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Gut microbial ecology of lizards: insights into diversity in the wild, effects of captivity, variation across gut regions and transmission

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Abstract

Animals maintain complex associations with a diverse microbiota living in their guts. Our understanding of the ecology of these associations is extremely limited in reptiles. Here, we report an in-depth study into the microbial ecology of gut communities in three syntopic and viviparous lizard species (two omnivores: *Liolaemus parvus* and *Liolaemus ruibali* and an herbivore: *Phymaturus williamsi*). Using 16S rRNA gene sequencing to inventory various bacterial communities, we elucidate four major findings: (i) closely related lizard species harbour distinct gut bacterial microbiota that remain distinguishable in captivity; a considerable portion of gut bacterial diversity (39.1%) in nature overlap with that found on plant material, (ii) captivity changes bacterial community composition, although host-specific communities are retained, (iii) faecal samples are largely representative of the hindgut bacterial community and thus represent acceptable sources for nondestructive sampling, and (iv) lizards born in captivity and separated from their mothers within 24 h shared 34.3% of their gut bacterial diversity with their mothers, suggestive of maternal or environmental transmission. Each of these findings represents the first time such a topic has been investigated in lizard hosts. Taken together, our findings provide a foundation for comparative analyses of the faecal and gastrointestinal microbiota of reptile hosts.

Keywords: captivity, gut microbiota, host–microbe interactions, reptiles

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Introduction

Animals maintain intimate and complex relationships with communities of microorganisms living within their gastrointestinal tracts (McFall-Ngai *et al.* 2013). These gut microbes can influence the ecology and evolution of their hosts through affecting behaviour (Archie & Theis 2011; Ezenwa *et al.* 2012), immune training (Hooper *et al.* 2001), nutrition (Mackie 2002), and reproductive isolation (Brucker & Bordenstein 2013; Shropshire & Bordenstein 2016). We are only recently beginning to understand the microbial ecology of these gut ecosystems, and how factors such as diet, physiological status or host genetics can interact to determine microbial community structure (Ley *et al.* 2008; David *et al.* 2014; Moeller *et al.* 2014; McKenney *et al.* 2015). Large-scale inventories of microbial diversity have been conducted

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across mammals (Ley *et al.* 2008), birds (Hird *et al.* 2015), fishes (Sullam *et al.* 2012; Clements *et al.* 2014) and to a lesser extent in amphibians (Vences *et al.* 2016). However, less than 10% of studies investigating the gut microbial communities of vertebrates are conducted on nonmammalian hosts (Colston & Jackson 2016). Reptiles represent a clade of vertebrate hosts that has been largely overlooked in terms of their gut microbial ecology.

Studying the gut microbial ecology of reptiles is imperative to begin understanding generalized patterns across vertebrate groups. Reptiles represent an ancient group with approximately 10 000 extant species, of which ~60% are within the clade Sauria, also known as lizards (Uetz & Hošek 2016). Lizards have diverse ecological, physiological and behavioural traits (Pianka & Vitt 2003), which may impact the ecology of their gut microbial communities. For example, the immune system plays a large role in determining gut microbial community structure, and several aspects of the reptilian immune system vary from those of other vertebrate classes (Zimmerman *et al.* 2010). Also, while herbivory is rare in lizards (<4% of species; Pough 1973) compared to mammals (~43% of species; Price *et al.* 2012), there are some lizards that consume primarily plant material (Espinoza *et al.* 2004). Do these herbivorous reptiles harbour similar fermentative microbes as mammalian herbivores? Last, in mammals, vaginal birth and maternal care are associated with transmitting the microbiota from mother to offspring (Dominguez-Bello *et al.* 2010; Funkhouser & Bordenstein 2013; Ardeshir *et al.* 2014). Conversely, lizards exhibit variation in birthing strategies from laying eggs (oviparity) to giving live birth (viviparity) and do not exhibit extensive parental care (Shine 1988), which may impact the fidelity of maternal transmission. The effects of captivity and maternal transmission on the gut microbiota may be of critical importance given that lizards are experiencing numerous population declines and extinctions (Sinervo *et al.* 2010), resulting in captive breeding and release programmes for some species (Alberts 2007; Connolly & Cree 2008). Studies regarding the success of captive-bred lizards in the wild have highlighted behavioural and physiological differences between wild and captive lizards (Alberts 2007; Connolly & Cree 2008). However, differences in host-associated microbial communities remain understudied, even though the gut microbiota has been suggested to play a role in conservation efforts (Redford *et al.* 2012). Overall, we still lack a basic understanding of the gut microbial ecology of lizards.

To expand our understanding of the gut microbial ecology of lizards, we performed a comprehensive set of studies using three syntopic species of lizards in the Southern Andes of Argentina, all within the family Liolaemidae (Fig. 1). We conducted bacterial diversity inventories on a large number of faecal and gut content samples to address four themes relating to the gut microbial ecology of lizards: (i) we collected faecal samples from three species in the wild over two seasons, corresponding to reproductive and postreproductive periods, as well as samples of plant material, invertebrate food items and soil. We investigated whether bacterial diversity varies across lizard species, sex and across seasons. We also investigated whether the environment (soil, food items) serves as a potential source of bacteria found in the guts of lizards. (ii) Next, we brought lizards into captivity and fed them laboratory diets for a period of 8 weeks. We then compared wild-collected samples to captive samples to understand the effects of captivity on the lizard gut microbiota and asked whether host-specific signatures are maintained. (iii) We then dissected animals to compare bacterial community structure across gut regions, which also allowed us to investigate the suitability of faecal samples as an index for the community in other gut regions. (iv) Finally, we collected samples from pregnant mothers and offspring born in captivity to understand the potential for maternal transmission in live-bearing lizard species. Independently, each of these studies represents the first time such a question has been investigated in lizard hosts. Collectively, these studies elucidate major factors associated with the gut microbial ecology of lizards.

