

Factors shaping the gut microbiome of five species of lizards from different habitats

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Abstract

Host-gut microbiota interactions are complex and can have a profound impact on the ecology and evolution of both counterparts. Several host traits such as systematics, diet and social behavior, and external factors such as prey availability and local environment are known to influence the composition and diversity of the gut microbiota. In this study, we investigate the influence of systematics, sex, host size, and locality/habitat on gut microbiota diversity in five lizard species from two different sites in Portugal: *Podarcis bocagei* and *Podarcis lusitanicus*, living in syntopy in a rural area in northern Portugal (Moledo); the invasive *Podarcis siculus* and the native *Podarcis virescens*, living in sympatry in urbanized environment (Lisbon); and the invasive *Teira dugesii* also living in an urban area (Lisbon). We also infer the potential microbial transmission occurring between species living in sympatry and syntopy. To accomplish these goals, we use a metabarcoding approach to characterize the bacterial communities from the cloaca of lizards, sequencing the V4 region of the 16S rRNA. Habitat/locality was an important factor explaining differences in gut bacterial composition and structure, with species from urbanized environments having higher bacterial diversity. Host systematics (i.e. species) influenced gut bacterial community structure only in lizards from the urbanized environment. We also detected a significant positive correlation between lizard size and gut bacterial alpha-diversity in the invasive species *P. siculus*, which could be due to its higher exploratory behavior. Moreover, estimates of bacterial transmission indicate that *P. siculus* may have acquired a high proportion of local microbiota after its introduction. These findings confirm that a diverse array of host and environmental factors can influence lizards gut microbiota.

Introduction

A myriad of microorganisms can be found living in the gastrointestinal tract of all animals. These microorganisms have a significant impact on host biology and can influence a variety of processes that affect host fitness (Cryan & Dinan, 2012; Thaïss et al. 2016). While certain variations in the composition of gut microbial communities can cause disease (Martin et al. 2014; Boursier et al. 2016), gut microbiome may also increase resistance to pathogens, besides being important for xenobiotics metabolism, nutrient uptake and energy acquisition [e.g. Vavre & Kremer, 2014; Rowland et al. 2018]. Moreover, gut microbiota may also contribute towards host adaptation to environment changes by enabling a response to new challenges, such as exploitation of novel food sources (Delsuc et al. 2014; Hammer & Bowers, 2015). Ultimately, gut microbiome can have a major impact on host development, behaviour and fitness, with cascading effects to the dynamics of ecosystems (Thaïss et al. 2016). In turn, it can also be modulated by several host traits, such as host evolutionary history, sex and size, as well as the external environment, such as habitat or prey availability (e.g., Muegge et al. 2011; Xavier et al. 2019). In addition, social interactions between hosts can

also influence the gut microbiome in many animal species, although these mechanisms remain less studied (see review by Archie & Tung, 2015).

Gut microbiome dynamics has been studied in many mammals (e.g., Thaiss et al. 2016), birds (e.g., Hird et al. 2015), fishes (e.g., Xavier et al. 2020) and amphibians (e.g., Bletz et al. 2016). Comparatively fewer studies have been performed in reptiles, and only a handful of these addressed lizards. Nevertheless, studies showed that maternal transmission of gut microbiota to offspring can occur in squamate reptiles (Kohl et al. 2017). Additionally, microbiota can be acquired by reptiles through horizontal transmission from the environment or through interaction with other organisms (e.g. predatory encounters, Colston, 2017). Host systematics and ecology were also seen to be important drivers of gut microbiota diversity in reptiles. For example, feeding habits influence the gut microbiota of the Chinese crocodile lizard, *Shinisaurus crocodilurus* Ahl 1930, with potential effects on host health due to the influence of diet on the abundances of pathogenic or opportunistic gut bacteria (Jiang et al. 2017). Diet and habitat of the Australian water dragon, *Intellagama lesueurii* (Gray, 1831), also have an effect on its gut microbiome, with lizards living in urban areas presenting higher bacterial diversity than populations living in natural habitats (Littleford-Colquhoun et al. 2019). Moreover, host systematics and habitat also influence the gut microbiota of venomous snakes (Smith et al. 2021).

