and is also a very successful invasive species (Carretero and Silva-Rocha, 2015; Damas-Moreira et al. 2019). Indeed, *P. siculus* is more aggressive, more exploratory, and bolder when exploiting food resources when compared to native *Podarcis*, including *P. virescens* (Damas-Moreira et al. 2018; Damas-Moreira et al. 2019). Furthermore, the displacement of *P. virescens* from gardens now inhabited by *P. siculus* (Ribeiro and Sá-Sousa, 2018), may be explained by the higher levels of aggression in *P. siculus* (Downes and Bauwens, 2002). This behaviour can lead to a larger ecological and trophic niche, which can result in a higher microbiome diversity in this species.

Finally, we performed a preliminary analysis regarding the bacterial transmission between the lizards living in sympatry. We hypothesized that the amount of bacterial transmission may reflect different non-exclusive aspects of the ecology of their host, ie., it could be reflecting the rate of physical encounters between species, but also their similarity in terms of dietary niches and habitat occupancy (i.e. the later would reflect bacterial transmission from the environment). We found high transmission values in both sympatric populations, but while in the semi natural habitat of Moledo transmission was more balanced between species, at Parque das Nações, we observed that the invasive *P. siculus* may have received a higher proportion of bacteria from the native *P. virescens*. These results are interesting and can be explained by the successful adaptation of the invasive to the new environment, having acquired a high quantity of local microbiota. They can also be reflecting an increased ability of exploiting a variety of food resources, or most likely a combination of both. The population of *P. siculus* and *P. virescens* (Parque das Nações) live in sympatry, occupying roughly the same area, but not in syntopy (occurrence of two species in the same habitat at the same time), and although the gardens where the individuals were captured for this study are 800 metres away, sightings of these two species within 50 m of each other have already been recorded (Ribeiro and Sá-Sousa, 2018; pers. obs.). On the other hand, in Moledo, both species besides living in the same area, and are also considered syntopics (Sannolo et al. 2018) and may explore their habitat in a similar manner. Besides this, they almost certainly consume the same prey items, and may also be encountering each other more frequently. This could possibly justify a much more bidirectional transmission between the two species. Due to the large difference in the number of samples in this population and to improve the results, this study could be repeated with a more balanced sample, allowing FEAST analysis to be more robust (currently it was limited to a n=8).

Conclusions

We were able to characterise the gut microbiome of five phylogenetically related species of lizards living in urban and non-urban habitats in Portugal. We conclude that the interactions between the host and its microbiome can be influenced by many factors such as type of habitat and species. These are some factors that have also been indicated as influencing the gut microbiota in other lizard studies (Littleford-Colquhoun et al. 2019). To expand this study, it would be informative to increase the sample size and to make collections in different times of the year, since this can also be an influencing factor. It would also be interesting to characterize and compare the diets of the lizards living in sympatry, to determine if it could be one of the factors leading to differences in their gut microbiota community. This type of study is very important to better understand the composition and diversity of the intestinal microbiota of these animals, and it provided baseline data that will aid futures studies of other aspects, such as the potential consequences to the level of parasitism. This would ultimately help us understand the effect and dynamics of gut microbiome on their hosts.

References

Amato, K. R., Yeoman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, A., Gaskins, H. R., Stumpf, R. M., Yildirim, S., Torralba, M., Gillis, M., Wilson, B. A., Nelson, K. E., White, B. A.,.. & Leigh, S. R. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. *The ISME journal*, 7(7), 1344-1353.

Bletz, M. C., Goedbloed, D. J., Sanchez, E., Reinhardt, T., Tebbe, C. C., Bhuju, S., Geffers, R., Jarek, M., Vences, M. & Steinfartz, S. (2016). Amphibian gut microbiota shifts differentially in community structure but converges on habitat-specific predicted functions. *Nature Communications*, 7(1), 1-12.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., Cope, E. K., Da Silva, R., Diener, C., Dorrestein, P. C., Douglas, G. M., Durall, D. M., Duvallet, C., Edwardson, C. F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J. M., Gibbons, S. M., Gibson, D. L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G. A., Janssen, S., Jarmusch, A. K., Jiang, L., Kaehler, B. D., Kang, K. B., Keefe, C. R., Keim, P., Kelley, S. T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M. G. I., Lee, J., Ley, R., Liu, Y. X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B. D., McDonald, D., McIver, L. J., Melnik, A. V., Metcalf, J. L., Morgan, S. C., Morton, J. T., Naimey, A. T., Navas-Molina, J. A., Nothias, L. F., Orchanian, S. B., Pearson, T., Peoples, S. L., Petras, D., Preuss, M. L., Pruesse, E., Rasmussen, L. B., Rivers, A., Robeson, M. S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S. J., Spear, J. R., Swafford, A. D., Thompson, L. R., Torres, P. J., Trinh, P., Tripathi, A., Turnbaugh, P. J., Ul-Hasan, S., van der Hooft, J. J. J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C. H. D., Willis, A. D., Xu, Z. Z., Zaneveld, J. R., Zhang, Y., Zhu, Q., Knight, R., & Caporaso J. G. (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* 37: 852–857. https://doi.org/10.1038/s41587-019-0209-9

Caeiro-Dias, G., Rocha, S., Couto, A., Pereira, C., Brelsford, A., Crochet, P. A., & Pinho, C. (2021). Nuclear phylogenies and genomics of a contact zone establish the species rank of *Podarcis lusitanicus* (Squamata, Lacertidae). *Molecular Phylogenetics and Evolution*, *164*, 107270.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nature methods*, 13(7), 581-583.

Carretero, M. A. & Silva-Rocha, I. (2015). La lagartija italiana (*Podarcis sicula*) en la Península Ibérica e islas Baleares. *Ecology* 22: 4829-4841.

Carretero, M. A., Galán, P., Salvador, A. (2015). Lagartija lusitana - *Podarcis guadarramae.* En: *Enciclopedia Virtual de los Vertebrados Españoles*. Salvador, A., Marco, A. (Eds.). Museo Nacional de Ciencias Naturales, Madrid. http://www.vertebradosibericos.org/

Chiarello M., Auguet J. C., Bettarel Y., Bouvier C., Claverie T., Graham N. A. J., & al. (2018). Skin microbiome of coral reef fish is highly variable and driven by host phylogeny and diet. *Microbiome* 6:147. 10.1186/s40168-018-0530-4

Colston, T. J., & Jackson, C. R. (2016). Microbiome evolution along divergent branches of the vertebrate tree of life: what is known and unknown. *Molecular ecology*, 25(16), 3776-3800.

Damas-Moreira, I., Oliveira, D., Santos, J. L., Riley, J. L., Harris, D. J., & Whiting, M. J. (2018). Learning from others: an invasive lizard uses social information from both conspecifics and heterospecifics*. Biology letters*, 14(10), 20180532.

Damas-Moreira, I., Riley, J. L., Harris, D. J., & Whiting, M. J. (2019). Can behaviour explain invasion success? A comparison between sympatric invasive and native lizards. *Animal Behaviour*, 151, 195-202.

Downes, S., & Bauwens, D. (2002). An experimental demonstration of direct behavioural interference in two Mediterranean lacertid lizard species. *Animal behaviour*, *63*(6), 1037-1046.

Freiría, F. M. (2005). Sobre o xénero *Podarcis* na Península e Baleares. Herpetoloxía. *Myotis* 1: 15-19.

González de la Vega, J.P., González-García, J.P., García-Pulido, T. & González-García, G. (2001). *Podarcis sicula* (Lagartija italiana), primera cita para Portugal. *Boletín de la Asociación Herpetológica Española*, 12: 9.

Hird, S. M., Sánchez, C., Carstens, B. C., & Brumfield, R. T. (2015). Comparative gut microbiota of 59 neotropical bird species. *Frontiers in microbiology*, 6, 1403.

Holmes, I. A., Monagan Jr, I. V., Rabosky, D. L., & Davis Rabosky, A. R. (2019). Metabolically similar cohorts of bacteria exhibit strong cooccurrence patterns with diet items and eukaryotic microbes in lizard guts. *Ecology and evolution*, *9*(22), 12471-12481.

Jiang, H. Y., Ma, J. E., Li, J., Zhang, X. J., Li, L. M., He, N., Liu, H. J., Wu, Z. J., Han, R. C. & Chen, J. P. (2017). Diets alter the gut microbiome of crocodile lizards. *Frontiers in Microbiology*, 8, 2073.

Juan, F. (1997). La lagartija ibérica (*Podarcis hispanica*) en la Sierra de Segura, Albacete: biometría, etología y folidosis. *Al-Basit: Revista de Estudios Albacetenses* 40: 111-134.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and environmental microbiology*, 79(17), 5112-5120.

Littleford-Colquhoun, B. L., Weyrich, L. S., Kent, N., & Frere, C. H. (2019). City life alters the gut microbiome and stable isotope profiling of the eastern water dragon (*Intellagama lesueurii*). *Molecular ecology*, *28*(20), 4592-4607.

Mačát, Z., Veselý, M. & Jablonski, D. (2015). New case of fruit eating observation in *Podarcis siculus* (Rafinesque-Schmaltz, 1810) (Lacertidae) from Croatia. *Biharean Biologist* 9(2): 158-159.

Martin, M. O., Gilman, F. R., & Weiss, S. L. (2010). Sex-specific asymmetry within the cloacal microbiota of the striped plateau lizard, *Sceloporus virgatus*. *Symbiosis*, *51*(1), 97-105.

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C., Carey, H. V., Domazet-Lošo, T., Douglas, A. E., Dubilier, N., Eberl, G., Fukami, T., Gilbert, S. F., Hentschel, U., King, N., Kjelleberg, S., Knoll, A. H., Kremer, N., Mazmanian, S. K., Metcalf, J. L., Nealson, K., Pierce, N. E., Rawls, J. F., Reid, A., Ruby, E. G., Rumpho, M., Sandre, J. G., Tautz, D., & Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences*, 110(9), 3229-3236.

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS one*, 8(4), e61217.

Moeller, A. H., Li, Y., Ngole, E. M., Ahuka-Mundeke, S., Lonsdorf, E. V., Pusey, A. E., Peeter, M., Hahn, B. H., & Ochman, H. (2014). Rapid changes in the gut microbiome during human evolution. *Proceedings of the National Academy of Sciences*, *111*(46), 16431-16435.

Moeller, A. H., Peeters, M., Ndjango, J. B., Li, Y., Hahn, B. H., & Ochman, H. (2013). Sympatric chimpanzees and gorillas harbor convergent gut microbial communities. *Genome research*, 23(10), 1715-1720.

Montoya-Ciriaco, N., Gómez-Acata, S., Muñoz-Arenas, L. C., Dendooven, L., Estrada-Torres, A., de la Vega, A. H. D., & Navarro-Noya, Y. E. (2020). Dietary effects on gut microbiota of the mesquite lizard *Sceloporus grammicus* (Wiegmann, 1828) across different altitudes. *Microbiome*, *8*(1), 1-19.

Muegge, B. D., Kuczynski, J., Knights, D., Clemente, J. C., González, A., Fontana & Gordon, J. I. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science*, 332(6032), 970-974.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D, Minchin, P. R, O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E. and Wagner, H. (2020). vegan: Community Ecology Package. *R package version* 2.5-7. https://CRAN.R-project.org/package=vegan

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., & Glöckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic acids research*, *35*(21), 7188-7196.

Quast C., Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucl. Acids Res*. 41 (D1): D590-D596.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ribeiro, R., & Sá-Sousa, P. (2018). Where to live in Lisbon: urban habitat used by the introduced Italian wall lizard (*Podarcis siculus*). *Basic and Applied Herpetology*, *32*, 57-70.

Sadek, R. A. (1981). The diet of the Madeiran lizard Lacerta dugesii. *Zoological Journal of the Linnean Society*, *73*(4), 313-341.

Salvador, A. & García-París, M. (2015). Lagartija italiana – *Podarcis siculus* (Rafinesque-Schmalz, 1810). In: Enciclopedia Virtual de los Vertebrados Españoles.

Sannolo, M., Barroso, F. M., & Carretero, M. A. (2018). Physiological differences in preferred temperatures and evaporative water loss rates in two sympatric lacertid species. *Zoology*, *126*, 58-64.

Sá-Sousa, P. (1995). The introduced Madeiran lizard, *Lacerta (Teira) dugesii* in Lisbon. *Amphibia-Reptilia*, 16(2), 211-214.

Shenhav, L., Thompson, M., Joseph, T. A., Briscoe, L., Furman, O., Bogumil, D., Mizrahi, I., Pe'er, I., & Halperin, E. (2019). FEAST: fast expectation-maximization for microbial source tracking. *Nature Methods*, *16*(7), 627-632.

Smith, S. N., Colston, T. J., & Siler, C. D. (2021). Venomous Snakes Reveal Ecological and Phylogenetic Factors Influencing Variation in Gut and Oral Microbiomes. *Frontiers in microbiology*, *12*, 603.

Thaiss, C. A., Zmora, N., Levy, M., & Elinav, E. (2016). The microbiome and innate immunity. *Nature*, 535(7610), 65-74.

Vervust, B., Grbac, I., & Van Damme, R. (2007). Differences in morphology, performance and behaviour between recently diverged populations of *Podarcis sicula* mirror differences in predation pressure. *Oikos*, *116*(8), 1343-1352.

Vervust, B., Pafilis, P., Valakos, E. D. & Van Damme, R. (2010). Anatomical and physiological changes associated with a recent dietary shift in the lizard *Podarcis sicula*. *Physiological and Biochemical Zoology* 83(4): 632-642.

Vogrin, M. (2005). Sexual Dimorphism in *Podarcis sicula campestris*. *Turkish Journal of Zoology*, 29(2), 189-191.

Xavier, R., Mazzei, R., Pérez-Losada, M., Rosado, D., Santos, J. L., Veríssimo, A., & Soares, M. C. (2019). A risky business? Habitat and social behavior impact skin and gut microbiomes in Caribbean cleaning gobies. *Frontiers in microbiology,* 10, 716.

Zuffi, M. & Giannelli, C. (2013). Trophic niche and feeding biology of the Italian wall lizard, *Podarcis siculus campestris* (De Betta, 1857) along western Mediterranean coast. *Acta Herpetologica* 8(1): 35-39.

MANUSCRIPT II

Looking to the mirror: do blood parasites affect behaviour in syntopic lizard species?

Abstract

Parasites can have a major impact on communities and on the structure of ecosystems. Blood parasites have obligate relationships with their hosts, and can influence host fitness, including their reproductive success, metabolism and also host behaviour. High parasite prevalence may result in reduced body condition and affect the ability to compete for resources. As such, uninfected organisms may be more active, exploratory and bolder when compared to infected individuals. For this study two lizard species that live in syntopy, *Podarcis bocagei* and *Podarcis lusitanicus* were studied. Behavioural tests were performed in the field using a mirror as visual stimulus and recording the responses of each individual when faced with the mirror. Blood smears were taken after the tests for subsequent parasite analysis under the microscope. Differences were found between the two species in terms of parasite prevalence, with a higher number of infected individuals in *P. bocagei*, but with *P. lusitanicus* having higher average parasite intensity. The species that interacted most with the mirror, and consequently is bolder, was *P. lusitanicus*. Unexpectedly, it was the most parasitised individuals that exhibited the boldest behaviour. These studies help understand the effect parasites can have on host behaviour.

.

KEYWORDS: Parasites, Blood smears, *Podarcis bocagei*, *Podarcis lusitanicus*, Boldness, visual stimulus

Introduction

Parasites can influence various parameters and behaviours of a population, and can have a significant impact on population dynamics (Hudson et al. 1998; Tome et al., 2021). For example, lizard hosts with higher infection levels may have lower reproductive output, slower running speed, and weaker competitive ability than uninfected individuals (Dawson and Bortolotti, 2000; Jiménez-Peñuela et al. 2019). Interestingly, in a recent study on the invasive lizard *P. siculus* almost no blood parasites were found (Tome et al. 2021), and this species was considered bolder, with a more exploratory and territorial behaviour, giving it a competitive advantage over the native *P. virescens*. (Damas-Moreira et al. 2019, 2020). Such contrasting results show it is necessary to understand the link between blood parasites and host behaviour.