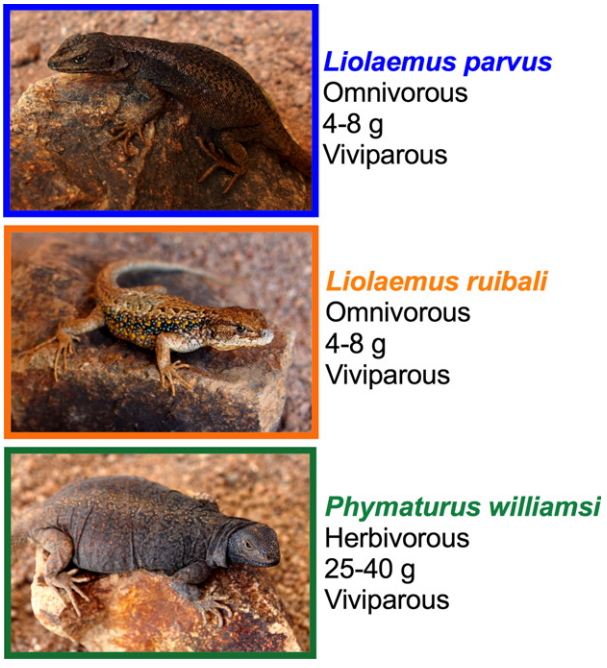

Fig. 1 Focal lizard species of this study and details of their biology. [Colour figure can be viewed at wileyonlinelibrary.com].

Methods

Ethics statement

This study was carried out under permission of Secretaría de Medio Ambiente and Dirección de Conservación y Áreas Protegidas, Provincia de San Juan (Exp.: 13004047, JCA). All methods were approved by the Institutional Committee of Animal Care and Use of the Universidad Nacional de San Luis under protocol #13185/14.

Animals maintenance and sample collection

Individuals of *Liolaemus parvus*, *L. ruibali* and *Phymaturus williamsi* (Fig. 1) were collected using lassos from Quebrada Vallecito, located in the Andes Mountains, 40 km W of Calingasta town, San Juan province, Argentina (31°11′21″S; 69°42′15″W, ~3000 m above sea level), in December 2014 (summer in the Southern Hemisphere). To collect wild faecal samples, animals were placed in individual, ethanol-sterilized plastic tubs overnight. Faeces were collected, placed in RNAlater and transported to the Universidad Nacional de San Luis, Argentina, and frozen at −20 °C. A second set of wild faecal samples was collected in March 2015 (autumn in the Southern Hemisphere), although here animals were released back into the wild after faecal samples were collected. We also collected six soil samples from the field site, 11 invertebrate samples (ants, spiders, small lepidopterans) and foliage from a number of plant species (three samples for each species of *Erodium cicutarium, Cerastium arvense, Phacelia secunda, Acaena magellanica, Mimulus depressus, Veronica anagallis-aquatica, Oenothera affinis, Adesmia pinnifolia, Senecio* spp., *Bacharis tola*), which are consumed by the lizard species (Villavicencio *et al.* 2005; Castro 2013; Castro *et al.* 2013; Pérez Mecado 2016). These samples were collected opportunistically in areas where lizards were captured (<20 m from point of capture) using ethanol-sterilized forceps or spatulas, placed in RNAlater and transported to the Universidad Nacional de San Luis, Argentina, and frozen at −20 °C.

Lizards collected in December 2014 were transported to the animal facility at the Universidad Nacional de San Luis, Argentina, and housed individually. Upon entering captivity, individuals of *L. parvus* and *L. ruibali* were fed a 'mixed' diet with a 50:50 mixture (dry weight/dry weight) of alfalfa-based rabbit chow and ground mealworms (water was added to create a diet of ~30% dry matter, 70% water). Lizards were kept in small plastic cages with autoclaved soil and given access to autoclaved water *ad libitum*. Further details for housing and feeding of *L. parvus* and *L. ruibali* can be found in Kohl *et al.* (2016). We fed *Phymaturus williamsi* in the same manner as described in Kohl *et al.* (2016), but used a diet of ground rabbit chow mixed with water (approximately 30% rabbit chow: 70% water) and an amount of ~9.8 mg dry food/gram body mass every other day.

A number of the female lizards that were captured were pregnant. Cages of pregnant females were checked daily for offspring, which were then moved into new cages. Thus, offspring were always removed from their mothers within 24 h of birth. Offspring lizards were also fed the same liquefied diets as adults daily for a period of 3 weeks. Faeces were collected daily, stored in RNAlater and frozen at −20 °C. Due to the extremely small size of faecal samples from offspring lizards, and the issues that small biomass samples can bring to interpreting microbial inventories (Salter *et al.* 2014), we pooled faecal samples over the first 3 weeks of life for all lizards within each group of siblings. Thus, each mother was an independent unit.

In February 2015, after approximately 8 weeks in captivity, lizards were again placed in individual, ethanol-sterilized plastic tubs overnight. Faeces were collected, placed in RNAlater and frozen at −20 °C. The following day, lizards were euthanized using isoflurane. Lizards were immediately dissected with stainless steel dissection tools, which were surface-sterilized with alcohol and bleach between animals. The gastrointestinal tracts of lizards were immediately opened, and the contents of the stomach, small intestine and hindgut were removed separately, stored in RNAlater and frozen at −20 °C. Hereafter, 'gut contents' refers to the luminal contents of the gut. We did not sample the mucosa-adherent microbiota, which may differ in composition (Dill-McFarland *et al.* 2014).

Microbial inventories

We extracted total DNA from all collected samples (faeces, gut contents, soil, plants, insects) using a MoBio PowerFecal DNA isolation kit. For invertebrate and plant samples, we extracted DNA from individual samples. For invertebrates, we used the entire specimen, and for plants, we measured a small amount of leaf material (~0.25 g). We also conducted nine 'blank' extractions to correct for contaminants found in DNA extraction kits (Salter *et al.* 2014). Extracted DNA was sent to Argonne National Laboratory (U.S. Department of Energy, Chicago, IL, USA) for amplification of the V4 region of the 16S rRNA gene with primers *515F* and *806R* and sequenced on the Illumina MiSeq platform (Caporaso *et al.* 2012).

Microbial sequences were analysed using QIIME version 1.9.1 (Caporaso *et al.* 2010). We grouped sequences into operational taxonomic units (OTUs) using an open reference method and a minimum sequence identity of 97% (He *et al.* 2015). Any OTUs present in the 'blank samples' were considered contaminants and were removed from all other samples (Salter *et al.* 2014). More details regarding sequence analysis can be found elsewhere (Kohl *et al.* 2016).