Here, we analyze and compare the diversity, composition and structure of gut bacterial communities of five related lacertid species captured in Portugal. Individuals of *Podarcis bocagei* (Lopez-Seoane, 1885) and *Podarcis lusitanicus* Geniez, Sá-Sousa, Guillaume, Cluchier and Crochet, 2014, were sampled in Moledo (North of Portugal) where they live in syntopy. Invasive *Podarcis siculus* (Rafinesque-Schmaltz, 1810) and native *Podarcis virescens* Geniez, Sá-Sousa, Guillaume, Cluchier and Crochet, 2014, were sampled from Parque das Nações (Lisbon) where they live in sympatry. Finally, a population of the invasive *Teira dugesii* (Milne-Edwards, 1829) was sampled in the Alcantara Docks in Lisbon. All five species exhibit sexual dimorphism, with males usually being larger than females, and they are mostly insectivorous (Geniez et al. 2014; Carretero et al. 2015), although *P. siculus* and *Teira dugesii* may also occasionally consume some fruits or flowers (Mačát et al. 2015). *Podarcis* species are considered model organisms to study ecotoxicology, immune/histochemical reactions, among other processes [e.g. Bicho et al. 2013; Luís et al. 2019]; however, microbiome studies are still largely lacking, with only three studies available to date. Two studies investigated two species endemic to the Balearic Islands (Spain), *Podarcis lilfordi* (Günther, 1874) and *Podarcis pityusensis* (Bosca, 1883), with results indicating that islet, time since separation from mainland and seasonality are significant factors contributing to their gut microbiome (Baldo et al. 2018, Alemany et al. 2022). Another recent study compared the gut microbiota of two Italian populations of *P. siculus* (mainland vs island) demonstrating that there were considerable differences between the two (Buglione et al., 2022).

Our main objective was to determine whether locality, which also corresponded to two different habitats (rural vs urbanized) and host factors such as species, size and sex modulate the gut bacterial diversity of these five lizards. To achieve this, we used cloacal swabs to obtain a proxy for gut bacterial communities which were characterized by sequencing the V4 region of the 16S rRNA gene. Swabs were preferred to fecal samples as these more accurately reflect microbial communities residing in lower gut and cloacal tissues (Bunker et al. 2021).

Methods

A total of 103 adult lizards from five different species were sampled in September 2020: *Podarcis bocagei* (males = 22; females = 9), *Podarcis lusitanicus* (males = 6; females = 2), *Podarcis siculus* (males = 13; females = 6), *Podarcis virescens* (males = 16; females = 6) and *Teira dugesii* (males = 7; females = 13).

All these lacertid species are small-sized, from about 4 cm to 6 cm snout-vent length in *P. virescens*, *P. bocagei* and *P. lusitanicus*, and up to 8 cm and 9 cm in the introduced species *P. siculus* and *Teira dugesii* (Arnold & Burton, 2002). *Podarcis bocagei* and *P. lusitanicus* were collected from a semi-natural habitat in Moledo, northern Portugal (Fig. 1a) (41°50'19.2"N 8°52'24.5"W), where they live in syntopy (i.e., occurrence of two species in the same habitat at the same time). This location has limited human disturbance and

has extensive vegetation with natural and artificial shelters (e.g., walls of agricultural properties) that can be used by lizards. Ecological adaptation is considered a major factor favoring the isolation between these two species; *P. lusitanicus* lives more on rocks, while *P. bocagei* is ground-dwelling (Carretero et al. 2015). The diet of these two species is mainly composed by prey belonging to Hemiptera, Coleoptera, Diptera, Hymenoptera and Araneae, with minimal differences between species or sexes (Kaliontzopoulou et al. 2011). *Podarcis siculus* and *P. virescens* were collected in Lisbon, at Parque das Nações (Fig. 1b, c) (38°76'22.4"N, 9°09'44.3 W), where both live in sympatry (sharing habitat type). This is a highly urbanized area near the Tejo River, characterized by large residential and commercial areas, with considerable daily human disturbance. While *P. virescens* is native to this location, *P. siculus* is an invasive species introduced about two decades ago (González de la Veja et al. 2001). Its plasticity in spatial use of habitat, morphology, behaviour, and diet explains its successful colonization of multiple locations outside its native range (Vervust et al. 2010; Carretero & Silva-Rocha, 2015; Damas-Moreira et al. 2019; Damas-Moreira et al. 2020). This invasive species can present a more versatile diet, as it can also consume fruits and nectar (Mačát et al. 2015; Vervust et al. 2010), while *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). Finally, we collected *Teira dugesii* in a nearby area in Lisbon, in the Alcantara docks, close to the city port area (38°70'33.8"N, 9°16'54.1"W). Similar to the other *Podarcis* spp. captured in Lisbon, *T. dugesii* occupies an anthropogenic area, although less busy, close to railway tracks with limited vegetation cover (Fig. 1d). This species is thought to have been accidentally introduced via transport ships from Madeira Island three decades ago, in 1992 (Sá-Sousa, 1995). *Teira dugesii* feeds preferentially on insects but also on small fruits (Sadek, 1981).