Among reptiles, there are many well-documented cases of territorialism for various species, including lizards (Pough et al., 2004). In an attempt to measure territorialism recent behavioural studies in lacertids, such as in *Podarcis muralis* (Scali et al. 2019), used individual's responses to different visual stimuli. A stimulus that is widely used in these studies are mirrors, which successfully reproduce images of another lizard, promoting social behaviours against the reflected image (Balzarini et al. 2014, Scali et al. 2019). Mirrors can be more effective than models mimicking other lizards, since they simulate another lizard with the same morphology, size and movements. Furthermore, mirror tests are easy to apply and can also be employed in the field (Hernández-Agüero and Megía-Palma, 2020).

For this study, two lizard species were used to test the relation between microparasite blood infections and performance in a mirror test: *P. bocagei* (Seoane, 1884) and *P. lusitanicus* Geniez et al. 2014, in Northern Portugal, Moledo, where they live in syntopy. Species that live in syntopy, are ideal to evaluate and compare, because their niches and the resources available are similar (Sillero et al. 2020). Furthermore, the differences that may exist between the levels of parasitism and between the responses to the behavioural tests may be important to understand further the dynamics between these populations and to determine if the parasites have any effect on the behaviour of the individuals. Both species are endemic and small-sized lizards with sexual dimorphism (Carretero et al. 2015). In this population there is a large overlap of microhabitats between both species, however *P. lusitanicus* is more likely to use open rocky habitats, while *P. bocagei* is found more on the ground (Sillero and Gonçalves-Seco, 2014; Carretero et al. 2015). This difference is not due to differences in locomotion, but attributed more to shelter use due to a greater flattening of the head in *P. lusitanicus*

(Kaliontzopoulou et al. 2012). There is certainly a territoriality between males of each species, where larger males dominate smaller males (Carretero et al. 2015). Moreover, these syntopic populations are also known for having high infection rates with the protozoan blood parasite *Hepatozoon sp.,* with *P. lusitanicus* having higher levels of parasite intensity and also higher prevalence (greater percentage of infected individuals) compared to *P. bocagei* (Maia et al. 2015).

The aim of this study was to assess the possible effect of blood parasites on the behaviour of these two syntopic lizard hosts, by performing a mirror test on both species in the field, and then recording infection rates with *Hepatozoon* blood parasites through examination of blood smears.

Methods

Behaviour experiment in the field

We conducted this study during June - August 2021, at the beach of Moledo (41°50'19.2 "N 8°52'24.5 "W). In total, we used 26 adult male lizards of *P. bocagei* (n=11) and *P. lusitanicus* (n=15). Only adult males were used, as there is greater territoriality between them and therefore they are expected to react more to the stimulus. In addition, the captures were made close to the breeding season to potentiate this response. Before each experimental day in the field, we previously prepared the set up (fig 22) to immediately receive the individuals in order to reduce as much as possible the stress that could interfere with the experience. The set up consisted of 4 individual boxes of the same dimensions (40 × 30 × 30 cm) with a refuge positioned in the middle of the box and a small container with water. A mirror (15 × 15 cm) was previously placed on the side of the box, covered with cardboard and only exposed at the beginning of the test. The videos were recorded with two GoPro, which were already positioned. A cardboard box was also used around the terrariums in order to reduce external stimuli. A maximum of 4 individuals were tested at the same time.

Fig. 22 - Images showing the set up used and previously prepared for the behavioural experiment.

The individuals were captured using the noose technique and placed in a terrarium for a period of acclimation (30 minutes). During the acclimation, lizards' terraria were partially exposed to sunlight, to keep them active, with *ad libitum* water to avoid dehydration. At the end of this acclimation period, we opened an umbrella (so the test would be conducted in the shade) without touching or moving the terrarium. Then, we started the test by removing the mirror covering and allowing the lizards 15 minutes without any disturbance. All experiments were recorded in video. At the end of the test, the lizard was removed from the terrarium and marked on the belly with a number. The terrarium was cleaned with ethanol between tests, and we maintained the conditions for all individuals. We photographed and measured the lizard with a digital caliper (± 0.01 mm), and collected a small part of tail, which was then stored in 96% ethanol. We collected blood smears in a slide with the blood that was released during the cut, for microscopic analysis, and also made blood spots on Whatman filter paper. All samples were stored in a small portable cooler in the field. At the end of the sampling, all lizards were released at the location they were caught.

Laboratorial analysis

Blood smears were examined under a microscope. We photographed the smears (fig 23) using the most recent EPview software® coupled with the OLYMPUS EP50 built-in camera with the 40x objective. We used ImageJ® software with a cell counter plug-in to score parasite intensity, by counting the number of infected erythrocytes. We counted at least 2,000 erythrocytes for each individual (Amo Fargallo, et al. 2005; Amo López et al. 2005).

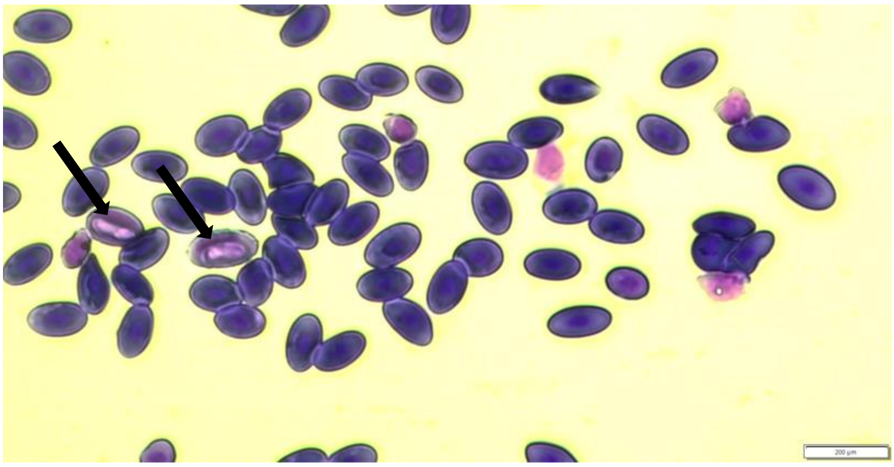

Fig. 23 - Picture of a blood smear from a *P. bocagei* individual, where a *Hepatozoon* sp. is indicated by the arrow. The photo was taken using OLYMPUS EP50 camera (40x objective) and the EPview software®. Picture by Diana Vasconcelos.

Behavioural video analysis

Videos were analysed using BORIS (Behavioral Observation Research Interactive Software; Friard and Gamba, 2016, freely available at www.boris.unito.it). Several variables were scored: whether or not the individual left the shelter (yes/no); the time of interaction with the mirror (0-900 seconds) and whether or not it touched the mirror (yes/no). These factors were used to define a score, based also on the previous study by Scali in 2019, that was established for each individual from 1 to 4 by means of their behaviours with the stimulus: 1- no interest (no interaction with the stimulus) ; 2- interest, but only observing (individuals who showed interest, but only observed the mirror) 3- interest and touched without aggression (observing and tongue-flicking or touching towards the stimulus, but without biting) 4 - individuals with overt aggression (biting against the stimulus

Statistical analyses

We used Spearman's correlation tests to calculated the relationship between the number of parasites and the SVL. We tested for differences in parasite intensity between species and SVL using the Permutational Multivariate Analysis of Variance, included in the "*vegan*" package. Using the Mann-Whitney U test (Kruskal, 1957), the difference in parasite number (intensity) between the two species was verified. To test the prevalence of parasites, we used a glm (generalised linear model) to find out what factors might affect the number of infected lizards. All data were analysed in R Software v.4.1.1 (R Core Team 2020).

Results

The correlation test indicates the number of parasites present in the lizards were associated with SVL, with smaller individuals hosting a higher number of parasites (p= 0.04, R = - 0.040) (fig 24). Size differences (SVL) between species (*P. bocagei* and *P. lusitanicus*) were not significant (p= 0.22).

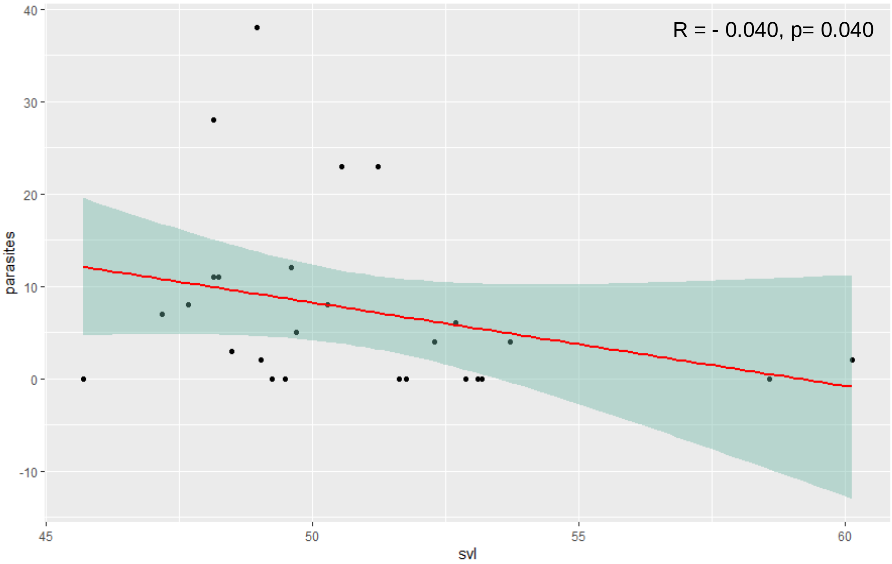

Figure 24 - Spearman's Correlation test between size (SVL) and parasite intensity. The coloured area represents the 95% confidence limit.

The prevalence of infection for the two species (*P. lusitanicus* and *P. bocagei*) was 65.4% (17 of the 26 individuals were infected). Within each species the prevalence was calculated, in *P. bocagei* the prevalence was 72.7% (8 infected in 11 individuals of the species) while in *P. lusitanicus* the prevalence was 60% (9 infected in 15 individuals of the species). The intensity of infection ranged from 0 to 38 infected cells in 2,000 erythrocytes. Among the 17 infected lizards, 10 showed an intensity of 0-10 infected cells, three lizards displayed an intensity of 10–20 infected cells and four exhibited an infection of >20 infected cells, in 2,000 erythrocytes. Significant differences in parasite intensity between the two species were found (wilcox.test(parasites ~ species) (W = 12, p-value = 0.0105) (fig 25), with *P. lusitanicus* having higher parasite intensity.

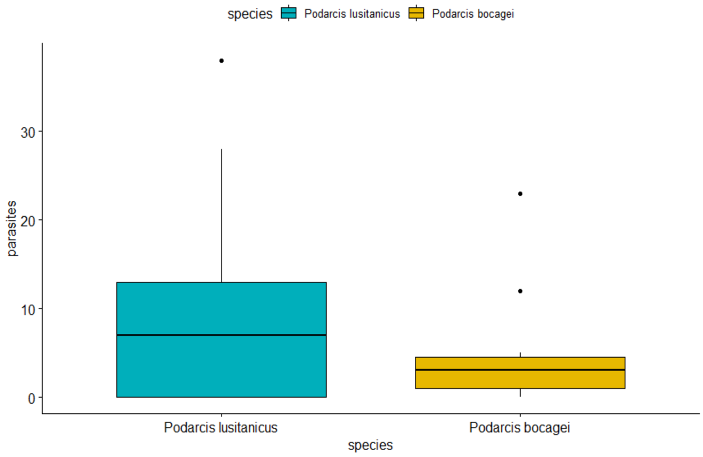

Fig. 25 - Boxplots with the relation between the species and parasite intensity.

Regarding to behaviour analysis, the highest score recorded for the mirror test was 3 (observed and touched the stimulus), since none of the individuals had an aggressive reaction to the mirror. 56% of the individuals (7 *P. bocagei* and 7 *P. lusitanicus*) did not interact with the mirror (score 1), one individual (1%) only observed the mirror without interacting (score 2) and finally 44% of the individuals (n=11) interacted and touched the mirror (score 3). Within these 11 individuals that were scored 3, eight were *P. lusitanicus* and three were *P. bocagei*. *Podarcis lusitanicus* was the species that had the highest interaction with the mirror (fig 26).

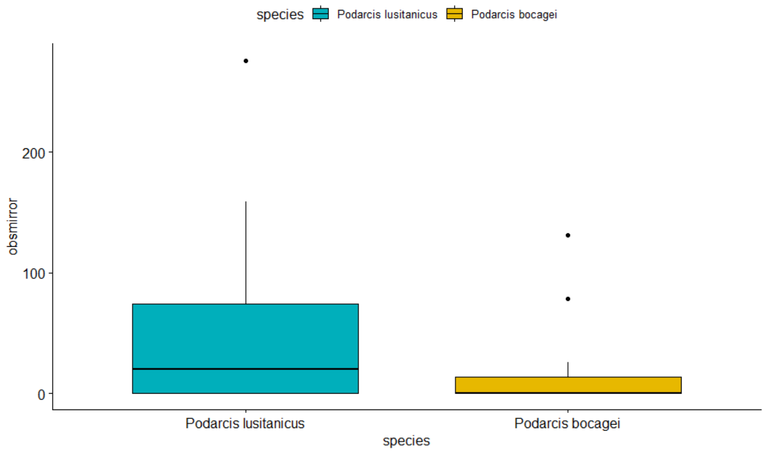

Figure 26 - Boxplots with the relation between the species and the time that each individual observed the mirror, during the trial.

Before analysing the effect of parasites on behaviour, we examined whether the sample was normally distributed using the Shapiro-Wilk test (Shapiro and Wilk, 1965) and homoscedasticity using Bartlett's test (Bartlett, 1937). The two tests were significant (in all of them $p<0.05$) which made us use non-parametric tests for the rest of the analysis, using the factors species, time of mirror observation, parasite intensity and parasite prevalence.

Using the function *adonis*, (adonis(formula=dados$parasites ~ dados$species * dados$SVL, permutations = 999, distance = "euclidean")) we found the infection intensity to be significant for the species (df=1, p = 0.02), indicating there is a difference between the species in the number of parasites, but the results were not significant for SVL. *Podarcis lusitanicus* had higher parasite infection level than *P. bocagei* (fig 6). Moreover, a Spearman correlation test showed a (nearly significant) positive correlation between the parasite intensity and the time that the individuals observed the mirror (p= 0.058, r=0.27), indicating that probably more parasitized individuals will have higher interaction with the mirror (fig 27). Also, a positive relationship was also found between prevalence and the parasite intensity using glm model (glm(infect~species*parasites, family=binomial(logit)) (p <0.005) and the time of observation of the mirror glm(infect~species*obsmirror, family=binomial(logit)) (p = 0.003).

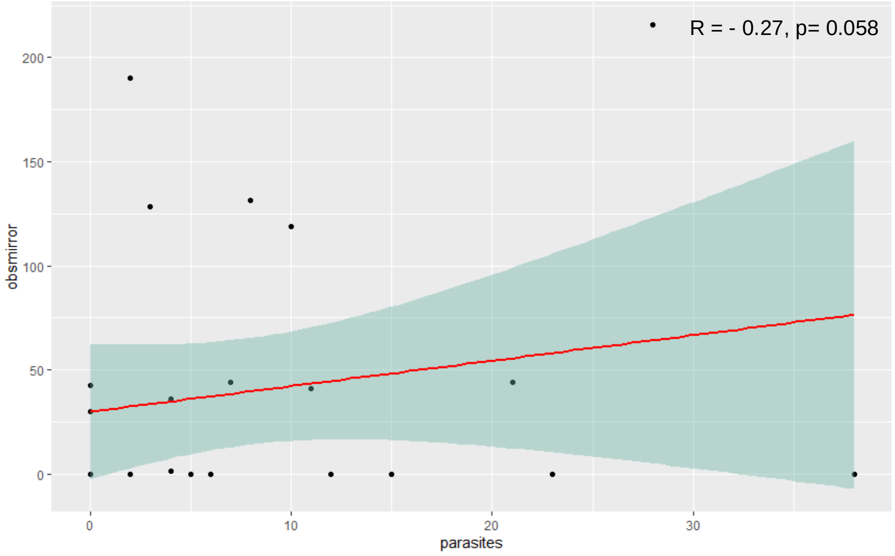

Figure 27 - Spearman's Correlation test between mirror-observation time and parasite intensity. The coloured area represents the 95% confidence limit.