We compared several aspects of gut bacterial community diversity and structure. First, we calculated several measurements of alpha diversity: Faith's phylogenetic diversity (Faith 1992), the Shannon index, evenness and the number of observed OTUs. For these diversity indices, we calculated the mean of 20 iterations for a subsampling of a determined number sequences depending on the comparison. For comparing diversity across species and seasons in the wild, as well as comparing wild samples to captive samples, we used 2000 sequences per sample. For comparisons across gut regions, we used 1000 sequences per sample. Last, to compare communities between mothers and offspring, we used 670 sequences per sample. These differences are due to variation in the number of sequences per sample in different comparisons. These sequence depths are sufficient for capturing differences in microbial communities (Caporaso *et al.* 2012). Further, rarefaction curves demonstrate that we captured a majority of microbial diversity (Fig. S1, Supporting information). Alpha diversity measurements were compared using ANOVAS.

We also investigated the effects of experimental variables (species, season, captivity, etc.) on the relative abundances of bacterial taxa across various groups. Relative abundances were transformed using a variance stabilizing transformation of arcsin(abundance$^{0.5}$) (Shchipkova *et al.* 2010; Kumar *et al.* 2012). We did not use rarefied data when comparing abundances of bacterial taxa (McMurdie & Holmes 2014). We used JMP®, version 12.0 (SAS Institute Inc., Cary, NC, USA) to compare relative abundances of bacterial phyla and genera using the response screening function with the robust fit option to conduct multiple Student's *t*-tests and correct *P*-values with the Benjamini–Hochberg false discovery rate (FDR) correction (Benjamini & Hochberg 1995). Sample sizes for all comparisons can be found in the Appendix S1, Supporting information and in Table 1.

Community membership and structure were compared by conducting principal coordinates analysis (PCoA) on unweighted and weighted UniFrac distances (Lozupone & Knight 2005). Distance matrices and PCoA plots were made using the same number of sequences as for measuring alpha diversity, depending on the analysis. Comparisons across groups were conducted using the adonis function in R on the distance matrices with 999 permutations (Clarke 1993). For the comparison across gut regions, we were specifically interested in how similar various gut regions were to each other, in order to test the validity of using faecal samples for bacterial inventories. We sampled 1000 random sequences per sample and pooled these within a particular type of sample (species and gut region). We then generated UPGMA trees (unweighted pair group method with arithmetic mean) of both unweighted and weighted UniFrac distance matrices using these pooled sequences.

Last, we used SourceTracker with default parameters (Knights *et al.* 2011) to compare the proportion of the faecal communities at the OTU level that were composed of either exogenous sources (soil, plant material, insects, food in captivity) or from the faecal microbiota of mothers.

All 16S rRNA sequences have been deposited in the Sequence Read Archive (SRA) under Accession nos PRJNA293117 and PRJNA312520.

Table 1 Sample sizes for various comparisons. M and F designate male and female samples for those collected in spring. Sex was not determined for samples collected from the wild in autumn

	Liolaemus parvus				*Liolaemus ruibali*				*Phymaturus williamsi*			
	Summer		Autumn		Summer		Autumn		Summer		Autumn	
	M	F			M	F			M	F		
N	10	11	10		7	8	8		11	18	4	
	Wild		Captive		Wild		Captive		Wild		Captive	
N	13		13		5		5		7		7	
	Stomach	SI	Hindgut	Faeces	Stomach	SI	Hindgut	Faeces	Stomach	SI	Hindgut	Faeces
N	14	11	14	14	9	5	9	9	4	4	6	6
	Mothers		Offspring		Mothers		Offspring		Mothers		Offspring	
N	4		4		5		5		2		2	

Results

Gut bacterial diversity in wild lizards

We hypothesized that gut bacterial communities would vary across host species, sex and season, with species having the largest effect on discriminating the gut microbiota. The basic reason is that although these lizard species are syntopic and may exchange microbial communities in close contact, they vary in their natural diet and size. *Liolaemus parvus* and *Liolaemus ruibali* are omnivores, whereas *Phymaturus williamsi* is a generalist herbivore. As expected, there was a significant effect of lizard species on faecal bacterial community membership (the presence or absence of species; Fig. 2A; adonis: $R^2 = 0.16$, $P < 0.001$) and community structure (which takes relative abundance into account; Fig. 2B; adonis: species effect: $R^2 = 0.36$, $P < 0.001$), with the greatest differentiation observed between the omnivores and generalist herbivore. Interestingly, the two omnivorous species (*L. parvus* and *L. ruibali*) also differed in bacterial community membership (adonis: $R^2 = 0.04$, $P < 0.001$) and structure ($R^2 = 0.05$, $P = 0.004$), which could be driven by host genetics, slightly different diets or a combination of these factors. However, it should be noted that only a small amount of variation was explained by species when comparing *L. parvus* and *L. ruibali*. In a variety of other comparisons, there were no effects of season or sex on bacterial community membership or structure (adonis: $P > 0.05$ for all) evaluated in each lizard species. Moreover, there were no significant effects of lizard species, sex or season on any measurements of alpha diversity (Shannon index, number of observed OTUs, Faith's phylogenetic diversity or evenness).

The striking differences in community membership and structure across syntopic lizard species were associated with variation in the underlying relative abundances of bacterial taxa. For example, we identified 10 bacterial phyla (52.6% of the observed phyla) and 25 bacterial genera (25.0% of the observed genera) that differed significantly in abundance across lizard species