All individuals were captured using nooses. Lizards were carefully immobilized, avoiding any human contact with the cloaca. We quickly inserted a sterile cotton swab into the entrance of the cloaca to obtain individual microbial samples. The tips of the swabs were cut into individual tubes and stored in ice boxes in the field, and then stored at -80°C upon arrival in the laboratory. After microbial sampling, each lizard was sexed, and the snout-vent length was measured (SVL; from head to cloaca) using a digital caliper (± 0.01 mm error).

In the laboratory, DNA was extracted from the swabs using the DNeasy® PowerSoil® Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA concentration and quality were measured with the Epoch Microplate Spectrophotometer (BioTek Instruments, Inc.; United States of America). DNA was shipped in dry ice to the Centre for Microbial Systems at the University of Michigan Medical School (USA) where the V4 region of the 16S rRNA gene (~ 250 bp) of the bacterial communities was amplified for each sample, along with the extraction blanks, PCR controls and a mock community (D6306 ZymoBIOMICS Microbial Community DNA Standard, Zymo Research, USA) using the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') and following the protocol of Kozich et al. 2013. The V4 region of this gene is widely used to characterize bacterial communities in various taxa, including reptiles (e.g. Colston and Jackson, 2016; Chiarello et al. 2018). Amplicons were sequenced in a single Illumina MiSeq run using a MiSeq Reagent Kit V2 500 cycles.

All analyses were performed using the R Software v.4.1.1 (Team, 2020). Raw FASTQ files were denoised using the DADA2 pipeline (Callahan et al. 2016). After an assessment of read quality plots, the parameters for trimming and filtering were set as: trimLeft = 20, truncLen = c(220, 200), maxN = 0, maxEE = c(2, 2), truncQ = 2. The SILVA 138 database (Pruesse et al. 2007; Quast et al. 2013) was chosen for taxonomic assignment. After quality control and taxonomic assignment, sequences identified as Archaea, Eukaryota, Mitochondria, Chloroplast, as well as sequences unassigned to bacteria were removed from the dataset. An Amplicon Sequence Variant (ASV) frequency table was constructed using the R package *phyloseq* (McMurdie & Holmes, 2013). Normalized read counts were obtained using the negative binomial distribution implemented in DESeq2 (Love et al. 2014; McMurdie and Holmes, 2014). ASVs not only with a count of less than 0.001% of the total number of reads (3586752 [total number of reads] x 0.001% = 36) but which were also present in a single sample were removed (Appendix 1). The composition and abundance of taxa in the mock community were similar to those described by the manufacturer.

Bacterial taxonomic diversity (alpha-diversity, calculated intra-sample) and structure (beta-diversity, calculated as the dissimilarity or distance between pairs of samples) were estimated using the *phyloseq* and the *picante* packages (McMurdie & Holmes, 2013; Kembel et al, 2010). Alpha-diversity was estimated using the number of observed ASVs, the Shannon index and Faith’s Phylogenetic Diversity (PD). Beta-diversity was measured using the Bray-Curtis index and the Unifrac phylogenetic weighted and unweighted distances. Principal Coordinate Analysis (PCoA) were used to visually assess dissimilarity among groups.

First, statistical differences in alpha-diversity between localities were assessed using species as a random factor using a linear mixed effects model ($\text{lmer}(\text{alpha-diversity} \sim \text{locality} + (1 | \text{species}))$). Given the significant effect of locality on alpha-diversity (see results section), differences in alpha-diversity among species and between sexes were further assessed using another linear mixed effects model with locality as a random factor ($\text{lmer}(\text{alpha-diversity} \sim \text{species} + \text{species}:\text{sex} + (1|\text{locality}))$) using the package *lme4* (Bates et al. 2014). The effects of locality and species on microbial beta-diversity were assessed using a permutational analysis of variance (PERMANOVA) with 9999 permutations, with the *adonis2* function of the *Rvegan* package (Oksanen et al. 2013), using the formula ($\text{adonis2}(\text{beta-diversity} \sim \text{locality} + \text{species})$). Since both locality and species significantly affected beta-diversity, the pairwise effects of species and sex were tested for each locality separately using the *pairwise.adonis2* function (Arbizu, 2020) using the model ($\text{pairwise.adonis2}(\text{beta-diversity} \sim \text{species} + \text{species}:\text{sex})$). P-values for multiple comparisons were adjusted with the Bonferroni correction. Differences in the proportions of the most abundant taxa at the phyla and genera levels (represented by [?]) 3% on average of all sequences) were assessed between species and sex for each locality separately using a linear model ($\text{lm}(\text{bacterial taxa} \sim \text{species} + \text{species}:\text{sex})$). Correlations between individual size and bacterial alpha-diversity were also tested using the Pearson correlation test for each species, using the *ggpubr* package (Kassambara & Kassambara, 2020).