Discussion

In this study, we evaluated the effect of blood parasites on behaviour using a mirror test, in two species of syntopic lizards, *P. bocagei* and *P. lusitanicus*. After analysing the videos, we noticed that the interactions that the lizards had with the mirror were not extensive. The lizards that interacted with the mirror were mostly exploring the terrarium and when they reached the mirror they showed only curiosity by touching it lightly and observing it. Despite the absence of an aggressive response, the response can still be considered a measure for boldness. Bolder behaviour (72%) was recorded in the species with the highest parasitic intensity, *P. lusitanicus*. However, this same species, *P. lusitanicus*, also has a lower percentage of infected individuals (prevalence) compared to *P. bocagei*. This was not entirely as expected, since the species with the most parasites was also the most responsive to the mirror. *P. bocagei* had very few individuals interacting with the mirror, but also had fewer parasitised individuals.

Despite living in the same habitat site and sharing similar resources, these two species differ in the levels of parasite infection. These can be justified by slight differences in habitat choices between them (Sillero and Gonçalves-Seco, 2014;

Carretero et al. 2015) and also in differences in exploratory behaviour between them. In a study conducted in 2015 (with data collected between 2011 and 2013) in this population with both species living in sympatry and where individuals of both sexes were captured, higher values were found in *P. lusitanicus* compared to *P. bocagei*, both in terms of parasite intensity and prevalence of infected (Maia et al. 2015). Regarding parasite intensity the results of our study are in line with the mentioned study (Maia et al. 2015) with the species *P. lusitanicus* having higher intensity than *P. bocagei.* However, the values of the prevalence of infected were different, with a higher prevalence of infected in *P. bocagei* than in *P. lusitanicus*. There were also no significant differences between the SVL of the species, but our sample only consisted of adult males. Maia et al. (2015) also reported significant differences in prevalence between individuals with regenerated tails compared to those with intact tails, and body size (SVL) was positively correlated with prevalence for both species, but not significantly correlated with intensity of infection. The relationship between prevalence and body size was different in our study, as it was not significant. This may be associated with the fact that the captures were made at a different time of the year, compared to this study, and this may affect these two factors. Relative to the tail condition, we did not make this analysis but it would be interesting to investigate this correlation with our data and compare it with the previous study. As such, in a future study it would be interesting to capture more individuals to investigate the influence that SVL has on both parasites and behavior. Our results indicate that males of *P. lusitanicus*, may be more daring and show higher exploratory behaviour than males of *P. bocagei*. However, considering the levels of parasitism, the species *P. lusitanicus* is also the one with higher average parasite intensity comparing to *P. bocagei*. This did not fit our initial expection, since, for example *P. siculus* which is more bold and with a more exploratory behavior (Damas-Moreira et al. 2019) had very few blood parasites compared to *P. virescens* (Tome at al. 2021). The positive correlation between the number of parasites and the time the lizards observed the mirror was not exactly significant, but it was close, but taking into account that our number of samples is relatively small, we can consider that there is reasonable evidence to indicate that more parasitized individuals interact more with the mirror. A larger sample size in this population would help clarify this result. Differences between our study and the results from Maia et al. (2015) may be due to the difference in sampling, both at the level of sexes (only males were used for this thesis) and because it is a smaller sample. Although sampling was carried out close to the breeding season, this small time difference may have already affected the results, compared to the study discussed above (Maia et al. 2015). On the other hand, a lizard with more exploratory behaviour is expected to move more over a larger area of habitat and may have more encounters with parasite individuals and vectors, which in turn may lead to contracting more parasites. *Podarcis lusitanicus* may show a bolder behaviour and have a higher intensity of parasites, but these may not have affected its physical condition, contrary to what happens in some cases (Dawson and Bortolotti, 2000; Jiménez-Peñuela et al. 2019). The same would need to be further investigated to test the pathogenicity of the parasites present in this individual and how they may or may not affect the lizard's behaviour.

After performing the tests and analyzing them, we can propose some improvements to obtain better data and increase the number of samples and responses, since some lizards had no interactions with the mirror. Despite the limitations of the study, our results suggest that mirrors seem to be a reasonable methodology to obtain behavioural responses in the field, thereby avoiding the need to transport lizards with all the associated risks and stress to the lizard. These studies are important to help us understand the effect that parasites can have on host behaviour and even on population dynamics.

References

Amo L, López P, Martín J (2005) Prevalence and intensity of haemogregarine blood parasites and their mite vectors in the common wall lizard, *Podarcis muralis*. *Parasitology Research*, 96, 378–381.

Amo, L., Fargallo, J. A., Martinez-Padilla, J., Millán, J., López, P., & Martín, J. (2005). Prevalence and intensity of blood and intestinal parasites in a field population of a Mediterranean lizard, *Lacerta lepida*. *Parasitology Research*, 96(6), 413-417.

Balzarini, V., Taborsky, M., Wanner, S., Koch, F., & Frommen, J. G. (2014). Mirror, mirror on the wall: the predictive value of mirror tests for measuring aggression in fish. *Behavioral Ecology and Sociobiology*, 68(5), 871-878.

Bartlett, M. S. (1937). Properties of sufficiency and statistical tests. *Proceedings of the Royal Society of London. Series A-Mathematical and Physical Sciences*, *160*(901), 268-282.

Caeiro-Dias, G., Rocha, S., Couto, A., Pereira, C., Brelsford, A., Crochet, P. A., & Pinho, C. (2021). Nuclear phylogenies and genomics of a contact zone establish the species rank of *Podarcis lusitanicus* (Squamata, Lacertidae). *Molecular Phylogenetics and Evolution*, *164*, 107270.

Carretero, M. A., Galán, P., Salvador, A. (2015). Lagartija lusitana - *Podarcis guadarramae.* En: *Enciclopedia Virtual de los Vertebrados Españoles*. Salvador, A., Marco, A. (Eds.). Museo Nacional de Ciencias Naturales, Madrid. http://www.vertebradosibericos.org/

Coladonato, A. J., Mangiacotti, M., Scali, S., Zuffi, M. A., Pasquariello, C., Matellini, C., Buratti, S., Battaiola, M., & Sacchi, R. (2020). Morph-specific seasonal variation of aggressive behaviour in a polymorphic lizard species. *PeerJ*, *8*, e10268.

Damas-Moreira, I., Riley, J. L., Carretero, M. A., Harris, D. J., & Whiting, M. J. (2020). Getting ahead: exploitative competition by an invasive lizard. *Behavioral Ecology and Sociobiology*, *74*(10), 1-12.

Damas-Moreira, I., Riley, J. L., Harris, D. J., & Whiting, M. J. (2019). Can behaviour explain invasion success? A comparison between sympatric invasive and native lizards. *Animal Behaviour*, *151*, 195-202.

Dawson, R. D., & Bortolotti, G. R. (2000). Effects of hematozoan parasites on condition and return rates of American kestrels. *The Auk*, *117*(2), 373-380.

Duckworth, R. A. (2008). Adaptive dispersal strategies and the dynamics of a range expansion. *The American Naturalist*, *172*(S1), S4-S17.

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations*. Methods in ecology and evolution*, 7(11), 1325-1330.

Hernández-Agüero, J. A., & Megía-Palma, R. Primer registro de respuesta agresiva de *Gallotia galloti* hacia su imagen especular. *Rivista Italiana Ornitologia*, *71*, 216-217.

Hudson, P., & Greenman, J. (1998). Competition mediated by parasites: biological and theoretical progress. *Trends in Ecology & Evolution*, *13*(10), 387-390.

Jiménez-Peñuela, J., Ferraguti, M., Martínez-de la Puente, J., Soriguer, R., & Figuerola, J. (2019). Urbanization and blood parasite infections affect the body condition of wild birds. *Science of the Total Environment*, *651*, 3015-3022.

Kaliontzopoulou, A., Carretero, M. A., & Llorente, G. A. (2012). Morphology of the Podarcis wall lizards (Squamata: Lacertidae) from the Iberian Peninsula and North Africa: patterns of variation in a putative cryptic species complex. *Zoological Journal of the Linnean Society*, 164(1), 173-193.

Kruskal, W. H. (1957). Historical notes on the Wilcoxon unpaired two-sample test. *Journal of the American Statistical Association*, *52*(279), 356-360.

Maia, J. P. (2015). *Diversity, infection patterns and host–parasite associations of apicomplexan parasites in reptiles* (Doctoral dissertation, Ph. D. Thesis. Universidade do Porto, Porto, Portugal).

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D, Minchin, P. R, O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E. and Wagner, H. (2020). vegan: Community Ecology Package. *R package version* 2.5-7. https://CRAN.R-project.org/package=vegan

Pough, F. H., Andrews, R. M., Cadle, J. E., Crump, M. L., Savitsky, A. L., & Wells K. D. (2004). *Herpetology*. Third Edition. Pearson Prentice Hall: Upper Saddle River, NJ.

R Core Team (2020). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. URL https://www.R-project.org/.

Scali, S., Mangiacotti, M., Sacchi, R., Coladonato, A. J., Falaschi, M., Saviano, L., Rampoldi, M. G., Crozi, M., Perotti, C., Zucca, F., Gozzo, E., & Zuffi, M. A. L. (2021). Close encounters of the three morphs: does color affect aggression in a polymorphic lizard?. *Aggressive Behavior*, *47*(4), 430-438.

Scali, S., Sacchi, R., Falaschi, M., Coladonato, A., Pozzi, S., Zuffi, M., & Mangiacotti, M. (2019). Mirrored images but not silicone models trigger aggressive responses in male Common wall lizards. *Acta Herpetologica*, 14(1), 35-41.

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, *52*(3–4), 591–611. https://doi.org/10.1093/biomet/52.3-4.591

Sillero, N., Argaña, E., Matos, C., Franch, M., Kaliontzopoulou, A., & Carretero, M. A. (2020). Local Segregation of Realised Niches in Lizards. *ISPRS International Journal of Geo-Information*, *9*(12), 764.

Sillero, N., Gonçalves-Seco, L. (2014). Spatial structure analysis of a reptile community with airborne LiDAR data. *Journal of Geographical Information Science*, 28:1709-1722.

Tomé, B., Harris, D. J., Perera, A., & Damas-Moreira, I. (2021). Invasive lizard has fewer parasites than native congener. *Parasitology research*, 1-5.

CHAPTER III

GENERAL DISCUSSION

3. General discussion

Throughout this thesis several factors that may influence or be a result of parasite intensity in the blood or changes in the gut microbima of Lizards have been addressed. It was possible to characterize the gut microbiome of five species from Portugal, *Podarcis siculus*, *Podarcis virescens, Teira dugessi, Podarcis bocagei* and *Podarcis lusitanicus*, for the first time. Comparing these species, which are located in different places of Portugal, we verified differences of diversity in the gut microbiome between the species from Lisbon (*P. siculus, P. virescens and T. dugesii*) and those from Moledo (*P. bocagei and P. lusitanicus*), with greater diversity in the Lisbon populations. In the invasive, *P. siculus*, a positive correlation was found between size and alpha bacterial diversity. Subsequently, by analysing and comparing only the two populations where the species coexist (Parque das Nações and Moledo), we were able to obtain preliminary results on the transmission of bacteria between the species. There were high levels of transmission in both populations, and possibly in Lisbon there was a greater transmission from the native *P. virescens* to the invasive *P. siculus*, while in Moledo there was a more bidirectional transmission between species.

Looking at the study between the levels of parasites in the blood and the possible behavioural changes it might generate, some answers were obtained, although not the expected ones. Differences between species were found in the prevalence and intensity of parasites. A higher prevalence of parasites was observed in *P. bocagei*, while in *P. lusitanicus* a higher intensity of parasites was found. In behavioural tests, 42% of the individuals reacted to the stimulus by observing and touching the mirror, with no aggressive behaviour against the stimulus by any lizard. There were differences between species in the mirror observation time, this being greater in *P. lusitanicus*. Now I will move on to the discussion and some explanations by topic to follow below.

3.1 What factors influence the gut microbiome?

The study of the gut microbiome of the species allows us to better understand the dynamics of the populations and the possible modifications that may alter their constitution. These gut microbiomes are being better characterised across animals through high-throughput, next-generation sequencing (NGS). In addition to this technology becoming more and more accessible, which facilitates its use, it also allows us to have a greater knowledge on how host ecology and environmental factors influence the gut microbial communities of the hosts (Shapira 2016). Due to this NGS study, we were able to characterize for the first time the gut microbiome of 5 lizard species that inhabit Portugal. This was a very important point to start a study that had not been done yet, but that brings us a lot of knowledge about these populations and their dynamics.

With this we were able to demonstrate that among the various factors that can affect the gut microbiome, with locality/habitat being the most influential factor for these species. A difference in gut bacterial composition was observed between populations from the North (Moledo) and Centre (Lisbon), where the Lisbon population presented a greater diversity of gut microbiome in its species. This may be due to the differences between localities and habitats, since the habitat of Moledo is semi-natural compared to Lisbon, which is clearly an urban habitat. Urban and non-urban habitats may differ in several factors, including food availability (Berlow et al. 2021), and environmental stressors, which can lead to divergences in the physiological and morphological characteristics of the animal (Baxter-Gilbert et al. 2021). Urban environments normally translate into higher rates of bacterial diversity (Berlow et al. 2021), and our data fits this trend, with diet also being a very influential factor in microbiome studies (Jiang et al. 2017). As such, this may be the case in this study where greater intestinal microbiome diversity exists in more urbanised populations, likely due to the food resources available and a greater diversity of bacteria in the surrounding environment.

Another significant result obtained was the correlation between SVL and alpha bacterial diversity, which was observed in the invasive species *P. siculus*. It has already been shown by researchers working with the same population that *P. siculus* as an invasive species exhibits higher territoriality and bolder behaviour. Their larger size is also potentially an influential factor in the way they compete (larger individuals will have a bolder behaviour) (Damas-Moreira et al. 2020). As such, this correlation found where larger individuals contain more bacterial diversity is in line with previous research. This could also be justified by the increased exploration of environments and contact with other individuals by these larger lizards, where they could acquire more bacteria. A wider ecological niche and a more diversified diet can also be associated to this higher diversity of bacteria. It would be necessary a more directed study probably adding and comparing faecal samples of the lizards over multiple seasons to analyze the diet of these individuals and environmental samples (Kohl et al. 2017), to study the influences of these factors and verify and characterise the niche where these species occur.

Little is still known about the rates at which hosts transmit and obtain the gut microbiome in lizards. This transmission can be horizontal, being acquired through direct contact with other individuals or from the environment (Colston, 2017). In this study, with the data obtained it was possible to make a preliminary study on the transmission of bacteria between individuals of species living in sympatry in both Moledo and PqN. The results indicate that there is a high bacterial transmission in both populations. The transmission found in the PqN population indicates that there is a possible greater transmission from the native *P. virescens* to the invasive *P. siculus* than vice versa. Given *P. siculus* is considered a successful invader (Damas-Moreira et al. 2020) and with a more exploratory behaviour, this may explain our finding. It should also be noted that a small population size at the beginning of the introduction would be expected and that the uptake of local microbiome would be non-uniform, but twenty years after the introduction of *P. siculus* and after its stabilization, perhaps a better balance between the microbiomes of these two species could be expected. In the case of Moledo, a more bidirectional transmission was found between the two species. However, the number of samples between the latter two species, *P. bocagei* and *P. lusitanicus*, was lower and therefore these results need to be interpreted cautiously. Furthermore, although the two populations are sympatric, in Moledo the two species also live in syntopy (Gomes et al. 2016; Sannolo et al. 2018). Syntopy is understood as species that occur in the same microhabitat, where they are observed in close proximity and at the same time (Rivas, 1964). This may justify more equality of gut microbiome diversity and more balanced transmission levels between species, due to greater cohabitation and interaction, relative to the species in Lisbon which are found in sympatry but rarely in syntopy.