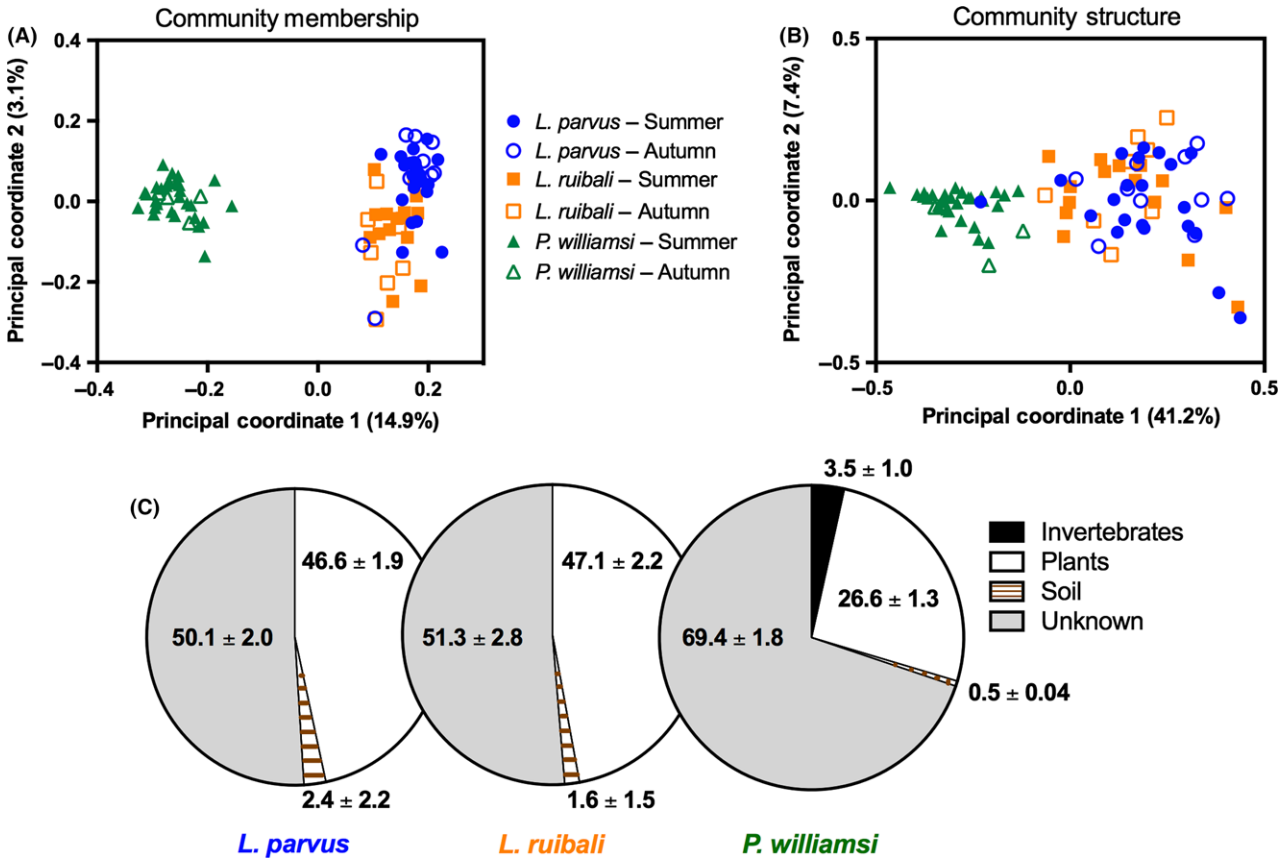

Fig. 2 Faecal microbial diversity in the wild. (A) Principal coordinates analysis of an unweighted UniFrac distance matrix. (B) Principal coordinates analysis of a weighted UniFrac distance matrix. (C) Source proportions for the faecal microbiota of wild lizards. Percentages and standard errors are placed over wedges. Overlap with invertebrate microbial communities was not detected in any individuals of *L. parvus* and only in a single individual of *L. ruibali* (0.03% of the community for that individual).

(Appendix S1, Supporting information). The phylum Deferribacteres was present in the two omnivorous lizard species, *L. parvus* and *L. ruibali*, but absent from the herbivorous species, *P. williamsi*. Additionally, the omnivorous species (*L. parvus* and *L. ruibali*) had lower abundances of Firmicutes (~48%) when compared to the herbivorous species (*P. williamsi*: 73% Firmicutes). At the genus-level, the omnivorous lizard species exhibited higher abundances of *Rikenella* and *Helicobacter*, while the herbivore, *P. williamsi*, exhibited higher abundances of *Caldicoprobacter*, *Coprococcus* and *Treponema* (among others; Appendix S1, Supporting information). The two omnivorous species (*L. parvus* and *L. ruibali*) exhibited highly similar bacterial abundances for the most part, with only three bacterial phyla (15.6% of observed bacterial phyla) and one bacterial genus (1.0% of observed bacterial genera) exhibiting significant differences between these two host species. The differential abundances of these bacterial taxa across lizard species may be adaptive for their various diets (see Discussion). We detected a significant effect of season on the relative abundances of four bacterial phyla, although these phyla also exhibited significant species × season interactions (Appendix S1, Supporting information). There were no detectable effects of sex on the relative abundances of any bacterial phyla or genera.

We also hypothesized that environmental sources (soil, plant material, invertebrates) contribute to the bacterial diversity in the lizard gut. We found that the gut communities of omnivorous species overlapped more with communities found on plant surfaces (46%) than the gut microbiota of the herbivorous species (26%; ANOVA: $P < 0.0001$; Fig. 2C). Unexpectedly, the gut bacterial microbiota of the herbivorous species, *P. williamsi*, overlapped significantly more with the microbiota from invertebrate prey (3.4%; ANOVA: $P = 0.0003$; Fig. 2C). In fact, 21 of the 34 *P. williamsi* faecal samples contained a portion of the bacterial taxa that overlapped with invertebrate bacterial taxa, while overlap was only detected in one of 55 samples from omnivorous lizards. Soil bacteria did not overlap with a large portion of the faecal bacterial microbiota in any lizard species. It is thought that local exposure to local pools of microbial species might underlie variation in the gut microbial communities of iguanas (Lankau *et al.* 2012).

Captivity significantly alters faecal bacterial communities

After a period of 8 weeks, we detected significant effects of captivity on both bacterial community membership (Fig. 3A,B; adonis: species effect: $R^2 = 0.15$, $P < 0.001$; captivity effect: $R^2 = 0.05$, $P < 0.001$; species × captivity effect: $R^2 = 0.04$, $P = 0.04$) and bacterial community structure (species effect: $R^2 = 0.22$, $P < 0.001$; captivity effect: $R^2 = 0.14$, $P < 0.001$; species × captivity effect: $R^2 = 0.06$, $P = 0.02$). The effects of captivity were more pronounced in the herbivorous species, *P. williamsi*, when compared to the omnivorous lizards, as demonstrated by larger UniFrac distances between wild and captive samples (ANOVA: unweighted UniFrac distances: $P = 0.03$; weighted UniFrac distances: $P = 0.005$; Fig. 3C,D). Notably, the omnivorous lizards (*L. parvus* and *L. ruibali*) still maintained distinct bacterial community membership in captivity ($R^2 = 0.05$, $P < 0.001$), although they did not exhibit distinct bacterial community structure ($R^2 = 0.07$, $P = 0.22$).