To further understand the levels of similarity between sympatric and syntopic species, bi-directional bacterial transmission between each pair of species from Moledo and Parque das Nacoes was estimated using the FEAST software (Shenhav et al. 2019), by testing the contribution of each species (source) to the microbial diversity to its sympatric congener (sink). To this end, the non-normalized ASV frequency table was used and, due to differences in the number of samples between *P. bocagei* and *P. lusitanicus*, only a fraction of the individuals of *P. bocagei* were included (the ones with the most similar sex and SVL ratios to the *P. lusitanicus* samples as possible), following the FEAST developers’ recommendations to avoid overestimation of transmission.

Results

After filtering, the final ASV table encompassed 3923 unique ASVs, included in a total of 39 bacteria phyla. The most abundant phyla among the species studied were Firmicutes, Bacteroidota, Actinobacteroidota, Proteobacteroidota and Campylobacterota.

Gut bacterial diversity, measured through alpha-diversity indices, was significantly different between localities considering the number of Observed ASVs and PD indices (F-statistics > 39.74; $p < 0.02$), with species from Lisbon showing consistently higher alpha-diversity indices than the ones from Moledo, (Fig. 2). No differences between localities were found with the Shannon index (F-statistics = 8.33; $p = 0.07$). Moreover, neither species or sex had a significant effect on microbial alpha-diversity ($F < 1.95$; $p > 0.11$), but *P. siculus* had higher diversity than the native *P. virescens*. Microbial structure, measured through beta-diversity indices, was significantly different between localities ($R^2 > 0.03$; $p < 0.04$) and species ($R^2 > 0.08$; $p < 0.03$) (Fig. 3). In general, pairwise differences in beta-diversity between species were found in species collected from Lisbon (Table 1), while no differences were found between sexes. In samples collected in Moledo, no differences were found in beta-diversity between species or sexes.

Although no differences were found in the proportion of the most abundant phyla between species or sexes, among the most abundant genera some differences were observed (Fig. 4). In the case of species in Moledo, sex influenced the proportion of the genus *Corynebacterium* (F-statistics = 4.46, $p = 0.02$) (Table 2). Differences in the proportion of the most abundant taxa between *P. siculus* and *P. virescens* were

found for an unidentified genus belonging to the order *Corynebacterium* (F-statistics = 6.66, $p = 0.003$) and for *Odoribacter* (F-statistics = 10.10, $p = 0.0002$) (Table 2).

Pearson correlation test only showed significantly positive correlations between SVL and bacterial alpha-diversity (for Shannon index) for males of the invasive species *P. siculus* (Fig. 5).

Results from FEAST software indicated that the level of bacterial transmission between sympatric species in both populations (Parque das Nacoes and Moledo) was high. Nevertheless, while bacterial transmission was balanced in both directions between the syntopic *P. lusitanicus* and *P. bocagei* (estimated transmission from *P. bocagei* towards *P. lusitanicus* was $\sim 71\%$ on average, and from *P. lusitanicus* towards *P. bocagei* was $\sim 69\%$ on average), between the two sympatric species in Lisbon there was a more biased transmission, with *P. virescens* seemingly having a higher contribution towards *P. siculus* gut microbiota (transmission estimates from *P. virescens* towards *P. siculus* was $\sim 72\%$ on average, and from *P. siculus* towards *P. virescens* it was about 55% on average).

Discussion

In this study, we characterized the gut bacterial microbiota of five lizard species from Portugal (the native *Podarcis virescens*, *P. bocagei* and *P. lusitanicus*, and the introduced *P. siculus* and *Teira dugesii*) using a metabarcoding approach. Our results showed that locality was a major predictor of microbial diversity and structure. Plausibly, differences in habitat may lead to differences in the composition and diversity of the gut microbiome of animals (e.g. Amato et al. 2013; Xavier et al. 2021). The two habitats in which the lizards from this study were captured are very different, with lizards from Lisbon living in a highly urbanized and artificial habitat, with greater environmental disturbance, compared to lizards from Moledo, which live in a semi-natural habitat. Specifically, we detected a consistently higher microbial diversity in the species from Lisbon which could be possibly explained by the higher variety of diet items consumed. *Podarcis siculus* diet is viewed as extremely opportunistic, and can include human food waste (e.g. cheese and pasta), fruits, other lizards and small carrion (Mo & Mo, 2021; Mačát et al. 2015; Capula & Aloise, 2011; personal observations).