3.2 Do blood parasites impact the behaviour of the Lizard?

Another objective of this thesis was to study and relate parasitism with behavioural changes in this population of lizards, comparing the two species *P. bocagei* and *P. lusitanicus.* Parasites are organisms increasingly recognized for having an important role in populations and being responsible for physiological, morphological and behavioral changes in hosts. This is particularly relevant given that in a recent study of *P. siculus* and *P. virescens* a lower number of parasites were observed in the invasive species, which was also considered the boldest (Tomé et al. 2021). For this reason, it was reasonable to hypothesize in this study that the species with the lowest number of parasites would also show bolder behaviour. Regarding the blood smears that were analyzed in this study, we found a higher number of infected lizards in the species*, P. bocagei*. But at the level of parasite intensity the highest number belongs to *P. lusitanicus*. Relating this result to the behavioural test results, the species with the highest interaction with the mirror is the one that should be considered as more bold and with a higher exploratory behaviour, a methodology inspired on the recent Scali study (Scali et al. 2019). In our study it was to observed that *P. lusitanicus* have the longest interaction time with the mirror and consequently, the boldest. Given this results we can consider that there is strong evidence to indicate that more parasitized individuals interact more with the mirror. These results do not fit our initial expectation, since *P. lusitanicus* was the boldest and would be expected to have fewer parasites, and vice versa. However, a lizard with more exploratory behaviour is expected to move more over a larger area of habitat and may also contract more parasites, due to the greater number of encounters with individuals and parasite vectors that may exist as a result of this boldness. Which may be the case for *P. lusitanicus* where it has a higher intensity of parasites coupled with a more daring allied behaviour, but these parasites may not have affected its physical condition, unlike what happens in some cases (Dawson and Bortolotti, 2000; Jiménez-Peñuela et al. 2019).

In conclusion, although we obtained some results from carrying out this study in the field (which can in some cases be the only possible approach), there were clearly notable limitations. Removing the shelter at the start of the test, at the same time of the cover of the mirror, could ensure that all the lizards could see the mirror and would all be in the same condition. Clearly testing much larger numbers of individuals might have improved these results, and possibly carrying out the experiments earlier in the year, at the beginning of the breeding season. Regardless, results suggest that mirrors seem to be a viable methodology to obtain behavioural responses. This study concerning these two components (parasites and behaviour) is also important and have implications for conservation. This is because parasites can cause adverse effects and affect host fitness, and as such play a crucial role at both individual and ecosystem levels.

3.3 Future Remarks

Even though the studies included in this thesis revealed interesting results and provided new information both on the gut microbiome of these five species, contributing to a better understanding of issues concerning host-gut microbiome and parasite-host behaviour interaction, there are still many questions that remain to be explored and justified. Although more researchers are focusing on the study of the lizard gut microbiome and influencing factors (Amato et al. 2013; Jiang et al. 2017; Littleford-Colquhoun et al. 2019), many of its relationships with host and environment remain unanswered. Particularly in Portugal where we have many populations coexisting in the same habitat (either sympatrically or syntopically) (Ribeiro e Sá-Sousa, 2018; Sannolo et al. 2018) it would be very interesting to take advantage of these conditions and try to better understand the interactions between them and the differences between species taking into account different factors. In the two populations that were used for the study of the gut microbiome in this thesis (Lisbon and PqN), it would be important to study the diets of each one and the microbiome that can be found in each specific environment, to try to see if it was possible to understand which would be the most effective source for the gut microbiome within the populations. Furthermore, since there are two species that have a higher level of interaction (syntopy in Moledo) than the populations in Parque das Nações, we could also understand which factors are more preponderant in each type of interaction. Additionally, in these two populations we are facing a population with two endemic species (Moledo) and in Parque das Nações we have the native-invasive relationship that will also be a good comparative factor. Furthermore, one of our objectives at the beginning of the thesis would also be to correlate the parasites of these individuals with their gut microbiome and although a microscopic analysis of the parasites and also molecular analysis to verify the existence of *Hepatozoon* in the species *Teira dugesii* was still carried out, however it was not possible to reach conclusions due to lack of material. Therefore, it would be interesting and important to have a new opportunity to try to understand this relationship.

In one of the studies of this thesis, we had the opportunity to explore a relationship between the parasite rates of individuals and possible changes in behaviour, but the results, although interesting, were not as expected. However, as only 26 individuals of both species were tested, these results can be regarded as a pilot study, but it already shows us good data and results that need more attention and deepening. It was also possible to understand, that although most behavioural studies with this methodology are done in mirrors (Scali et al. 2019), it is possible to perform this type of studies with positive responses from the individuals, without causing too much stress to the lizard (which could influence the results). Regarding the results obtained in this study, it would be necessary to further investigate why the higher the levels of parasites resulted in an increase in boldness behaviour and try to understand if it can be justified with these parasites not compromising the physical conditions of the species, and development of a certain resistance. To do so, a more detailed investigation of the types of parasites present in these species simultaneously would be fundamental to assess this possibility, using the metabarcoding approach to be able to analyse multiple parasites. And the fact that this work was done in a syntopic population is interesting, since these populations share the same environmental characteristics and resources and these significant differences in parasite taxa can be explained by possible particularities in microhabitats, differences in vectors and possible resistance to some parasites.

In conclusion, further research is needed in both studies presented in this thesis to clarify the results and to better understand these interactions, as well as their causes and consequences

General references

Adl, S. M., Simpson, A. G., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R., Bowser, S. S., Brugerolle, G., Fensome, R. A., Fredericq, S., James, T. Y., Karpov, S., Kugrens, P., Krug, J., Lane, C. E., Lewis, L. A., Lodge, J., Lynn, D. H., Mann, D. G., Mccourt, R. M., Mendoza, L., Moestrup, O., Mozley-Standridge, S. E., Nerad, T. A., Shearer, C. A., Smirnov, A. V., Spiegel, W. F., & Taylor, M. F. (2005). The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *Journal of Eukaryotic Microbiology*, *52*(5), 399-451.

Alm, E. W., Daniels-Witt, Q. R., Learman, D. R., Ryu, H., Jordan, D. W., Gehring, T. M., & Santo Domingo, J. (2018). Potential for gulls to transport bacteria from human waste sites to beaches. *Science of The Total Environment*, 615, 123-130.

Amato, K. R., Yeoman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, A., Gaskins, H. R., Stumpf, R. M., Yildirim, S., Torralba, M., Gillis, M., Wilson, B. A., Nelson, K. E., White, B. A.,. & Leigh, S. R. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. *The ISME journal*, 7(7), 1344-1353.

Amo L, López P, Martín J (2005) Prevalence and intensity of haemogregarine blood parasites and their mite vectors in the common wall lizard, *Podarcis muralis*. *Parasitology Research*, 96, 378–381.

Amo, L., Fargallo, J. A., Martinez-Padilla, J., Millán, J., López, P., & Martín, J. (2005). Prevalence and intensity of blood and intestinal parasites in a field population of a Mediterranean lizard, *Lacerta lepida*. *Parasitology Research*, 96(6), 413-417.

Angilletta, M. J. (2009). *Thermal Adaptation. A Theoretical And Empirical Synthesis*. Oxford University Press, Oxford.

Arizza, V., Vecchioni, L., Caracappa, S., Sciurba, G., Berlinghieri, F., Gentile, A., ... & Alduina, R. (2019). New insights into the gut microbiome in loggerhead sea turtles *Caretta caretta* stranded on the Mediterranean coast. *PloS one*, 14(8), e0220329.

Arnold, E. N. (1973). Relationships of the Palaearctic lizards assigned to the genera *Lacerta*, *Podarcis*, *Algyroides* and *Psammodromus*. Bulletin of the British Museum (Natural History), *Zoology* 25 (8): 289-366.

Balzarini, V., Taborsky, M., Wanner, S., Koch, F., & Frommen, J. G. (2014). Mirror, mirror on the wall: the predictive value of mirror tests for measuring aggression in fish. *Behavioral Ecology and Sociobiology*, 68(5), 871-878.

Barbosa, D., Font, E., Desfilis, E., & Carretero, M. A. (2006). Chemically mediated species recognition in closely related *Podarcis* wall lizards. Journal of chemical ecology, 32(7), 1587-1598.

Barelli, C., Albanese, D., Donati, C., Pindo, M., Dallago, C., Rovero, F., Tuohy, K. M., Hauffe, H. C., & De Filippo, C. (2015). Habitat fragmentation is associated to gut microbiota diversity of an endangered primate: implications for conservation. *Scientific reports*, *5*(1), 1-12.

Barrios-O'Neill, D., Bertolini, C., & Collins, P. C. (2017). Trophic cascades and the transient keystone concept. *Biological Conservation*, *212*, 191-195.

Bartlett, M. S. (1937). Properties of sufficiency and statistical tests. *Proceedings of the Royal Society of London. Series A-Mathematical and Physical Sciences*, *160*(901), 268-282.

Baxter-Gilbert, J., Riley, J. L., Frère, C. H., & Whiting, M. J. (2021). Shrinking into the big city: influence of genetic and environmental factors on urban dragon lizard morphology and performance capacity. *Urban Ecosystems*, *24*(4), 661-674.

Berlow, M., Phillips, J. N., & Derryberry, E. P. (2021). Effects of urbanization and landscape on gut microbiomes in white-crowned sparrows. *Microbial Ecology*, *81*(1), 253-266.

Bharwani, A., Mian, M. F., Surette, M. G., Bienenstock, J., & Forsythe, P. (2017). Oral treatment with *Lactobacillus rhamnosus* attenuates behavioural deficits and immune changes in chronic social stress. *BMC medicine*, 15(1), 1-14.

Biondi, L. M., Fuentes, G. M., Córdoba, R. S., Bó, M. S., Cavalli, M., Paterlini, C. A., Castano, M. V., & García, G. O. (2020). Variation in boldness and novelty response between rural and urban predatory birds: The Chimango Caracara, *Milvago chimango* as study case. *Behavioural processes*, 173, 104064.

Blackburn, T. M., Pyšek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarošík, V., Wilson, J. R. U., & Richardson, D. M. (2011). A proposed unified framework for biological invasions. *Trends in ecology & evolution*, 26(7), 333-339.

Bletz, M. C., Goedbloed, D. J., Sanchez, E., Reinhardt, T., Tebbe, C. C., Bhuju, S., Geffers, R., Jarek, M., Vences, M. & Steinfartz, S. (2016). Amphibian gut microbiota shifts differentially in community structure but converges on habitat-specific predicted functions. *Nature Communications*, 7(1), 1-12.

Blumstein, D. T. (2016). Habituation and sensitization: new thoughts about old ideas. *Animal Behaviour*, 120, 255-262.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., Cope, E. K., Da Silva, R., Diener, C., Dorrestein, P. C., Douglas, G. M., Durall, D. M., Duvallet, C., Edwardson, C. F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J. M., Gibbons, S. M., Gibson, D. L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G. A., Janssen, S., Jarmusch, A. K., Jiang, L., Kaehler, B. D., Kang, K. B., Keefe, C. R., Keim, P., Kelley, S. T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M. G. I., Lee, J., Ley, R., Liu, Y. X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B. D., McDonald, D., McIver, L. J., Melnik, A. V., Metcalf, J. L., Morgan, S. C., Morton, J. T., Naimey, A. T., Navas-Molina, J. A., Nothias, L. F., Orchanian, S. B., Pearson, T., Peoples, S. L., Petras, D., Preuss, M. L., Pruesse, E., Rasmussen, L. B., Rivers, A., Robeson, M. S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S. J., Spear, J. R., Swafford, A. D., Thompson, L. R., Torres, P. J., Trinh, P., Tripathi, A., Turnbaugh, P. J., Ul-Hasan, S., van der Hooft, J. J. J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C. H. D., Willis, A. D., Xu, Z. Z., Zaneveld, J. R., Zhang, Y., Zhu, Q., Knight, R., & Caporaso J. G. (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* 37: 852–857. https://doi.org/10.1038/s41587-019-0209-9

Boursier, J., Mueller, O., Barret, M., Machado, M., Fizanne, L., Araujo-Perez, F.,Guy, C. D., Seed, P. C., Rawls, J. F., David, L. A., Hunault, G., Oberti, F., Calès, P., & Diehl, A. M. (2016). The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology*, *63*(3), 764-775.

Broom, L. J., & Kogut, M. H. (2018). The role of the gut microbiome in shaping the immune system of chickens. *Veterinary immunology and immunopathology*, 204, 44-51.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of parasitology*, 575-583.

Caeiro-Dias, G., Rocha, S., Couto, A., Pereira, C., Brelsford, A., Crochet, P. A., & Pinho, C. (2021). Nuclear phylogenies and genomics of a contact zone establish the species rank of *Podarcis lusitanicus* (Squamata, Lacertidae). *Molecular Phylogenetics and Evolution*, *164*, 107270.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nature methods,* 13(7), 581-583.

Capula, M., Luiselli, L., & Rugiero, L. (1993). Comparative ecology in sympatric *Podarcis muralis* and *P. sicula* (Reptilia: Lacertidae) from the historical centre of Rome: What about competition and niche segregation in an urban habitat?. *Italian Journal of Zoology*, 60(3), 287-291.

Carretero, M. A. & Silva-Rocha, I. (2015). La lagartija italiana (*Podarcis sicula*) en la Península Ibérica e islas Baleares. *Ecology* 22: 4829-4841.

Carretero, M. Á., & Salvador Milla, A. (2016). Lagartija verdosa–*Podarcis virescens* Geniez, Sá-Sousa, Guillaume, Cluchier y Crochet, 2014. En: *Enciclopedia Virtual de los Vertebrados Españoles.*

Carretero, M. Á., Galán, P., & Salvador Milla, A. (2015). Lagartija lusitana–*Podarcis guadarrama*e (Boscá, 1916).

Carlson, C. J., Dallas, T. A., Alexander, L. W., Phelan, A. L., & Phillips, A. J. (2020). What would it take to describe the global diversity of parasites?. *Proceedings of the Royal Society B*, *287*(1939), 20201841.

Carthey, A. J., & Banks, P. B. (2018). Naïve, bold, or just hungry? An invasive exotic prey species recognises but does not respond to its predators. *Biological Invasions*, 20(12), 3417-3429.

Chiarello M., Auguet J. C., Bettarel Y., Bouvier C., Claverie T., Graham N. A. J., & al. (2018). Skin microbiome of coral reef fish is highly variable and driven by host phylogeny and diet. *Microbiome* 6:147. 10.1186/s40168-018-0530-4

Coladonato, A. J., Mangiacotti, M., Scali, S., Zuffi, M. A., Pasquariello, C., Matellini, C., Buratti, S., Battaiola, M., & Sacchi, R. (2020). Morph-specific seasonal variation of aggressive behaviour in a polymorphic lizard species. *PeerJ*, *8*, e10268.

Colston, T. J. (2017). Gut microbiome transmission in lizards. *Molecular Ecology* (2017) 26, 972–974

Colston, T. J., & Jackson, C. R. (2016). Microbiome evolution along divergent branches of the vertebrate tree of life: what is known and unknown. *Molecular ecology*, 25(16), 3776-3800.

Colston, T. J., Noonan, B. P., & Jackson, C. R. (2015). Phylogenetic analysis of bacterial communities in different regions of the gastrointestinal tract of *Agkistrodon piscivorus*, the cottonmouth snake. *PLoS One,* 10(6), e0128793.

Combes, C. (2001). *Parasitism: the ecology and evolution of intimate interactions*. University of Chicago Press.

Concepción, E. D., Moretti, M., Altermatt, F., Nobis, M. P., & Obrist, M. K. (2015). Impacts of urbanisation on biodiversity: the role of species mobility, degree of specialisation and spatial scale. *Oikos*, 124(12), 1571-1582.

Coyte, K. Z., Schluter, J., & Foster, K. R. (2015). The ecology of the microbiome: networks, competition, and stability. *Science*, *350*(6261), 663-666.

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature reviews neuroscience*, 13(10), 701-712.

Damas-Moreira, I., Oliveira, D., Santos, J. L., Riley, J. L., Harris, D. J., & Whiting, M. J. (2018). Learning from others: an invasive lizard uses social information from both conspecifics and heterospecifics*. Biology letters*, 14(10), 20180532.

Damas-Moreira, I., Riley, J. L., Carretero, M. A., Harris, D. J., & Whiting, M. J. (2020). Getting ahead: exploitative competition by an invasive lizard. *Behavioral Ecology and Sociobiology*, *74*(10), 1-12.