Specifically, captivity resulted in changes in the relative abundances of three bacterial phyla (23.1% of observed phyla; Fig. 3E–G) and 20 genera (25.3% of observed genera; Appendix S1, Supporting information). Captivity also corresponded with a loss of the genera *Butyrivibrio* and *Christensenella*, as these genera were not detected in any captive samples from any lizard species (Appendix S1, Supporting information). Additionally, captivity resulted in the introduction of *Enterobacter*, *Salmonella* and *Trabulsiella* to the lizard gut microbiota. These genera were not detected in any of the wild-collected samples, but were each present in at least 75% or more of the captive samples from all three lizard hosts. Although captivity significantly altered bacterial community membership, there were no differences in any measurements of alpha diversity between wild and captive lizards (Shannon index, number of observed OTUs, Faith's phylogenetic diversity or evenness).

Importantly, a majority of the microbiota in captive samples overlapped with those detected in the wild (Fig. 3H), suggesting that lizards retain their natural bacterial microbiota in captivity. However, this overlap varied across lizard species, such that 70 ± 1% of the captive bacterial microbiota of omnivorous species (*L. parvus* and *L. ruibali*) overlapped with the wild microbiota, while only 60 ± 4% of the captive bacterial microbiota of *P. williamsi* overlapped with wild-collected samples (ANOVA: $P = 0.014$). The bacteria present in laboratory food did not compose a significant portion of the captive faecal microbiota (<1%; Fig. 3H).

Bacterial diversity varies across gut regions

We hypothesized that gut bacterial communities would vary across gut regions, given that different gut chambers vary in their pH, nutrient composition and other physiological characteristics. Measurements of Faith's phylogenetic diversity varied significantly across gut regions ($F_{3,93} = 68.50$; $P < 0.0001$) such that the small

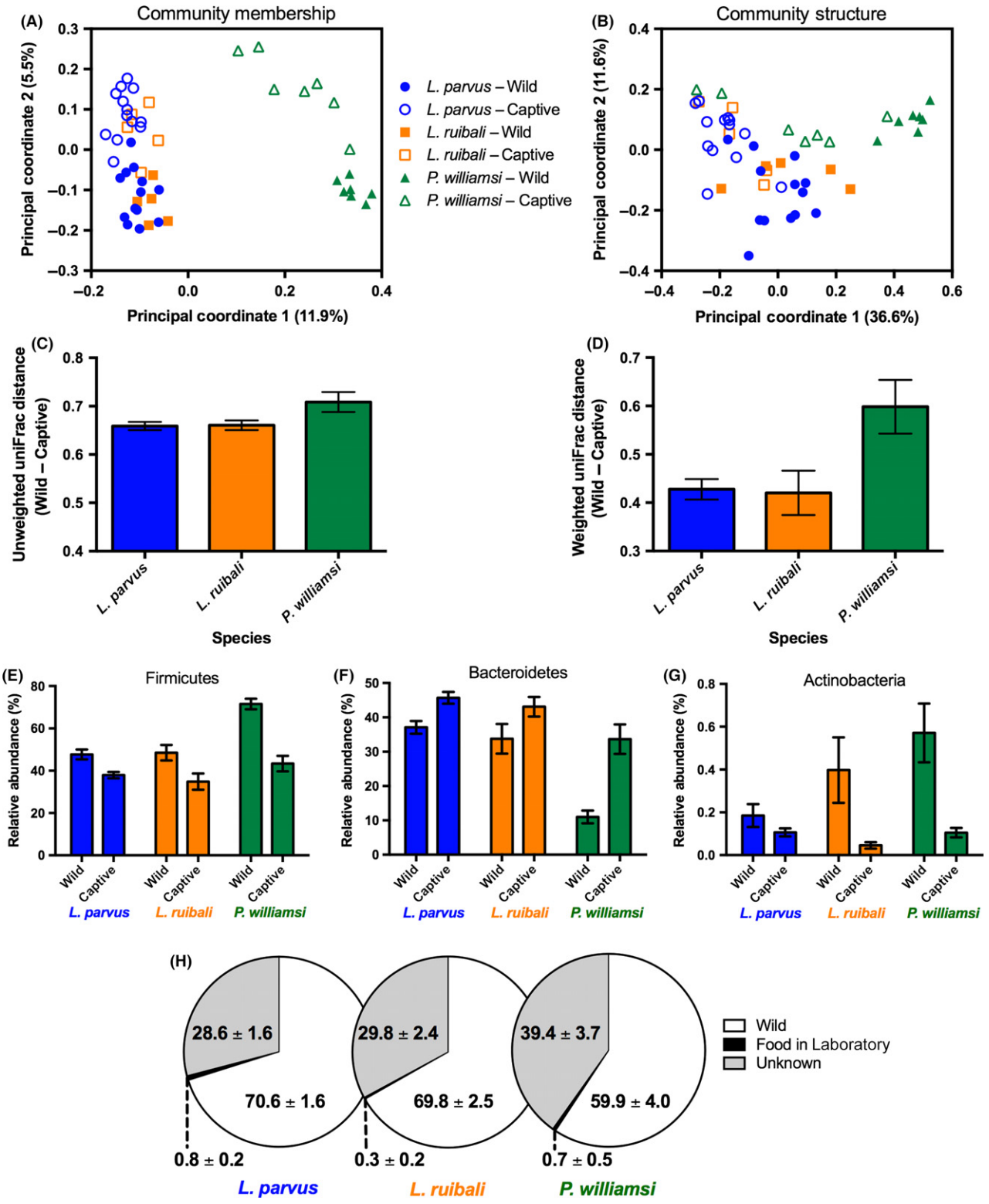

Fig. 3 Effects of captivity on the lizard gut microbiota. (A) Principal coordinates analysis of an unweighted UniFrac distance matrix. (B) Principal coordinates analysis of a weighted UniFrac distance matrix. (C) Mean ± s.e.m. pairwise unweighted UniFrac distances between wild and captive samples within a lizard individual. (D) Mean ± s.e.m. pairwise weighted UniFrac distances between wild and captive samples within a lizard individual. (E-G) Mean ± s.e.m. relative abundances of microbial phyla in the faeces of lizards in the wild and captivity. (H) Source proportions for the faecal microbial communities of captive lizards. Percentages and standard errors are placed over wedges.

intestine had the lowest measurement of phylogenetic diversity (Fig. 4A). While phylogenetic diversity did not vary across species ($F_{2,93}$ = 0.53; P = 0.59), there was a significant species × gut region interaction ($F_{6,93}$ = 2.23, P = 0.049). Other measurements of alpha diversity (Shannon index, number of observed OTUs, evenness) exhibited the same trends (Fig. S2, Supporting information).