Although the species we sampled in urban areas can also reside in rural habitats, and vice-versa, our sampling design did not allow comparisons of gut bacterial communities between conspecifics residing in these two habitats. Nevertheless, urbanization is frequently seen to restructure the gut microbiome of animals (e.g. Stothart et al. 2019), with increases in microbiome alpha diversity reported for some reptiles (avian and non-avian) and mammals (Dillard et al. 2022). For example, higher gut bacterial diversities were previously reported in a study from populations of the Australian water dragon residing urban environments when compared to those inhabiting natural environments, presumably driven by differences in the diet (Littleford-Colquhoun et al. 2019). Additionally, authors hypothesized that environmental microbiota, which may be horizontally transferred to lizards, could also be more diverse in urban habitat than in semi-natural ones (Littleford-Colquhoun et al. 2019). A similar pattern was observed in urban crested anole lizards, white-crowned sparrows as well as coyotes (Dillard et al., 2022; Phillips et al. 2018; Sugden et al. 2020). Interestingly, Dillard et al. (2022) found increased similarities between the gut microbiota of these three animals and human populations in urbanized habitats. Different hypothesis have been put forward to explain this trend, including that it could be caused by increased heterogeneity of urban land cover (Phillips et al. 2018), higher consumption of human food waste (Sugden et al. 2020) and acquisition of human microbiota in urban habitats (Dillard et al. 2022). We hypothesize that the higher microbiome diversity in lizards from the urban environment could also be related with the aforementioned factors, but further studies including conspecific lizards from urban and natural habitats are needed to determine the generality of this pattern.

Gut microbial diversity (alpha-diversity) did not significantly differ between lizard species and there were no differences in bacterial community structure (beta-diversity) between the two syntopic species, *P. bocagei* and *P. lusitanicus*, sampled at Moledo. Additionally, our analysis of potential bacterial transmission between these two syntopic lizards indicates a high and balanced bi-directional transmission of bacteria between the two species (ca. 70%), indicating a high similarity between their gut microbiota (Shenhav et al. 2019). This is not surprising as the two species have high dietary overlap and similarity in their habitat occupancy.

Moreover, it is likely they consume the same or very similar prey items (Kaliontzopoulou et al. 2011), and also encounter each other frequently. On the contrary, there were significant differences between the structure of gut bacterial communities (beta-diversity) of the different species of lizards sampled in Lisbon, with lower and unbalanced estimates for bacterial transmission between the two sympatric species, *P. siculus* and *P. virescens*. The invasive *P. siculus* was estimated to receive a higher proportion of bacteria from the native *P. virescens* than vice versa (ca. 72% vs 55% on average). These differences could be related to an increased habitat occupancy and successful adaptation to the environment by the invasive species, which facilitated the acquisition of a higher quantity of local microbiota upon its arrival. These results could also be reflecting an increased ability to exploit a variety of food resources, or most likely a combination of both. Although the populations of *P. siculus* and *P. virescens* are found living in sympatry, occupying roughly the same area, they are rarely in syntopy, although sightings of these two species within 50 m of each other have been recorded (Ribeiro & Sá-Sousa, 2018; personal observations).

The proportion of some of the most abundant bacterial genera found in our study also differed between *P. virescens* and *P. siculus*, but not between *P. lusitanicus* and *P. bocagei*. The influence of host taxonomy in gut microbiota, which is a proxy not only for host genetics but also its general ecology, has been reported in many animals (Moeller et al. 2013; Moeller et al. 2014), including reptiles (Smith et al. 2021). It is also interesting to note that the gut microbiota of individuals of *P. siculus* sequenced herein and those from Italy by Buglione et al. (2022) share the most abundant bacterial phyla but differ at the level of the most represented bacterial genera.

Comparatively, host sex had a negligible effect on gut microbiota of the studied lizards, albeit it significantly affected the abundance of *Corynebacterium* in lizards from Moledo. These results agree with those from a previous study on two *Podarcis* species from the Balearic islands, in which sex had no influence on the gut microbial structure (Alemany et al. 2022). However, an interesting result was that size of males of *P. siculus* was positively related with bacterial diversity. This lizard can reach larger sizes than the other studied species (Carretero & Silva-Rocha, 2015, Damas-Moreira et al. 2019). Furthermore, *Podarcis siculus* can be bolder and more aggressive than native *Podarcis* species (Downes & Bauwens, 2002), and also more exploratory and better at exploiting food resources when compared to the native *P. virescens* at our study location (Damas-Moreira et al. 2019, 2020). These behaviors can be associated with the displacement of *P. virescens* from gardens now inhabited by *P. siculus* (Ribeiro & Sá-Sousa, 2018) and can also be leading to a wider ecological and trophic niche, and consequently to the correlation found as well as to the slightly higher average microbiome diversity observed for *P. siculus*.