Damas-Moreira, I., Riley, J. L., Harris, D. J., & Whiting, M. J. (2019). Can behaviour explain invasion success? A comparison between sympatric invasive and native lizards. *Animal Behaviour*, 151, 195-202.

Davis, D. J., Bryda, E. C., Gillespie, C. H., & Ericsson, A. C. (2016). Microbial modulation of behavior and stress responses in zebrafish larvae. *Behavioural brain research*, 311, 219-227.

Dawson, R. D., & Bortolotti, G. R. (2000). Effects of hematozoan parasites on condition and return rates of American kestrels. *The Auk*, *117*(2), 373-380.

de la Cruz, F. R. M., Manríquez, N. L., Ríos, E. A., & Ibargüengoytía, N. (2014). Male reproductive cycles in lizards. *Reproductive Biology and Phylogeny of Lizards and Tuatara. CRC Press: Taylor & Francis Group, Boca Raton*, 314-351.

De Palma, G., Blennerhassett, P., Lu, J., Deng, Y., Park, A. J., Green, W., Denou, E., Silva, M. A., Santacruz, A., Sanz, Y., Surette, M. G., Verdu, E. F., Collins, S. M., & Bercik,

P. (2015). Microbiota and host determinants of behavioural phenotype in maternally separated mice. *Nature communications*, 6(1), 1-13.

de Waal, T. (2012) Advances in diagnosis of protozoan diseases. *Veterinary Parasitology,* 189, 65–74.

Delsuc, F., Metcalf, J. L., Wegener Parfrey, L., Song, S. J., González, A., & Knight, R. (2014). Convergence of gut microbiomes in myrmecophagous mammals. *Molecular ecology*, *23*(6), 1301-1317.

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J. F., & Dinan, T. G. (2010). Effects of the probiotic *Bifidobacterium* infantis in the maternal separation model of depression. *Neuroscience*, *170*(4), 1179-1188.

Dias, G., Luis, C., Pinho, C., & Kaliontzopoulou, A. (2016). A case of *Podarcis carbonelli* intake by *Podarcis virescens*. *Herpetology Notes*, 9, 105-108.

Diego-Rasilla, F. J., Pérez-Mellado, V. (2003). Home range and habitat selection by *Podarcis hispanica* (Squamata, Lacertidae) in Western Spain. *Folia Zoologica*, 52 (1): 87-98.

Douglas, A. E., & Werren, J. H. (2016). Holes in the hologenome: why host-microbe symbioses are not holobionts. *MBio*, *7*(2), e02099-15.

Downes, S., & Bauwens, D. (2002). An experimental demonstration of direct behavioural interference in two Mediterranean lacertid lizard species. *Animal behaviour*, *63*(6), 1037-1046.

Ducatez, S., Sayol, F., Sol, D., & Lefebvre, L. (2018). Are urban vertebrates city specialists, artificial habitat exploiters, or environmental generalists? *Integrative and Comparative Biology*, *58*(5), 929-938.

Duckworth, R. A. (2008). Adaptive dispersal strategies and the dynamics of a range expansion. *The American Naturalist*, *172*(S1), S4-S17.

Dunlap, K. D., & Mathies, T. (1993). Effects of nymphal ticks and their interaction with malaria on the physiology of male fence lizards. *Copeia*, 1045-1048.

Dunlap, K. D., & Schall, J. J. (1995). Hormonal alterations and reproductive inhibition in male fence lizards (*Sceloporus occidentalis*) infected with the malarial parasite *Plasmodium* mexicanum. *Physiological Zoology*, *68*(4), 608-621.

Ebert, D. (2005). *Ecology, epidemiology, and evolution of parasitism in Daphnia*. National Library of Medicine.

Farrell, W. J., & Wilczynski, W. (2006). Aggressive experience alters place preference in green anole lizards, *Anolis carolinensis*. *Animal Behaviour*, 71(5), 1155-1164.

Filippo, C. D., Cavalieri, D., Paola, M. D., Ramazzotti, M., Poullet, J. B., Massart, S., ... & Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(33), 14691-14696.

Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., Chapin, F. S., Coet, M. T., Daily, G- C., Gibbs, H. K., Helkowski, J. H., Hollowav, T., Howard, E.

A., Kucharik, C. J., Monfred, C., Patz, J. A., Prentice, I. C., Ramankutty, N., & Snyder, P. K. (2005). Global consequences of land use. *science*, 309(5734), 570-574.

Foster, J. A., Rinaman, L., & Cryan, J. F. (2017b). Stress & the gut-brain axis: regulation by the microbiome. *Neurobiology of stress*, 7, 124-136.

Foster, K. R., Schluter, J., Coyte, K. Z., & Rakoff-Nahoum, S. (2017a). The evolution of the host microbiome as an ecosystem on a leash. *Nature*, *548*(7665), 43-51.

Freiría, F. M. (2005). Sobre o xénero *Podarcis* na Península e Baleares. Herpetoloxía. *Myotis* 1: 15-19.

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations*. Methods in ecology and evolution*, 7(11), 1325-1330.

Fuller, R. C., & Endler, J. A. (2018). A perspective on sensory drive. *Current zoology*, 64(4), 465-470.

Galán, P. (1986). Morfología y distribución del género *Podarcis*, Wagler, 1830 (Sauria, Lacertidae) en el Noroeste de la Península Ibérica. *Revista Española de Herpetología*, *1*, 85-142.

Geffroy, B., Samia, D. S., Bessa, E., & Blumstein, D. T. (2015). How nature-based tourism might increase prey vulnerability to predators. *Trends in Ecology & Evolution*, 30(12), 755-765.

Geniez, P., Sa-Sousa, P., Guillaume, C. P., Cluchier, A., & Crochet, P. A. (2014). Systematics of the *Podarcis hispanicus* complex (Sauria, Lacertidae) III: valid nomina of the western and central Iberian forms*. Zootaxa, 3794*(1), 1-51.

Godfrey Jr, R. D., Fedynich, A. M., & Pence, D. B. (1987). Quantification of hematozoa in blood smears. *Journal of wildlife diseases*, *23*(4), 558-565.

Godfrey, S. S., Bull, C. M., James, R., & Murray, K. (2009). Network structure and parasite transmission in a group living lizard, the gidgee skink, *Egernia stokesii*. *Behavioral Ecology and Sociobiology*, *63*(7), 1045-1056.

Godfrey, S. S., Bull, C. M., Murray, K., & Gardner, M. G. (2006). Transmission mode and distribution of parasites among groups of the social lizard Egernia stokesii. *Parasitology research*, *99*(3), 223-230.

Gomes, V., Carretero, M. A., & Kaliontzopoulou, A. (2016). The relevance of morphology for habitat use and locomotion in two species of wall lizards. *Acta Oecologica*, 70, 87-95.

González de la Vega, J. P., González-García, J. P., García-Pulido, T. & González-García, G. (2001). *Podarcis sicula* (Lagartija italiana), primera cita para Portugal. *Boletín de la Asociación Herpetológica Española* 12(1): 9.

Grimm, N. B., Foster, D., Groffman, P., Grove, J. M., Hopkinson, C. S., Nadelhoffer, K. J., Pataki, D. E., & Peters, D. P. (2008). The changing landscape: ecosystem responses to urbanization and pollution across climatic and societal gradients. *Frontiers in Ecology and the Environment*, 6(5), 264-272.

Gubbels, M. J., & Duraisingh, M. T. (2012). Evolution of apicomplexan secretory organelles. *International journal for parasitology*, *42*(12), 1071-1081.

Hack, M. A. (1997). The energetic costs of fighting in the house cricket, *Acheta domesticus* L. *Behavioral Ecology*, 8(1), 28-36.

Haklová-Kočíková, B., Hižňanová, A., Majláth, I., Račka, K., Harris, D. J., Földvári, G., Tryjanowski, P., Kokošová, N., Malčeková, B., & Majláthová, V. (2014). Morphological and molecular characterization of *Karyolysus*–a neglected but common parasite infecting some European lizards. *Parasites & vectors*, *7*(1), 1-12.

Hammer, T. J., & Bowers, M. D. (2015). Gut microbes may facilitate insect herbivory of chemically defended plants. *Oecologia*, *179*(1), 1-14.

Harris, D. J. & Sá-Sousa, P. (2001). Species distinction and relationships of the Western Iberian *Podarcis* lizards (Reptilia, Lacertidae) based on morphology and mitochondrial DNA sequences. *Herpetological Journal* 11(1): 129-136.

Harris, D. J. & Sá-Sousa, P. (2002). Molecular phylogenetics of Iberian wall lizards (*Podarcis*): Is *Podarcis hispanica* a species complex?. *Molecular Phylogenetics and Evolution* 23(1): 75–81. DOI: 10.1006/mpev.2001.1079

Harris, D. J., Pinho, C., Carretero, M. A., Corti, C., & Bohme, W. (2005). Determination of genetic diversity within the insular lizard *Podarcis tiliguerta* using mtDNA sequence data, with a reassessment of the phylogeny of *Podarcis*. *Amphibia Reptilia*, 26(3), 401-407.

Hatcher, M. J., Dick, J. T., & Dunn, A. M. (2012). Diverse effects of parasites in ecosystems: linking interdependent processes. *Frontiers in Ecology and the Environment*, *10*(4), 186-194.

Hazlett, B. A., Acquistapace, P., & Gherardi, F. (2002). Differences in memory capabilities in invasive and native crayfish. *Journal of Crustacean Biology*, 22(2), 439-448.

Hernández-Agüero, J. A., & Megía-Palma, R. Primer registro de respuesta agresiva de *Gallotia galloti* hacia su imagen especular. *Rivista Italiana Ornitologia*, *71*, 216-217.

Hird, S. M., Sánchez, C., Carstens, B. C., & Brumfield, R. T. (2015). Comparative gut microbiota of 59 neotropical bird species. *Frontiers in microbiology*, 6, 1403.

Holmes, I. A., Monagan Jr, I. V., Rabosky, D. L., & Davis Rabosky, A. R. (2019). Metabolically similar cohorts of bacteria exhibit strong cooccurrence patterns with diet items and eukaryotic microbes in lizard guts. *Ecology and evolution*, *9*(22), 12471-12481.

Hsiao, E. Y., McBride, S. W., Hsien, S., Sharon, G., Hyde, E. R., McCue, T., Codelli, J. A., Chow, J., Reisman, S. E., Petrosino, J. F., Patterson, P. H., & Mazmanian, S. K. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell, 155(7), 1451-1463.

Hudson, P. J., Dobson, A. P., & Lafferty, K. D. (2006). Is a healthy ecosystem one that is rich in parasites?. *Trends in ecology & evolution*, 21(7), 381-385.

Hudson, P. J., Rizzoli, A. P., Grenfell, B. T., Heesterbeek, J. A. P., & Dobson, A. P. (2002). Ecology of wildlife diseases.

Hudson, P., & Greenman, J. (1998). Competition mediated by parasites: biological and theoretical progress. *Trends in Ecology & Evolution*, *13*(10), 387-390.

Hufbauer, R. A., Facon, B., Ravigne, V., Turgeon, J., Foucaud, J., Lee, C. E., Rey, O., & Estoup, A. (2012). Anthropogenically induced adaptation to invade (AIAI): contemporary adaptation to human-altered habitats within the native range can promote invasions. *Evolutionary applications*, 5(1), 89-101.

Ilmberger, N., Güllert, S., Dannenberg, J., Rabausch, U., Torres, J., Wemheuer, B., ... & Streit, W. R. (2014). A comparative metagenome survey of the fecal microbiota of a breast-and a plant-fed Asian elephant reveals an unexpectedly high diversity of glycoside hydrolase family enzymes. *PloS one*, 9(9), e106707.

Imhof, D., Muller, J., Hemphill, A., Ferre, I., Sanchez-Sanchez, R., Ortega-Mora, L. M., & Ojo, K. K. (2021). One health therapeutics: Target-Based drug development for cryptosporidiosis and other apicomplexa diseases. *Veterinary parasitology*, *289*, 109336.

Jakes, K., O'donoghue, P. J., & Cameron, S. L. (2003). Phylogenetic relationships of *Hepatozoon* (*Haemogregarina*) boigae, *Hepatozoon* sp., *Haemogregarina clelandi* and *Haemoproteus chelodina* from Australian reptiles to other Apicomplexa based on cladistic analyses of ultrastructural and life-cycle characters. *Parasitology*, *126*(6), 555-559.

Jiang, H. Y., Ma, J. E., Li, J., Zhang, X. J., Li, L. M., He, N., Liu, H. J., Wu, Z. J., Han, R. C. & Chen, J. P. (2017). Diets alter the gut microbiome of crocodile lizards. *Frontiers in Microbiology*, 8, 2073.

Jiménez-Peñuela, J., Ferraguti, M., Martínez-de la Puente, J., Soriguer, R., & Figuerola, J. (2019). Urbanization and blood parasite infections affect the body condition of wild birds. *Science of the Total Environment*, *651*, 3015-3022.

Juan, F. (1997). La lagartija ibérica (*Podarcis hispanica*) en la Sierra de Segura, Albacete: biometría, etología y folidosis. *Al-Basit: Revista de Estudios Albacetenses* 40: 111-134.

Kaliontzopoulou, A., Carretero, M. A., & Llorente, G. A. (2012). Morphology of the Podarcis wall lizards (Squamata: Lacertidae) from the Iberian Peninsula and North Africa: patterns of variation in a putative cryptic species complex. *Zoological Journal of the Linnean Society*, 164(1), 173-193.

Kaliontzopoulou, A., Pinho, C., Harris, D. J. & Carretero, M. A. (2011). When cryptic diversity blurs the picture: a cautionary tale from Iberian and North African *Podarcis* wall lizards. *Biological Journal of the Linnean Society* 103(4): 779-800.

Khiav, L. A., & Zahmatkesh, A. (2021). Vaccination against pathogenic *clostridia* in animals: a review. *Tropical Animal Health and Production*, 53(2), 1-12.

Kluger, M. J. (1991). Fever: role of pyrogens and cryogens. *Physiological reviews*, *71*(1), 93-127.

Kohl, K. D., Brun, A., Magallanes, M., Brinkerhoff, J., Laspiur, A., Acosta, J. C., Caviede-Vidal, E., & Bordenstein, S. R. (2017). Gut microbial ecology of lizards: insights into diversity in the wild, effects of captivity, variation across gut regions and transmission. *Molecular ecology*, *26*(4), 1175-1189.

Kohl, K. D., Cary, T. L., Karasov, W. H., & Dearing, M. D. (2013). Restructuring of the amphibian gut microbiota through metamorphosis. *Environmental microbiology reports*, *5*(6), 899-903.

Kolbe, J. J., Lavin, B. R., Burke, R. L., Rugiero, L., Capula, M. & Luiselli, L. (2013). The desire for variety: Italian wall lizard (*Podarcis siculus*) populations introduced to the United States via the pet trade are derived from multiple native-range sources. *Biological Invasions* 15(4): 775-783.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and environmental microbiology*, 79(17), 5112-5120.

Kruskal, W. H. (1957). Historical notes on the Wilcoxon unpaired two-sample test. *Journal of the American Statistical Association*, *52*(279), 356-360.

Lange, H., & Leimar, O. (2001). The influence of predation risk on threat display in great tits. *Behavioral Ecology*, 12(4), 375-380.

Littleford-Colquhoun, B. L., Weyrich, L. S., Kent, N., & Frere, C. H. (2019). City life alters the gut microbiome and stable isotope profiling of the eastern water dragon (*Intellagama lesueurii*). *Molecular ecology*, *28*(20), 4592-4607.

Loureiro, A., Ferrand de Almeida, N., Carretero, M. A. & Paulo, O. S. (eds.) (2008). *Atlas dos anfíbios e répteis de Portugal*. Instituto da Conservação da Natureza e da Biodiversidade, Lisboa.

Lowry, H., Lill, A., & Wong, B. B. (2013). Behavioural responses of wildlife to urban environments*. Biological reviews*, 88(3), 537-549.

Mačát, Z., Veselý, M. & Jablonski, D. (2015). New case of fruit eating observation in *Podarcis siculus* (Rafinesque-Schmaltz, 1810) (Lacertidae) from Croatia. *Biharean Biologist* 9(2): 158-159.