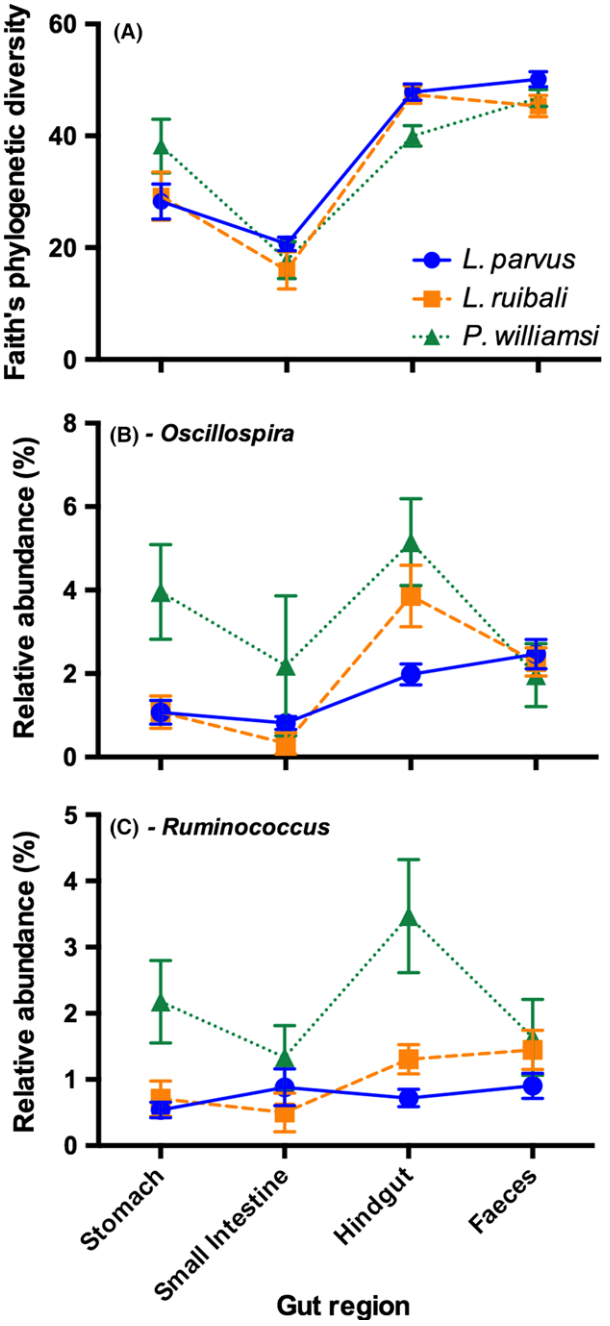

Fig. 4 Microbial diversity across gut regions. (A) Mean ± s.e.m. Faith's phylogenetic across gut regions. (B+C) Mean ± s.e.m. relative abundances of microbial genera across gut regions.

We also observed differential relative abundances of several bacterial taxa across gut regions. Six bacterial phyla exhibited differential abundances across gut regions (26.1% of the observed phyla; Appendix S1, Supporting information). For example, Actinobacteria composed 13.7 ± 3.5% of the small intestinal communities of *L. parvus* and *L. ruibali*, but only than 0.03% of the hindgut communities. Additionally, we detected 49 bacterial genera that exhibited significant differences in relative abundances across gut regions (44.9% of the observed genera). For example, the genera *Oscillospira* and *Ruminococcus* exhibited significant differences across gut regions (Fig. 4B,C, Statistics in Appendix S1, Supporting information). These differences across gut regions may provide insight into the functions of the bacterial communities in these regions (see Discussion).

We were also interested in whether faecal samples were suitable as an index for the bacterial community in other gut regions. The use of UPGMA clustering revealed that faecal and hindgut communities clustered within each species in terms of community membership (Fig. 5A). Additionally, small intestinal samples all clustered together, suggesting similar community membership in this region across all three lizard species (Fig. 5A). In terms of community structure, faeces and hindgut samples clustered together for *L. parvus* and *L. ruibali*, but not for *P. williamsi* (Fig. 5B). The bacterial community membership and structure of hindgut and faeces were not distinguishable within any lizard species (ANOSIM: $P > 0.05$ for all three species). Instead, most of the variation in community membership and structure was driven by the individual lizard that samples were collected from (ANOSIM $P < 0.01$ for all except community structure in *P. williamsi*, where $P > 0.1$). Overall, it seems that faeces are representative of the hindgut communities of lizards.

A portion of the gut microbiota is shared between mother and offspring

Last, we hypothesized that live-bearing lizards would transmit portions of their gut microbiota from mothers to offspring. Consistent with maternal transmission or a common rearing environment, a significant proportion (34.3 ± 5.6%) of the faecal bacterial communities of captive-born lizards overlapped with those of their mothers (Fig. 6A). The majority of overlapping microbes were identified as Enterobacteriaceae, particularly *Trabulsiella* and *Enterococcus*, the former of which was notably introduced into adults during captivity. However, there were still some marked differences in the bacterial communities of mothers and offspring. Lizard offspring