The present study contributes to the existing knowledge on the effects of the environment and host factors on the dynamics of the gut microbiome of lizards. Our results also set the stage for future research exploring the influence of diet and urbanization on the microbiome of *Podarcis* and the use of sympatric lizards as models to test the effects of behavior on lizard microbial composition.

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Statements & Declarations

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Data Availability: Data will be deposited in NCBI’s Short Read Archive (SRA) upon acceptance.

Ethics approval: Experimental protocols and research were approved by the Portuguese Institute for Conservation of Nature and Forests (ICNF) (License 703/2021/CAPT).

Table 1 - Results from the pairwise PERMANOVA testing the effect of species and sex in gut microbial beta-diversity (R^2 and respective adjusted p-values). Significant results are depicted in bold.

				Unifrac phylo
Lisbon	<i>Podarcis siculus</i> vs. <i>Podarcis virescens</i>	<i>Podarcis siculus</i> vs. <i>Podarcis virescens</i>	species	$R^2 = 0.10$; p =
			sex	$R^2 = 0.01$; p =
	<i>Podarcis siculus</i> vs. <i>Teira dugesii</i>	<i>Podarcis siculus</i> vs. <i>Teira dugesii</i>	species	$R^2 = 0.03$; p =
			sex	$R^2 = 0.01$; p =
	<i>Podarcis virescens</i> vs. <i>Teira dugesii</i>	<i>Podarcis virescens</i> vs. <i>Teira dugesii</i>	species	$R^2 = 0.10$; p =
			sex	$R^2 = 0.02$; p =
Moledo	<i>Podarcis bocagei</i> vs. <i>Podarcis lusitanicus</i>	<i>Podarcis bocagei</i> vs. <i>Podarcis lusitanicus</i>	species	$R^2 = 0.02$; p =
			sex	$R^2 = 0.05$; p =

Table 2 - Results from the linear models testing the effect of species and sex in the proportion of the most abundant genera for each locality. Order/Family of genera that remained unclassified is presented between brackets. Significant results are depicted in bold.

		Lisbon	Lisbon
		species	species
Odoribacter	Odoribacter	F = 10.10; p = 0.0002	F = 10.10; p = 0.000
Corynebacterium	Corynebacterium	F = 6.66; p = 0.003	F = 6.66; p = 0.003
<i>Helicobacter</i>	<i>Helicobacter</i>	F = 2.96; p = 0.06	F = 2.96; p = 0.06
<i>Parabacteroides</i>	<i>Parabacteroides</i>	F = 2.11; p = 0.13	F = 2.11; p = 0.13
<i>Bacteroides</i>	<i>Bacteroides</i>	F = 2.43; p = 0.10	F = 2.43; p = 0.10
<i>Pseudomonas</i>	<i>Pseudomonas</i>	NA	NA
Unclassified (<i>Selenomonadaceae</i>)	Unclassified (<i>Selenomonadaceae</i>)	NA	NA
Unclassified (<i>Lachnospiraceae</i>)	Unclassified (<i>Lachnospiraceae</i>)	F = 1.42; p = 0.25	F = 1.42; p = 0.25

Legends

Figure 1 : Aerial photographs of sampling sites for A) *P. siculus*, B) *P. viriscens*, C) *Teira dugesii* and D) *P. bocagei* and *P. lusitanicus*. Specific collection areas are delimited by yellow lines). Map data ©2021 Google