Maia, J. P., Álvares, F., Boratyński, Z., Brito, J. C., Leite, J. V., & Harris, D. J. (2014). Molecular assessment of Hepatozoon (Apicomplexa: Adeleorina) infections in wild canids and rodents from North Africa, with implications for transmission dynamics across taxonomic groups. *Journal of Wildlife Diseases*, *50*(4), 837-848.

Maia, J. P. (2015). *Diversity, infection patterns and host–parasite associations of apicomplexan parasites in reptiles* (Doctoral dissertation, Ph. D. Thesis. Universidade do Porto, Porto, Portugal).

Maia, J. P., Perera, A., & Harris, D. J. (2012). Molecular survey and microscopic examination of Hepatozoon Miller, 1908 (Apicomplexa: Adeleorina) in lacertid lizards from the western Mediterranean. *Folia Parasitologica*, 59(4), 241.

Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M., & Schad, G. (1982). The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *The Journal of parasitology*, *68*(1), 131-133.

Martin, M. O., Gilman, F. R., & Weiss, S. L. (2010). Sex-specific asymmetry within the cloacal microbiota of the striped plateau lizard, *Sceloporus virgatus*. *Symbiosis*, *51*(1), 97-105.

Martín, R., Miquel, S., Langella, P., & Bermúdez-Humarán, L. G. (2014). The role of metagenomics in understanding the human microbiome in health and disease. *Virulence*, *5*(3), 413-423.

Mateus, C. (2014). The impact of repeated-fire regimes on the reptile community at the Peneda-Gerês National Park. Internship Report. University of Porto, Porto, Portugal.

Mathipa, M. G., & Thantsha, M. S. (2017). Probiotic engineering: towards development of robust probiotic strains with enhanced functional properties and for targeted control of enteric pathogens. *Gut pathogens*, 9(1), 1-17.

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C., Carey, H. V., Domazet-Lošo, T., Douglas, A. E., Dubilier, N., Eberl, G., Fukami, T., Gilbert, S. F., Hentschel, U., King, N., Kjelleberg, S., Knoll, A. H., Kremer, N., Mazmanian, S. K., Metcalf, J. L., Nealson, K., Pierce, N. E., Rawls, J. F., Reid, A., Ruby, E. G., Rumpho, M., Sandre, J. G., Tautz, D., & Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences*, 110(9), 3229-3236.

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS one*, 8(4), e61217.

McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS computational biology*, *10*(4), e1003531.

Mennen G. J., & Laskowski, K.L. (2018) Defence is the best offence: invasive prey behaviour is more important than native predator behaviour. Animal Behaviour 138, 157–164. (doi:10.1016/j.anbehav.2018.02.017)

Moeller, A. H., Ivey, K., Cornwall, M. B., Herr, K., Rede, J., Taylor, E. N., & Gunderson, A. R. (2020). The lizard gut microbiome changes with temperature and is associated with heat tolerance. *Applied and environmental microbiology*, 86(17), e01181-20.

Moeller, A. H., Li, Y., Ngole, E. M., Ahuka-Mundeke, S., Lonsdorf, E. V., Pusey, A. E., Peeter, M., Hahn, B. H., & Ochman, H. (2014). Rapid changes in the gut microbiome during human evolution. *Proceedings of the National Academy of Sciences*, *111*(46), 16431-16435.

Moeller, A. H., Peeters, M., Ndjango, J. B., Li, Y., Hahn, B. H., & Ochman, H. (2013). Sympatric chimpanzees and gorillas harbor convergent gut microbial communities. *Genome research*, 23(10), 1715-1720.

Montoya-Ciriaco, N., Gómez-Acata, S., Muñoz-Arenas, L. C., Dendooven, L., Estrada-Torres, A., de la Vega, A. H. D., & Navarro-Noya, Y. E. (2020). Dietary effects on gut microbiota of the mesquite lizard *Sceloporus grammicus* (Wiegmann, 1828) across different altitudes. *Microbiome*, *8*(1), 1-19.

Moody, A. (2002). Rapid diagnostic tests for malaria parasites. *Clinical microbiology reviews*, *15*(1), 66-78.

Mooney, H. A., & Cleland, E. E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences*, 98(10), 5446-5451.

Morrison, D. A. (2009). Evolution of the Apicomplexa: where are we now?. *Trends in parasitology*, *25*(8), 375-382.

Muegge, B. D., Kuczynski, J., Knights, D., Clemente, J. C., González, A., Fontana & Gordon, J. I. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science*, 332(6032), 970-974.

Müller, C. B., & Schmid-Hempel, P. (1993). Exploitation of cold temperature as defence against parasitoids in bumblebees. *Nature*, *363*(6424), 65-67.

Myers, R. E., & Hyman, J. (2016). Differences in measures of boldness even when underlying behavioral syndromes are present in two populations of the song sparrow (*Melospiza melodia*). *Journal of ethology*, 34(3), 197-206.

Neufeld, K. M., Kang, N., Bienenstock, J., & Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology & Motility*, *23*(3), 255-e119.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D, Minchin, P. R, O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E. and Wagner, H. (2020). vegan: Community Ecology Package. *R package version* 2.5-7. https://CRAN.R-project.org/package=vegan

Oliverio, M., Bologna, M. A., & Mariottini, P. (2000). Molecular biogeography of the Mediterranean lizards *Podarcis* Wagler, 1830 and *Teira* Gray, 1838 (Reptilia, Lacertidae). *Journal of Biogeography*, 27(6), 1403-1420.

Oppliger, A., Celerier, M. L., & Clobert, J. (1996). Physiological and behaviour changes in common lizards parasitized by haemogregarines. *Parasitology*, *113*(5), 433-438.

Orosz, F. (2021). Apicortin, a Constituent of Apicomplexan Conoid/Apical Complex and Its Tentative Role in Pathogen—Host Interaction. *Tropical medicine and infectious disease*, *6*(3), 118.

Osborne, L., Umbers, K. D., Backwell, P. R., & Keogh, J. S. (2012). Male tawny dragons use throat patterns to recognize rivals. *Naturwissenschaften*, 99(10), 869-872.

Pedersen, A. B., & Fenton, A. (2007). Emphasizing the ecology in parasite community ecology. *Trends in ecology & evolution*, *22*(3), 133-139.

Pedersen, A. B., & Greives, T. J. (2008). The interaction of parasites and resources cause crashes in a wild mouse population. *Journal of Animal Ecology*, 77(2), 370-377.

Pellitteri-Rosa, D., Liuzzi, C. & Bellati, A. (2015). First report of adult *Podarcis siculus* (Rafinesque-Schmaltz, 1810) predation by *Tarentola mauritanica* (Linnaeus, 1758). *Herpetozoa* 28 (1/2): 89-92.

Perkins, S. L., Martinsen, E. S., Falk, B. G. (2011) Do molecules matter more than morphology? Promises and pitfalls in parasites. *Parasitology*, 138, 1664–1674.

Pettit, L., Brown, G. P., Ward-Fear, G., & Shine, R. (2021). Anthropogenically modified habitats favor bigger and bolder lizards. *Ecology and Evolution*, 11(4), 1586-1597.

Pinho, C., Ferrand, N. & Harris, D. J. (2006). Reexamination of the Iberian and North African *Podarcis* (Squamata: Lacertidae) phylogeny based on increased mitochondrial DNA sequencing. *Molecular Phylogenetics and Evolution* 38(1): 266-273.

Pinho, C., Harris, D. J., & Ferrand, N. (2008). Non-equilibrium estimates of gene flow inferred from nuclear genealogies suggest that Iberian and North African wall lizards (*Podarcis* spp.) are an assemblage of incipient species. *BMC Evolutionary Biology*, 8(1), 1-20.

Pinho, C., Kaliontzopoulou, A., Harris, D. J., & Ferrand, N. (2011). Recent evolutionary history of the Iberian endemic lizards *Podarcis bocagei* (Seoane, 1884) and *Podarcis carbonelli* Pérez-Mellado, 1981 (Squamata: Lacertidae) revealed by allozyme and microsatellite markers. *Zoological Journal of the Linnean Society*, 162(1), 184-200.

Pleguezuelos, J. M. (2002). Atlas y libro rojo de los anfibios y reptiles de España (pp. 501-532). R. Márquez, & M. Lizana (Eds.). Spain: Dirección General de Conservación de la Naturaleza.

Podnar, M., Mayer, W., & Tvrtković, N. (2005). Phylogeography of the Italian wall lizard, *Podarcis sicula*, as revealed by mitochondrial DNA sequences. *Molecular Ecology*, 14(2), 575-588.

Pough, F. H., Andrews, R. M., Cadle, J. E., Crump, M. L., Savitsky, A. L., & Wells K. D. (2004). *Herpetology*. Third Edition. Pearson Prentice Hall: Upper Saddle River, NJ.

Pough, F. H., R. M. Andrews, f. E. Cadle, M. L. Crump, A. H. SaviElry, K. D. Wells. (1998). *Herpetology. Prectice-hall*, Upper Saddle Rive4, NJ, USA

Poulakakis, N. P. A. D. E. M. E., Lymberakis, P., Antoniou, A., Chalkia, D., Zouros, E., Mylonas, M., & Valakos, E. (2003). Molecular phylogeny and biogeography of the wall-lizard *Podarcis erhardii* (Squamata: Lacertidae). *Molecular Phylogenetics and Evolution*, 28(1), 38-46.

Poulin R (2007) *Evolutionary ecology of parasites*. Princeton University Press, New Jersey.

Poulin, R. (2017). Invasion ecology meets parasitology: Advances and challenges. International Journal for Parasitology: *Parasites and Wildlife*, 6(3), 361-363.

Prenter, J., MacNeil, C., Dick, J. T., & Dunn, A. M. (2004). Roles of parasites in animal invasions. *Trends in ecology & evolution*, 19(7), 385-390.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., & Glöckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic acids research*, *35*(21), 7188-7196.

Qian, G., & Ho, J. W. (2020). Challenges and emerging systems biology approaches to discover how the human gut microbiome impact host physiology. *Biophysical Reviews*, 1-13.

Quast C., Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucl. Acids Res*. 41 (D1): D590-D596.

R Core Team (2020). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. URL https://www.R-project.org/.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., Dingemanse, N. J. (2007) Integrating animal temperament within ecology and evolution. *Biological Reviews* 82:291–318.

Ribeiro, R., & Sá-Sousa, P. (2018). Where to live in Lisbon: urban habitat used by the introduced Italian wall lizard (*Podarcis siculus*). *Basic and Applied Herpetology*, *32*, 57-70.

Richards-Zawacki, C. L. (2010). Thermoregulatory behaviour affects prevalence of chytrid fungal infection in a wild population of Panamanian golden frogs. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1681), 519-528.

Richter, K.., (1980) - *Lacerta dugesii* Milne-Edwards, 1829 und *Lacerta perspicillata* Duméril et Bibron, 1839 gehören zur Gattung *Podarcis* Wagler, Subgenus *Teira* Gray, 1838 (Reptilia, Lacertidae). Zool. Abh. Mus. Tierk. Dresden, 36. 1-9

Rivas, L. R. (1964). A reinterpretation or the concepts “Sympatric” and “Allopatric” with proposal or the additional terms “Syntopic” and “Allotopic”. *Systematic Zoology*, *13*(1), 42-43.

Rivera, X., Arribas, O., Carranza, S. & Maluquer-Margalef, J. (2011). An introduction of *Podarcis sicula* in Catalonia (NE Iberian Peninsula) on imported olive trees. Butlletí de la Societat Catalana d’Herpetologia 19: 79-85.

Roca, V., & Galdón, M. A. (2010). Haemogregarine blood parasites in the lizards *Podarcis bocagei* (Seoane) and *P. carbonelli (*Pérez-Mellado) (Sauria: Lacertidae) from NW Portugal. *Systematic Parasitology*, *75*(1), 75-79.

Rocca, J. D., Simonin, M., Blaszczak, J. R., Ernakovich, J. G., Gibbons, S. M., Midani, F. S., & Washburne, A. D. (2019). The microbiome stress project: toward a global meta-analysis of environmental stressors and their effects on microbial communities. *Frontiers in microbiology,* 9, 3272.

Routtu, J., & Ebert, D. (2015). Genetic architecture of resistance in *Daphnia* hosts against two species of host-specific parasites. *Heredity*, *114*(2), 241-248.

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., & Tuohy, K. (2018). Gut microbiota functions: metabolism of nutrients and other food components. *European journal of nutrition*, *57*(1), 1-24.

te

Sadek, R. A. (1981). The diet of the Madeiran lizard Lacerta dugesii. *Zoological Journal of the Linnean Society*, *73*(4), 313-341.

Salvador, A. & García-París, M. (2015). Lagartija italiana – *Podarcis siculus* (Rafinesque-Schmalz, 1810). In: Enciclopedia Virtual de los Vertebrados Españoles.

Salvi, D., Pinho, C., Mendes, J., & Harris, D. J. (2021). Fossil-calibrated time tree of *Podarcis* wall lizards provides limited support for biogeographic calibration models. *Molecular Phylogenetics and Evolution*, 161, 107169.

Sambrook, J., Fritsch, E. F., Maniatis, T. (1989) Molecular cloning (Vol. 2). *Cold spring harbor laboratory press*, New York.

Sampson, T. R., & Mazmanian, S. K. (2015). Control of brain development, function, and behavior by the microbiome. *Cell host & microbe,* 17(5), 565-576.

Sannolo, M., Barroso, F. M., & Carretero, M. A. (2018). Physiological differences in preferred temperatures and evaporative water loss rates in two sympatric lacertid species. *Zoology*, *126*, 58-64.

Santos, M., Cruz, C. S., Alves, F. L., Metelo, I., Bogalho, V., Pereira, H. M., ... & Henriques, D. (2012). Biodiversidade na cidade de Lisboa: uma estratégia para 2020. *Documento Técnico*. Câmara Municipal de Lisboa, Lisboa

Sá-Sousa, P. (1995). The introduced Madeiran lizard, *Lacerta (Teira) dugesii* in Lisbon. *Amphibia-Reptilia*, 16(2), 211-214.

Sá-Sousa, P. (2001). Comparative chorology between *Podarcis bocagei* and *P. carbonelli* (Sauria: Lacertidae) in Portugal. *Rev. Esp. Herp*, *15*, 85-97.

Scali, S., Mangiacotti, M., Sacchi, R., Coladonato, A. J., Falaschi, M., Saviano, L., Rampoldi, M. G., Crozi, M., Perotti, C., Zucca, F., Gozzo, E., & Zuffi, M. A. L. (2021). Close encounters of the three morphs: does color affect aggression in a polymorphic lizard?. *Aggressive Behavior*, *47*(4), 430-438.

Scali, S., Sacchi, R., Falaschi, M., Coladonato, A., Pozzi, S., Zuffi, M., & Mangiacotti, M. (2019). Mirrored images but not silicone models trigger aggressive responses in male Common wall lizards. *Acta Herpetologica*, 14(1), 35-41.

Scepanovic, P., Hodel, F., Mondot, S., Partula, V., Byrd, A., Hammer, C., Alanio, C., Bergstedt, J., Patin, E., Touvier, M., Lantz, O., Albert, M. L., Duffy, B., Quintana-Murci, L., Fellay, J., & Fellay, J. (2019). A comprehensive assessment of demographic, environmental, and host genetic associations with gut microbiome diversity in healthy individuals. *Microbiome*, *7*(1), 1-15.

Schmid-Hempel P (2011) *Evolutionary Parasitology: The integrated study of infections, immunology, ecology and genetics*. Oxford University Press, New York.

Schmid-Hempel, P. (2003). Variation in immune defence as a question of evolutionary ecology. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1513), 357-366.

Scott, K. P., Gratz, S. W., Sheridan, P. O., Flint, H. J., & Duncan, S. H. (2013). The influence of diet on the gut microbiota. *Pharmacological research*, *69*(1), 52-60.