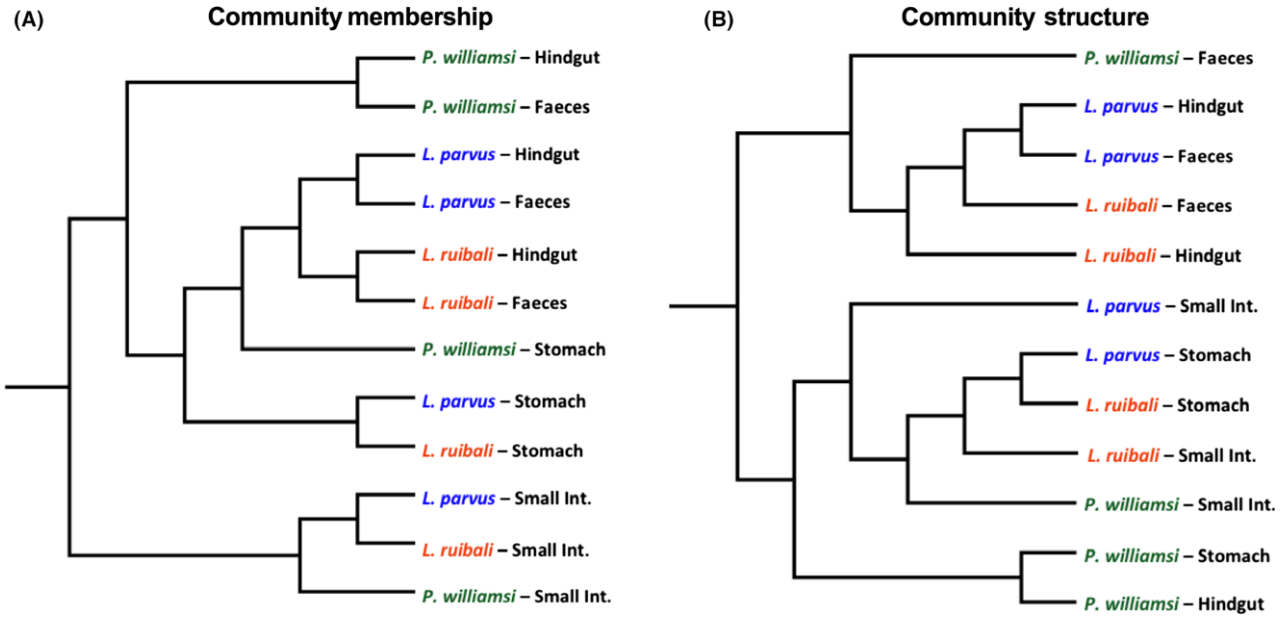

Fig. 5 Microbial diversity across gut regions. (A) UPGMA tree of unweighted UniFrac distances. (B) UPGMA tree of weighted UniFrac distances. Sequences were evenly pooled across individuals (1000 sequences per sample) within a sample type prior to analysis.

harboured significantly less diverse bacterial communities than their mothers (*e.g.* 61.8% lower diversity in offspring compared to mothers as estimated by Faith's phylogenetic diversity; Fig. S3, Supporting information). Additionally, we identified six bacterial phyla (50.0% of observed phyla) and 11 bacterial genera (12.9% of observed genera) that were present in differential abundances between mothers and offspring (Appendix S1, Supporting information). We also identified several common genera that did not seem to be transmitted from mothers to offspring. For example, the genera *Lawsonia* and *Desulfovibrio* were detected in all adult samples, but were only detected in one offspring sample from *L. ruibali*.

Thus, despite some maternal or environmental transmission, the lower diversity and differential bacterial abundances of lizard offspring caused these animals to exhibit distinct community membership and community structure from their mothers (Fig. 6B+C). While differentiation between mothers and offspring was easily visualized using principal coordinate 1 (Fig 6B+C), there were similarities between mothers and offspring within species when removing food samples from the analysis and visualizing using principal coordinates 2 and 3, especially in terms of community membership (Fig. 6D+E). Offspring of the two omnivorous lizard species (*L. parvus* and *L. ruibali*) harboured bacterial microbiota with distinct community membership ($R^2 = 0.16$, $P = 0.015$), but not different community structure ($R^2 = 0.16$, $P = 0.14$), suggesting that these offspring were inoculated with distinct bacterial communities. However, it should also be noted that some of the differences between mothers and offspring are confounded by the fact that the offspring lizards in our study were still young and developing. Their bacterial communities may have stabilized towards a more adult-like community later in life (see Discussion).

Discussion

The gut bacterial communities of our focal species varied significantly across lizard species. The two omnivorous species (*Liolaemus parvus* and *Liolaemus ruibali*) exhibited more similar communities when compared to the herbivorous species, *Phymaturus williamsi*. This finding could be an effect of diet and/or evolutionary history, given that both of these factors influence the gut microbial communities of mammals (Ley *et al.* 2008), or the larger size of *P. williamsi*, given that body size can influence microbial diversity (Godon *et al.* 2016). However, several of the bacterial taxa enriched in the herbivorous species may be adaptations for digesting a high fibre diet. For example, the xylanolytic genus *Caldicoprobacter* (Yokoyama *et al.* 2010) was only detected in the faeces of *P. williamsi*. Additionally, the faeces of *P. williamsi* were enriched in *Treponema*, which can degrade plant polysaccharides (Paster & Canale-Parola 1985; Piknova *et al.* 2008) and enhance fibre fermentation by other cellulolytic bacteria (Kudo *et al.* 1987). The independent evolution of herbivory is often associated with unique metabolic machinery from distinct microbes (Pope *et al.* 2010; Hong *et al.* 2015). Thus, it