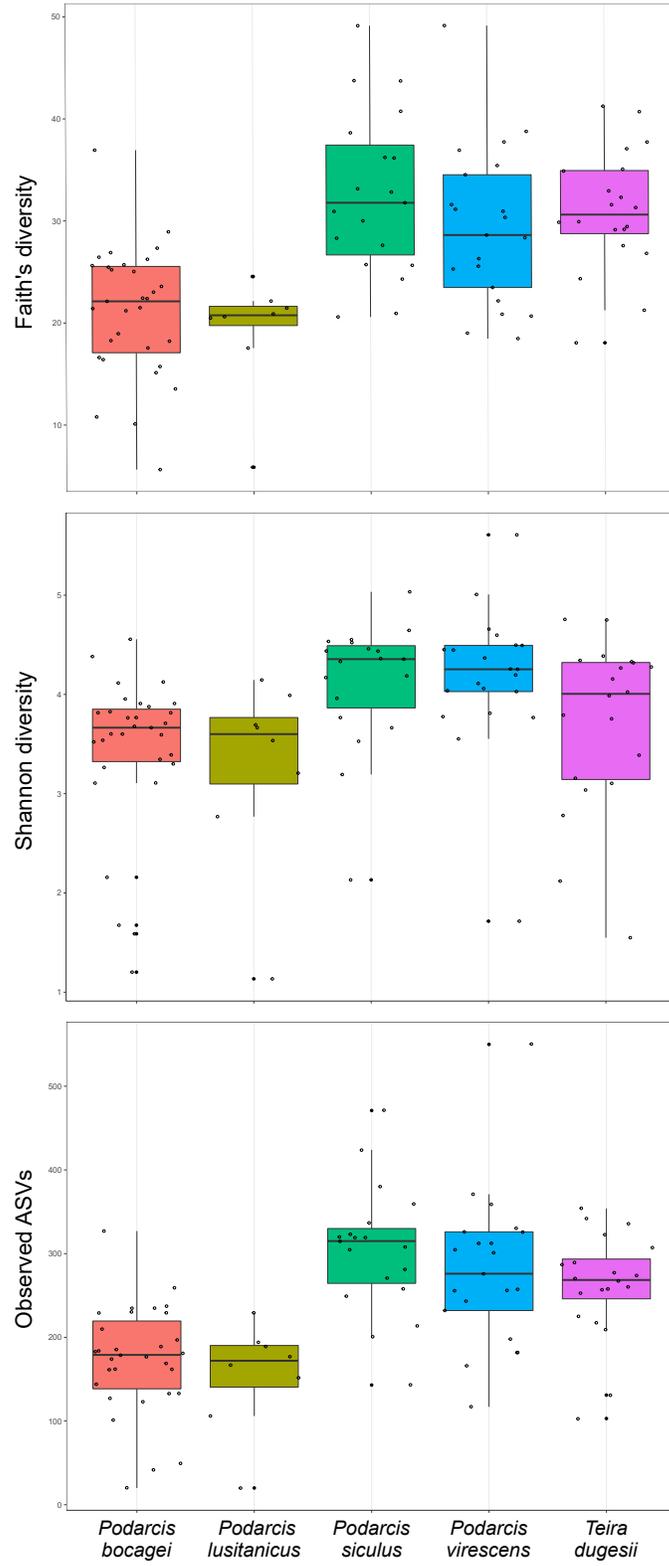
Figure 2: Boxplots of the alpha-diversity indices (Faith's phylogenetic diversity, Shannon diversity and the number of observed ASVs) for the gut microbiome of the studied lizards.

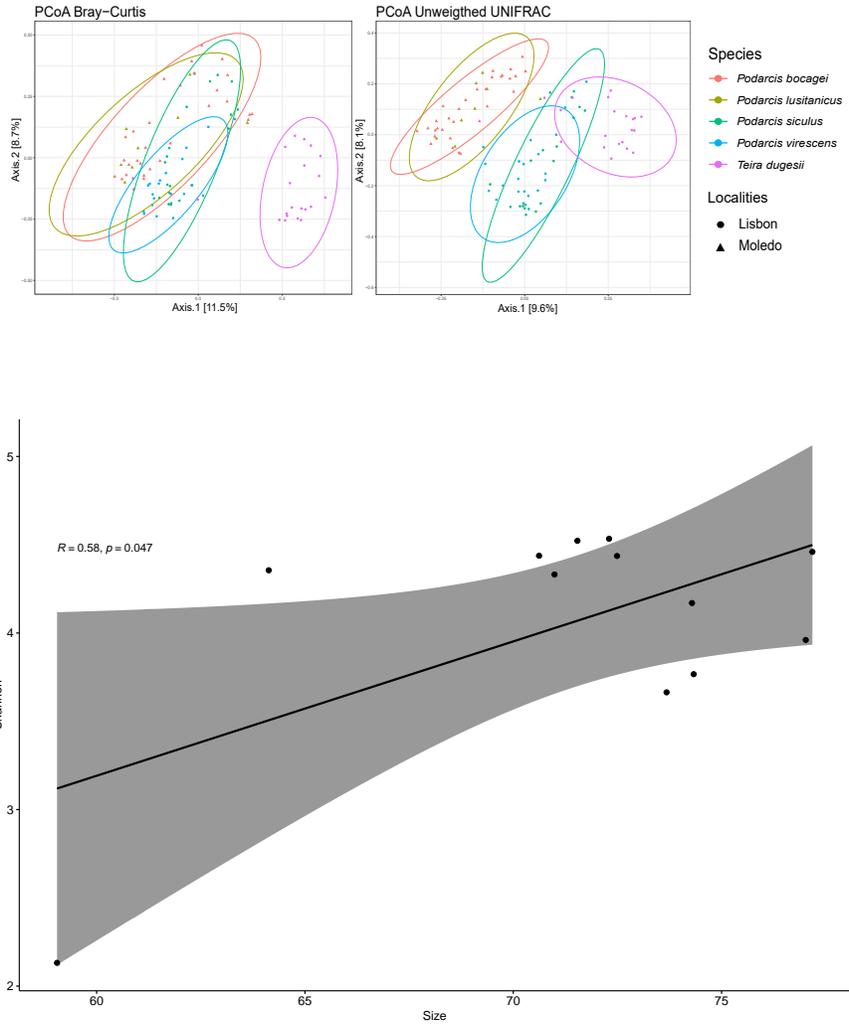
Figure 3: PCoA plots representing Bray–Curtis and Unweighted Unifrac distances, grouped by species with 95% confidence interval ellipse.