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., Pagad, S., Pyšek, P., Kleunen, M. V., Winter, M., Ansong, M., Arianoutsou, M., Bacher, S., Blasius, B., Brockerhoff, E. G., Brundu, G., Capinha, C., . Causton, C. E., Celesti-Grapow, L., Dawson, W., Dullinger, S., Economo, E. P., Fuentes, N., Guénard, B., Jäger, H., Kartesz, J., Kenis, M., Kühn, I., Lenzner, A., Liebhold B.,, Mosena, A., Moser, D., Nentwig, W., Nishino, M., Pearman, D., Pergl, A. M. J., Rabitsch, W., Rojas-Sandoval, J., Roques, A., Rorke, S., Rossinelli, S., Roy, H. E., Scalera, R., Schindler, S., Štajerová, K., Tokarska-Guzik, B., Walker, K., Ward, D. F., Yamanaka, T., & Essl, F. (2018). Global rise in emerging alien species results from increased accessibility of new source pools. *Proceedings of the National Academy of Sciences*, 115(10), E2264-E2273.

Seto, K. C., Güneralp, B., & Hutyra, L. R. (2012). Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools. *Proceedings of the National Academy of Sciences*, 109(40), 16083-16088.

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika*, *52*(3–4), 591–611. https://doi.org/10.1093/biomet/52.3-4.591

Sharon, G., Segal, D., Ringo, J. M., Hefetz, A., Zilber-Rosenberg, I., & Rosenberg, E. (2010). Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences*, 107(46), 20051-20056.

Shenhav, L., Thompson, M., Joseph, T. A., Briscoe, L., Furman, O., Bogumil, D., Mizrahi, I., Pe'er, I., & Halperin, E. (2019). FEAST: fast expectation-maximization for microbial source tracking. *Nature Methods*, *16*(7), 627-632.

Shivaramaiah, C., Barta, J. R., Hernandez-Velasco, X., Téllez, G., & Hargis, B. M. (2014). Coccidiosis: recent advancements in the immunobiology of *Eimeria* species, preventive measures, and the importance of vaccination as a control tool against these Apicomplexan parasites. *Veterinary Medicine: Research and Reports*, *5*, 23.

Short, K. H., & Petren, K. (2008). Boldness underlies foraging success of invasive *Lepidodactylus lugubris* geckos in the human landscape. *Animal Behaviour*, 76(2), 429-437.

Sillero, N., Argaña, E., Matos, C., Franch, M., Kaliontzopoulou, A., & Carretero, M. A. (2020). Local Segregation of Realised Niches in Lizards. *ISPRS International Journal of Geo-Information*, *9*(12), 764.

Sillero, N., Gonçalves-Seco, L. (2014). Spatial structure analysis of a reptile community with airborne LiDAR data. *Journal of Geographical Information Science*, 28:1709-1722.

Silva-Rocha, I., Salvi, D. & Carretero, M. A. (2012): Genetic data reveal a multiple origin for the populations of the Italian wall lizard *Podarcis sicula* (Squamata: Lacertidae) introduced in the Iberian Peninsula and Balearic islands. *Italian Journal of Zoology* 79(4): 502-510.

Silva-Rocha, I., Salvi, D., Harris, D. J., Freitas, S., Davis, C., Foster, J., Deichsel, G., Adamopoulou, C. & Carretero, M. A. (2014). Molecular assessment of *Podarcis sicula* populations in Britain, Greece and Turkey reinforces a multiple-origin invasion pattern in this species. *Acta Herpetologica* 9(2): 253-258.

Silva-Rocha, I., Sá-Sousa, P., Fariña, B., & Carretero, M. A. (2016). Molecular analysis confirms Madeira as source for insular and continental introduced populations of *Teira dugesii (*Sauria: Lacertidae). *Salamandra*, 52(3), 269-272.

Sloboda, M., Kamler, M., Bulantová, J., Votýpka, J., & Modrý, D. (2007). A new species of *Hepatozoon* (Apicomplexa: Adeleorina) from *Python regius* (Serpentes: Pythonidae) and its experimental transmission by a mosquito vector. *Journal of Parasitology*, *93*(5), 1189-1198.

Smith, S. N., Colston, T. J., & Siler, C. D. (2021). Venomous Snakes Reveal Ecological and Phylogenetic Factors Influencing Variation in Gut and Oral Microbiomes. *Frontiers in microbiology*, *12*, 603.

Sorci, G., Clobert, J., & Michalakis, Y. (1996). Cost of reproduction and cost of parasitism in the common lizard, *Lacerta vivipara*. *Oikos*, 121-130.

Speybroeck, J., Beukema, W., Bok, B., & Van Der Voort, J. (2016). *Field guide to the amphibians and reptiles of Britain and Europe*. Bloomsbury publishing.

Speybroeck, J., Beukema, W., Dufresnes, C., Fritz, U., Jablonski, D., Lymberakis, P., Martínez-Solano, M., Razzetti, E., Vamberger, M., Vences, M., Vörös, J., & Crochet, P. A. (2020). Species list of the European herpetofauna–2020 update by the Taxonomic Committee of the Societas Europaea Herpetologica. *Amphibia-Reptilia,* 41(2), 139-189.

Sugden, S., Sanderson, D., Ford, K., Stein, L. Y., & Clair, C. C. S. (2020). An altered microbiome in urban coyotes mediates relationships between anthropogenic diet and poor health. *Scientific reports,* 10(1), 1-14.

Sylvia, K. E., Jewell, C. P., Rendon, N. M., John, E. A. S., & Demas, G. E. (2017). Sex-specific modulation of the gut microbiome and behavior in Siberian hamsters. *Brain, Behavior, and Immunity*, 60, 51-62.

Tamate, S., Iwasaki, W.M., Krysko, K.L., Camposano, B. J., Mori, H., Funayama, R., Nakayama, K., Makino, T., & Kawata, M. (2017). Inferring evolutionary responses of *Anolis carolinensis* introduced into the Ogasawara archipelago using whole genome sequence data. *Scientific Reports* 7, 18008 https://doi.org/10.1038/s41598-017-17852-7

Telford, S. R. (2009) *Hemoparasites of the Reptilia*: Color atlas and text. CRC Press, Boca Raton, Florida.

Thaiss, C. A., Zmora, N., Levy, M., & Elinav, E. (2016). The microbiome and innate immunity. *Nature*, 535(7610), 65-74.

Tomé, B., Harris, D. J., Perera, A., & Damas-Moreira, I. (2021). Invasive lizard has fewer parasites than native congener. *Parasitology research*, 1-5.

Torchin, M. E., Lafferty, K. D., Dobson, A. P., McKenzie, V. J., & Kuris, A. M. (2003). Introduced species and their missing parasites. *Nature*, 421(6923), 628-630.

Tosini, G., Foà, A., & Avery, R. A. (1992). Body temperatures and exposure to sunshine of ruin lizards *Podarcis sicula* in central Italy. *Amphibia-Reptilia*, 13(2), 169-175.

Uetz, P., Freed, P., & Hošek, J. (2016). The reptile database. 2016. *Available from: www. reptile-database. org*.

Ujvari B, Madsen M, Olsson M (2004) High prevalence of *Hepatozoon* spp. (Apicomplexa, Hepatozoidae) infection in Water Pythons (*Liasis fuscus*) from Tropical Australia. *Journal of Parasitology*, 90, 670–672.

Valdeón, A., Perera, A., Costa, S., Sampaio, F. & Carretero, M. A. (2010). Evidencia de una introducción de *Podarcis sicula* desde Italia a España asociada a una importación de olivos (Olea europaea). *Boletín de la Asociación Herpetológica Española* 21: 122-126.

Valkiūnas, G., Iezhova, T. A., Križanauskienė, A., Palinauskas, V., Sehgal, R. N., & Bensch, S. (2008). A comparative analysis of microscopy and PCR-based detection methods for blood parasites. *Journal of Parasitology*, *94*(6), 1395-1401.

Van Kleunen, M., Dawson, W., Schlaepfer, D., Jeschke, J. M., & Fischer, M. (2010). Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. *Ecology letters*, *13*(8), 947-958.

Van Voorhis, W. C., Hulverson, M. A., Choi, R., Huang, W., Arnold, S. L., Schaefer, D. A., Betzer, D. P., Vidadala, R. S. R., Lee, S., Whitman, G. R., Barrett, L. K., Maly, D. J., Riggs, M. W., Fan, E., Kennedy, T. J., Tzipori, S., Doggett, J. S., Winzer, P., Anghel, N., Vavre, F., & Kremer, N. (2014). Microbial impacts on insect evolutionary diversification: from patterns to mechanisms. *Current opinion in insect science*, *4*, 29-34.

Verderame, M., & Scudiero, R. (2019). Health status of the lizard *Podarcis siculus* (Rafinesque-Schmaltz, 1810) subject to different anthropogenic pressures. Comptes rendus biologies, 342(3-4), 81-89.

Vervust, B., Grbac, I., & Van Damme, R. (2007). Differences in morphology, performance and behaviour between recently diverged populations of *Podarcis sicula* mirror differences in predation pressure. *Oikos*, *116*(8), 1343-1352.

Vervust, B., Pafilis, P., Valakos, E. D. & Van Damme, R. (2010). Anatomical and physiological changes associated with a recent dietary shift in the lizard *Podarcis sicula*. *Physiological and Biochemical Zoology* 83(4): 632-642.

Vighi, G., Marcucci, F., Sensi, L., Di Cara, G., & Frati, F. (2008). Allergy and the gastrointestinal system. *Clinical & Experimental Immunology*, 153, 3-6.

Vignoli, L., Vuerich, V., & Bologna, M. A. (2012). Experimental study of dispersal behaviour in a wall lizard species (*Podarcis sicula*)(Sauria Lacertidae). E*thology Ecology & Evolution,* 24(3), 244-256.

Vogrin, M. (2005). Sexual Dimorphism in *Podarcis sicula campestris*. *Turkish Journal of Zoology*, 29(2), 189-191.

Walter, J. (2008). Ecological role of lactobacilli in the gastrointestinal tract: implications for fundamental and biomedical research. *Applied and environmental microbiology*, *74*(16), 4985-4996.

Wang, T. P., Shrivastava, J., Johansen, M. V., Zhang, S. Q., Wang, F. F., & Webster, J. P. (2006). Does multiple hosts mean multiple parasites? Population genetic structure of

Schistosoma japonicum between definitive host species. *International Journal for Parasitology*, *36*(12), 1317-1325.

Weiser, E. L., & Powell, A. N. (2010). Does garbage in the diet improve reproductive output of Glaucous Gulls?. *The Condor*, 112(3), 530-538.

White, N. J. (2008). *Plasmodium knowlesi*: the fifth human malaria parasite.

Wichmann, D., Panning, M., Quack, T., Kramme, S., Burchard, G. D., Grevelding, C., & Drosten, C. (2009). Diagnosing schistosomiasis by detection of cell-free parasite DNA in human plasma. *PLoS neglected tropical diseases*, *3*(4), e422.

Willson, N. L., Van, T. T., Lever, J., Moore, R. J., & Stanley, D. (2019). Characterisation of the intestinal microbiota of commercially farmed saltwater crocodiles, *Crocodylus porosus*. *Applied microbiology and biotechnology*, 103(21), 8977-8985.

Wong, S. & Rawls, J. F. (2012), Intestinal microbiota composition in fishes is influenced by host ecology and environment. *Molecular Ecology*, 21: 3100-3102. https://doi.org/10.1111/j.1365-294X.2012.05646.x

Xavier, R., Mazzei, R., Pérez-Losada, M., Rosado, D., Santos, J. L., Veríssimo, A., & Soares, M. C. (2019). A risky business? Habitat and social behavior impact skin and gut microbiomes in Caribbean cleaning gobies. *Frontiers in microbiology,* 10, 716.

Xavier, R., Pereira, A., Pagan, A., Hendrick, G. C., Nicholson, M. D., Rosado, D., ... & Sikkel, P. C. (2020). The effects of environment and ontogeny on the skin microbiome of two *Stegastes* damselfishes (Pomacentridae) from the eastern Caribbean Sea. *Marine Biology*, *167*(7), 1-12.

Yang, W., Feiner, N., Pinho, C., While, G. M., Kaliontzopoulou, A., Harris, D. J., Salvi, D., & Uller, T. (2021). Extensive introgression and mosaic genomes of Mediterranean endemic lizards. *Nature communications*, 12(1), 1-8.

Yoshimura, M., & Okochi, I. (2005). A decrease in endemic odonates in the Ogasawara Islands, Japan. *Bulletin of FFPRI*, 4(1), 45-51.

Zozaya, S. M., Alford, R. A., & Schwarzkopf, L. (2015). Invasive house geckos are more willing to use artificial lights than are native geckos. *Austral ecology*, 40(8), 982-987.

Zuffi, M. & Giannelli, C. (2013). Trophic niche and feeding biology of the Italian wall lizard, *Podarcis siculus campestris* (De Betta, 1857) along western Mediterranean coast. *Acta Herpetologica* 8(1): 35-39.

CHAPTER IV

APPENDICES

Table A1 – Gut microbiome studies in different species of lizards.

Study	Reference	Species	Local study	Sample type	Method (region ampfified)	Observations
Sex-specific asymmetry within the cloacal microbiota of the striped plateau lizard, Sceloporus virgatus	(Martin et al. 2010)	*Sceloporus virgatus*	Arizona, USA	cloaca swabs	16S rRNA gene	Significant sex difference in microbial community structure, with females having significantly lower microbial diversity and richness than do males
Phylogenetic analysis of the fecal microbial community in herbivorous land and marine iguanas of the Galápagos Islands using 16S rRNA-based pyrosequencing	(Hong et al. 2011)	*Amblyrynchus cristatus, Conolophus subscristatus* and *C. pallidus*	Galápagos archipelago	faecal samples	16S rRNA gene	Dietary differences contribute to the differentiation of the gut microbial community
Does adaptive radiation of a host lineage promote ecological diversity of its bacterial communities? A test using gut microbiota of Anolis lizards	(Ren et al. 2016)	*Anolis sagrei, A. cristatellus, A. stratulus, A. evermani, A. krugi, A. pulchellus, A. gundlachi*	Puerto Rico and Florida	faecal samples	V1-V3 regions of the bacterial 16S rRNA gene	Sympatric members of the same ecomorph class, differed in bacterial beta diversity, despite their similarity in habitat use
Diets alter the gut microbiome of crocodile lizards	(Jiang et al. 2017)	*Shinisaurus crocodilurus*	Guangdong Luokeng *S. crocodilurus* National Nature Reserve	cloaca swabs	V4 region of the bacterial 16S rRNA gene	Significant differences in community richness and membership between groups of different diets; effect of captivity in shaping of gut microbiota was depend on diet
Processes shaping gut microbiota diversity in allopatric populations of the endemic lizard Podarcis lilfordi from Menorcan islets (Balearic Islands)	(Baldo et al. 2018)	*Podarcis lilfordi*	Menorca island	gastrointestinal tracts	V3-V4 regions of the bacterial 16S rRNA gene	Differences de microbioma among islets potentially following population fragmentation and bottlenecks
Effects of acute exposures of 2,4,6-trinitrotoluene and inorganic lead on the fecal microbiome of the green anole (Anolis carolinensis)	(Indest et al. 2018)	*Anolis carolinensis*	Environmental Toxicology Research Facility at the US Army Engineer Research and Development Center (Vicksburg, MS)	faecal samples	V3-V4 regions of the bacterial 16S rRNA gene	Environmental and health impacts of relevant military compounds on host microbiological composition and metabolism
City life alters the gut microbiome and stable isotope profiling of the eastern water dragon (Intellagama lesueurii)	(Littleford-Colquhoun et al. 2019)	water dragon (Intellagama lesueurii)	Queensland, Australia	faecal samples	V3-V4 regions of the bacterial 16S rRNA gene	Diet leads to differences in gut microbiome profile
Metabolically similar cohorts of bacteria exhibit strong cooccurrence patterns with diet items and eukaryotic microbes in lizard guts	(Holmes et al. 2019)	*Anolis sericeus, Holcosus undulatus*	Chiapas, Mexico	faecal samples	V4 region of the bacterial 16S rRNA gene	Taxonomic changes in the bacterial microbiome that may occur by dietary changes in host lineages over evolutionary time
The lizard gut microbiome changes with temperature and is associated with heat tolerance	(Moeller et al. 2020)	*Sceloporus occidentalis*	Campus of the California Polytechnic State University (Cal Poly)	faecal samples	V4-V5 regions of the bacterial 16S rRNA gene	Temperature significantly affects the gut microbiota of lizards
Captivity influences gut microbiota in crocodile lizards (Shinisaurus crocodilurus)	(Tang et al. 2020)	*Shinisaurus crocodilurus*	Crocodile Lizard National Nature Reserve, Guangxi Province, China	faecal samples	V3-V4 regions of the bacterial 16S rRNA gene	Significant effect of captivity was found on the composition of gut microbiota of the host
The Role of Environmental Stress in Determining Gut Microbiome: Case Study of Two Sympatric Toad-headed Lizards	(Qi et al. 2020)	*Phrynocephalus axillaris , P. forsythii*	Korla (southwest corner of the Tarim Basin)	gastrointestinal tracts	V3-V4 regions of the bacterial 16S rRNA gene	Hereditary and environmental factors influence the gut microbiota, depending on the environmental stresses involved
Dietary effects on gut microbiota of the mesquite lizard Sceloporus grammicus (Wiegmann, 1828) across different altitudes	(Montoya-Ciriaco et al, 2020)	*Sceloporus grammicus*	National Park "La Malinche", México	feacal samples and gastrointestinal tracts	V3-V4 regions of the bacterial 16S rRNA gene	The limitation of food resources at high elevations, forcing them to exploit other food resources and their gut microbiota is affected
Gut and oral bacterial diversity of the lizard Diploderma splendidum investigated using metagenomic analysis.	(Tian et al. 2020)	*Diploderma splendidum*	Quebrada (Laojun mountains)	cloaca swabs		Geography, species and diet factors show differences in bacterial communities
Environment-Dependent Variation in Gut Microbiota of an Oviparous Lizard (Calotes versicolor)	(Zhang et al. 2021)	*Calotes versicolor*	Hainan, China	cloaca swabs	V3-V4 regions of the bacterial 16S rRNA gene	Variations of gut microbiota composition and metabolic pathway in different habitat
Recovered microbiome of an oviparous lizard differs across gut and reproductive tissues, cloacal swabs, and feces	(Bunker et al. 2021)	*Sceloporus virgatus*	Madrean Sky Islands (southwestern United States)	cloaca swabs and faecal samples	V4 region of the bacterial 16S rRNA gene	Transfer of antifungal microbes from the mother to the eggshell
Gut microbial ecology of the Critically Endangered Fijian crested iguana (Brachylophus vitiensis): Effects of captivity status and host reintroduction on endogenous microbiomes	(Eliades et al. 2021)	*Brachylophus vitiensis*	Monuriki Island, Fiji Islands	cloacal swabs and faecal samples	V4 region of the bacterial 16S rRNA gene	Captivity can lead to differences in microbial composition compared to wild individuals