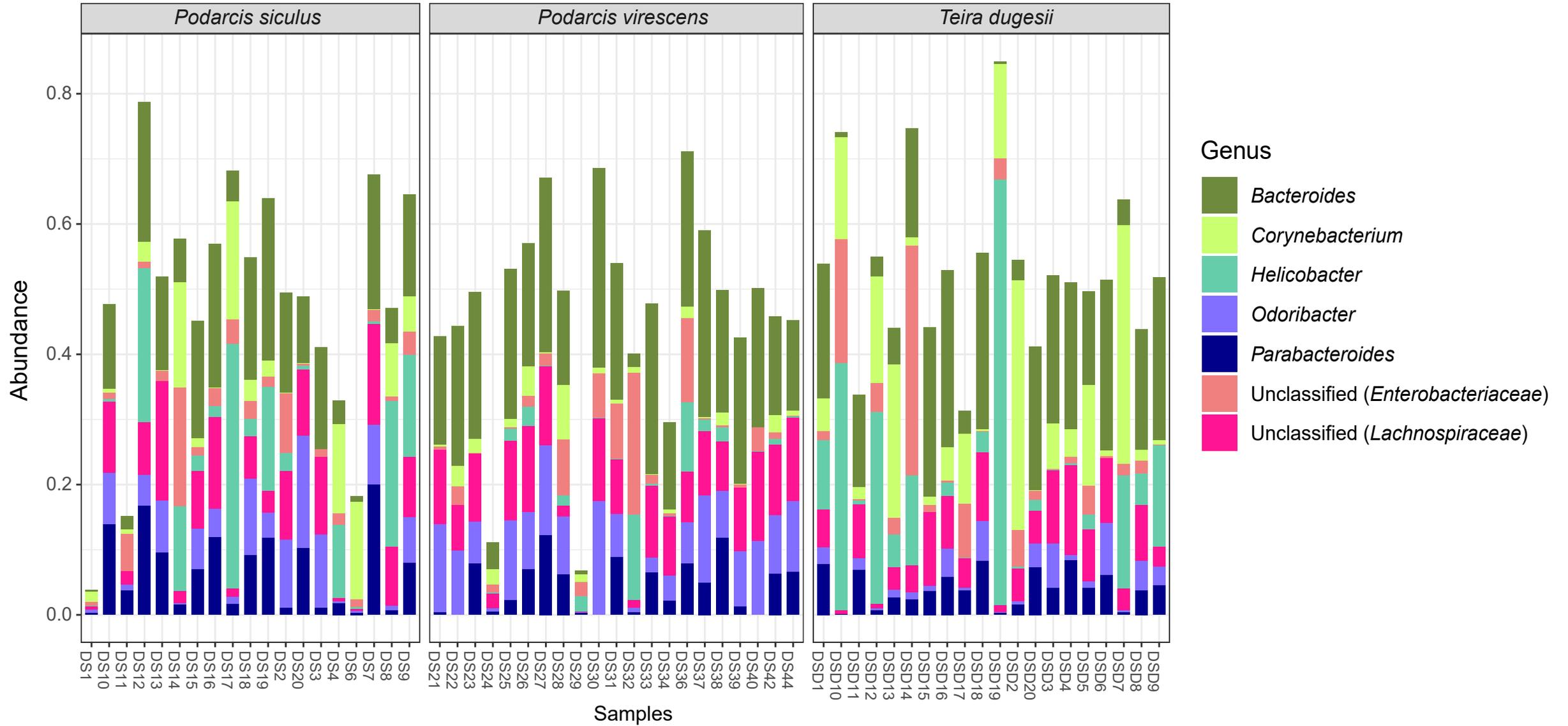
Figure 4: Relative abundance of the most abundant bacterial genera in the gut microbiome of the studied lizard species from Lisboa (A) and from Moledo (B).

Figure 5 : Linear regression plot between size (SVL) and gut bacterial alpha-diversity (Shannon index) for *Podarcis siculus*. The coloured area represents the 95% confidence limit.







A

B