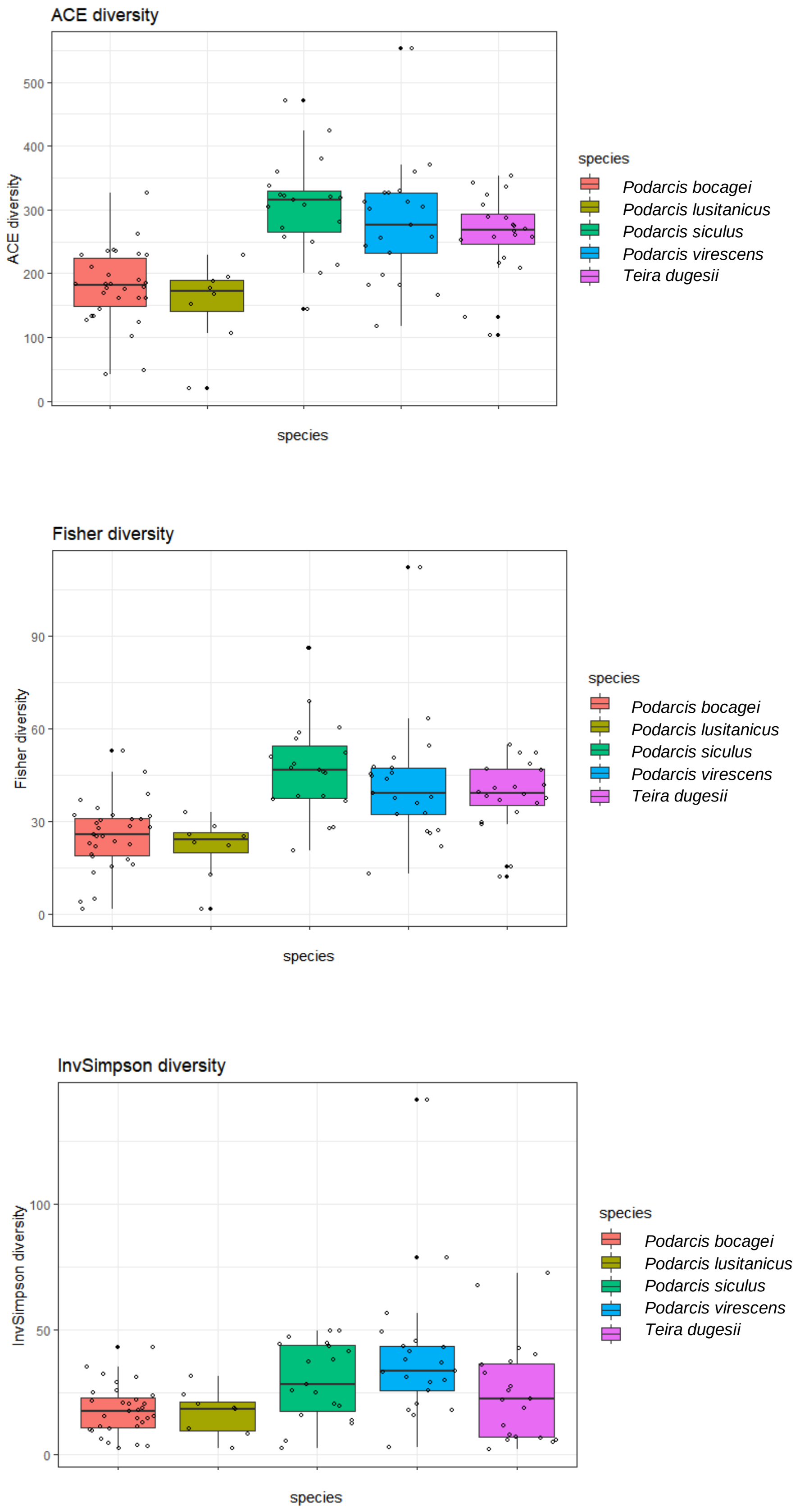

Fig. A1 - Boxplots showing the median values and interquartile ranges for the alpha diversity indices for the gut microbiome of the five species from the Microbiome study (Manuscript I). Samples are represented by open circles and outliers are shown as black circles

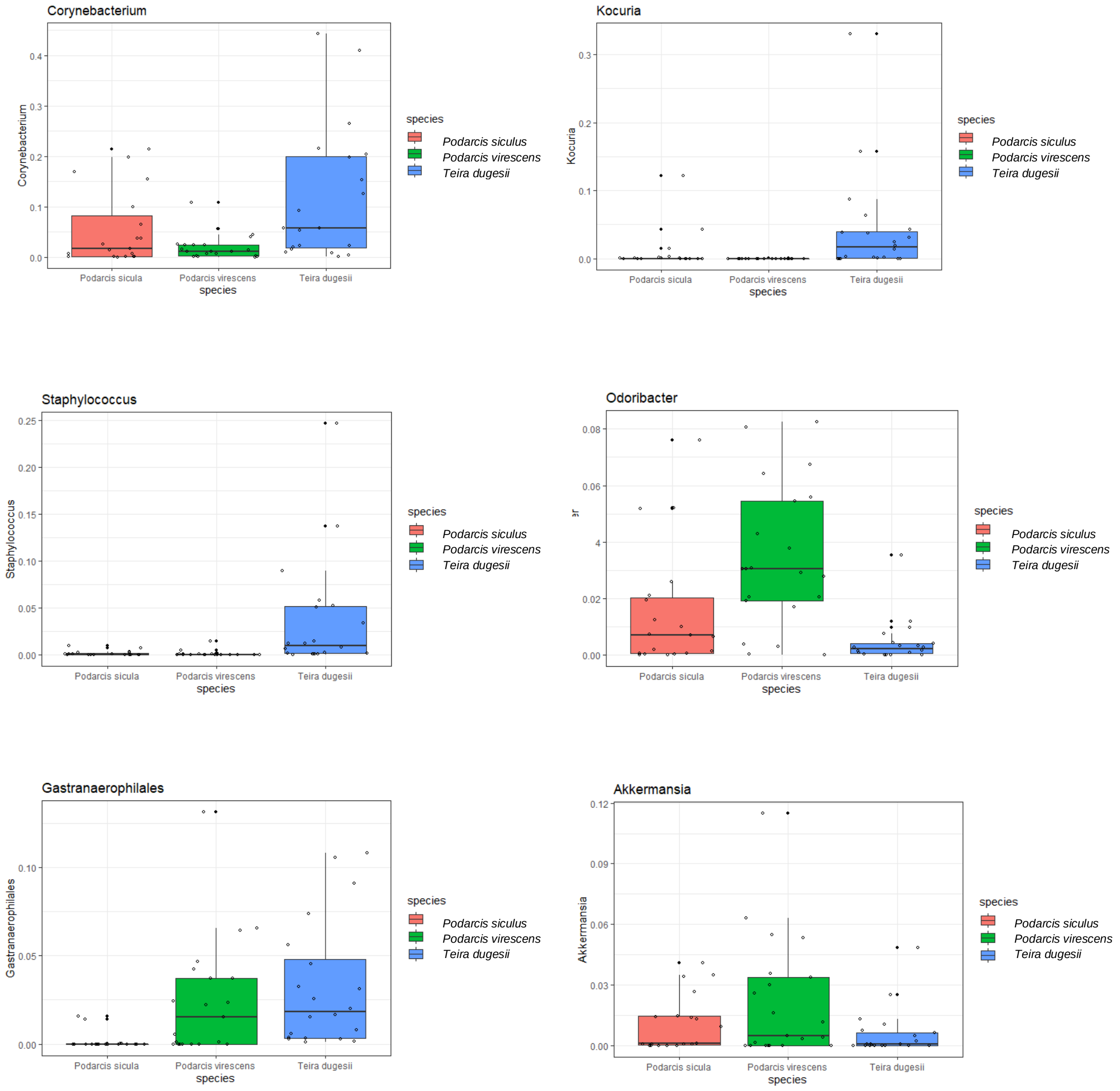

Fig. A2 – Boxplots showing the median values and interquartile ranges for the proportions of the most abundant genera significantly different between species in the Lisbon population (P. siculus, P. virescens and T. dugesii). Samples are represented by open circles and outliers are shown as black circles.

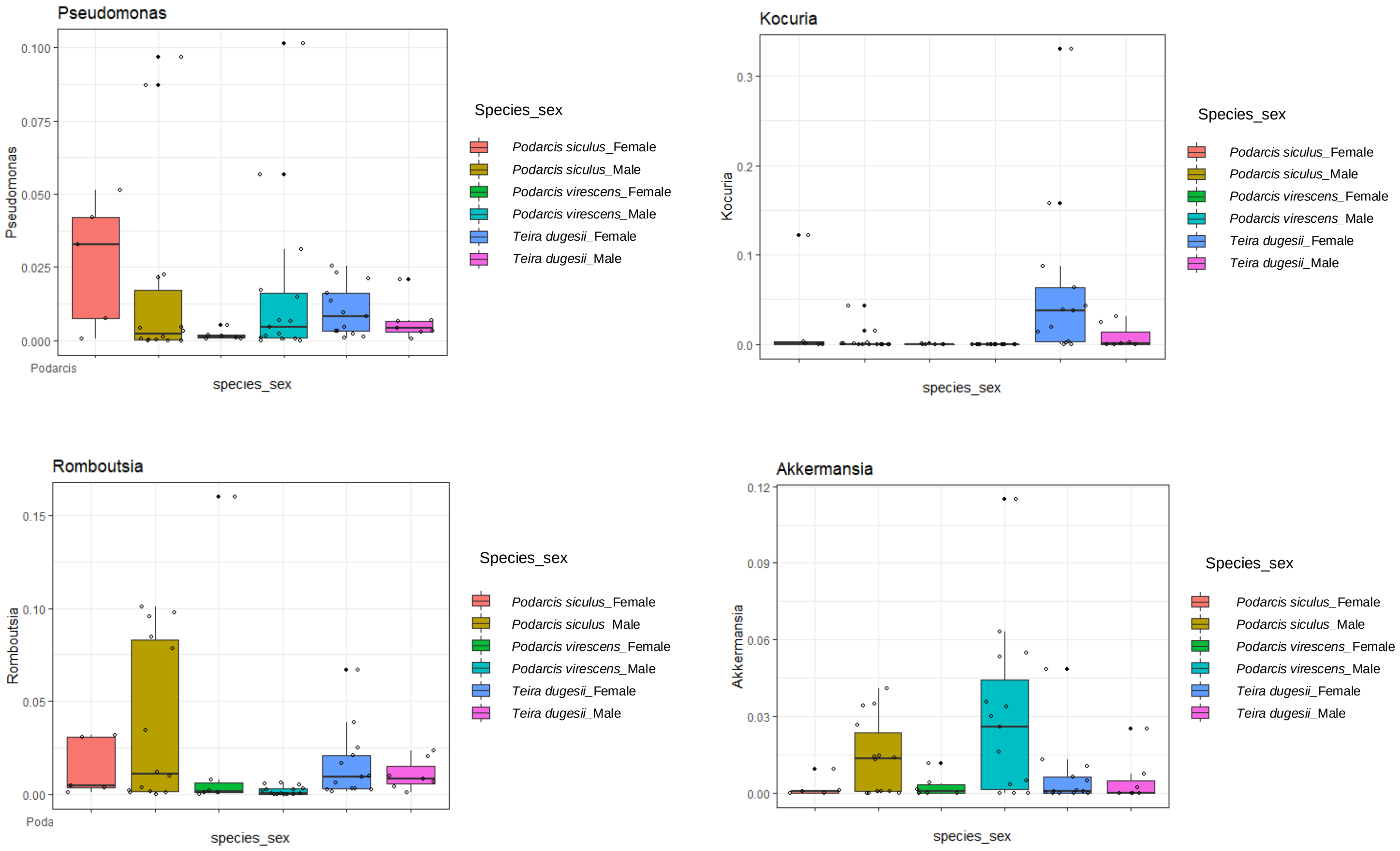

Fig. A3 - Boxplots showing the median values and interquartile ranges for the proportions of the most abundant genera significantly different between sexes in the Lisbon population (P. siculus, P. virescens and T. dugesii). Samples are represented by open circles and outliers are shown as black circles.

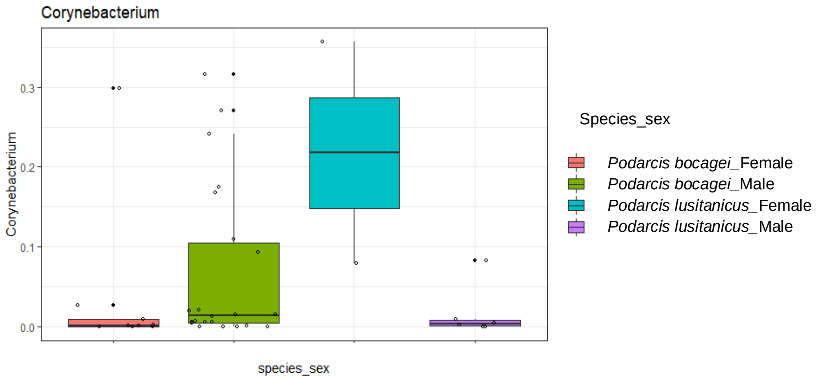

Fig. A4 – Boxplots showing the median values and interquartile ranges for the proportions of the most abundant in the proportion of the most abundant genera (species*sex) in Moledo populations (*P. bocagei* and *P. lusitanicus*). Samples are represented by open circles and outliers are shown as black circles.