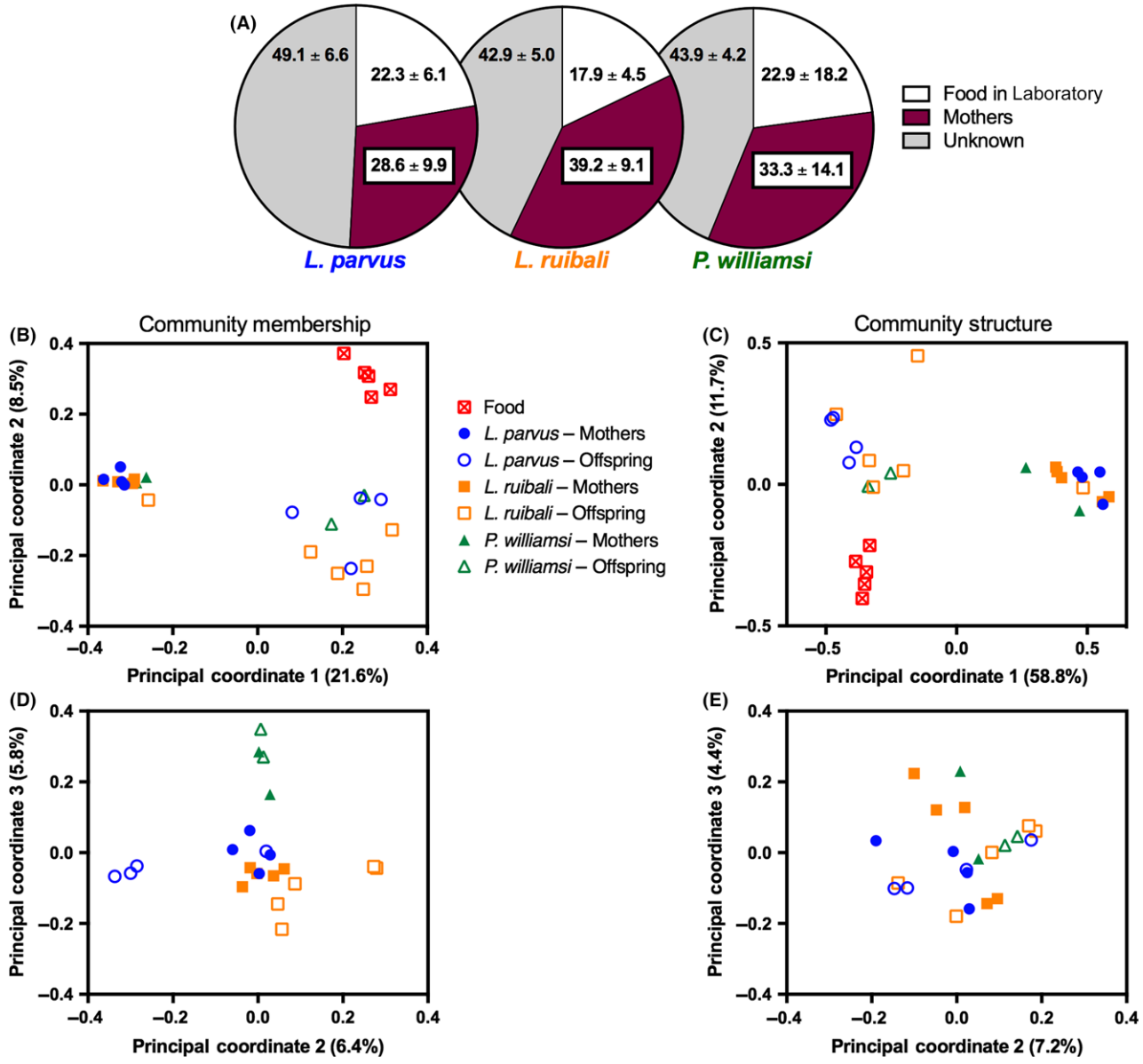

Fig. 6 Gut microbiota of mothers and offspring. (A) Source proportions for the gut microbial communities of lizards born in captivity. Percentages and standard errors are placed over wedges. (B+D) Principal coordinates analysis of an unweighted UniFrac distance matrix. (C+E) Principal coordinates analysis of a weighted UniFrac distance matrix.

would be interesting to further study the microbes that permit herbivory in *Phymaturus* lizards.

The effects of sex and season on the gut microbiota were less pronounced. We did not detect any effect of sex on bacterial diversity or the relative abundances of any taxa. Sex has been documented to influence the gut microbiota of rodents (Maurice *et al.* 2015) and to some extent in the striped plateau lizard (*Sceloporus virgatus*; Martin *et al.* 2010). There were seasonal differences in the relative abundances of several bacterial taxa in the lizard gut, although all of these taxa also exhibited significant species × season interactions. The gut microbial communities of wild mice vary seasonally due to changes in diet, reproductive status and other physiological parameters (Maurice *et al.* 2015). The lizard species studied here exhibit seasonal variation in diet composition (Castro 2013; Lobo *et al.* 2013; Pérez Mecado 2016), which may drive the species-specific seasonal changes in relative abundances of microbial taxa. Moreover, the lizards studied here live in the high Andes and thus undergo periods of winter hibernation (Cartes *et al.* 2010). Hibernating mammals exhibit marked seasonal restructuring of the gut microbiota (Dill-McFarland *et al.* 2014); in the future, one could

compare the shifts in microbial community structure that occur in hibernating mammals and herptiles.

We also investigated environmental sources that might contribute to the gut microbial communities of wild lizards. Soil bacteria did not contribute significantly to the gut communities of lizards, even though these lizards occupy a variety of microhabitats, such as rocky outcrops, shrub patches, rock crevices and small burrows dug in bare soil (Halloy *et al.* 2007). We found that a significant amount of the lizard gut bacterial communities overlapped with microbes found on or in the plants that these animals consume. This result is similar to a previous study in herbivorous desert wood rats (*Neotoma lepida*), where there was substantial overlap between their gut microbiota and the phyllosphere microbiota of their dietary plants (Kohl & Dearing 2014). The microbes of invertebrate diet items did not contribute significantly to the gut bacterial microbiota of lizards, similar to Burmese pythons (*Python molurus*), where microbes from their rodent meal compose less than 1% of their gut community (Costello *et al.* 2010). It is unclear why phyllosphere microbes might occupy the gut niche so well, while the insect microbiota do not. It is also puzzling why the insect bacterial microbiota seems to overlap more with the gut community of the herbivorous lizard compared to the omnivorous hosts. One potential explanation is that herbivores have potentially less restrictive filters against consumed microbes (Beasley *et al.* 2015) and the fact that many herbivores are opportunistically carnivorous (Dudley *et al.* 2016). Thus, the herbivorous *P. williamsi* may occasionally consume insect prey items and become inoculated with an insect microbiota. Further investigations into the ecology of allochthonous microbes in various animals and wild systems would provide insight into the assembly of the gut microbiota.

A number of previous studies have documented that the gut microbiota of captive animals differ from their wild counterparts (Uenishi *et al.* 2007; Scupham *et al.* 2008; Villers *et al.* 2008; Xenoulis *et al.* 2010; Wienemann *et al.* 2011; Nelson *et al.* 2013; Kohl & Dearing 2014). A recent study documented a shift in the gut microbiota of insectivorous *Anolis sagrei* lizards brought into captivity (Ren *et al.* 2016). Our current work builds upon this study by investigating omnivorous and herbivorous lizards and demonstrating that species-specific bacterial signatures are retained in captivity. In our study, captive lizards retained a majority of their wild bacterial microbiota (~65%), suggesting that captive studies on the lizard gut microbiota may still have ecological relevance. However, we found that similar to other hosts, the bacterial microbiota of wild and captive lizards differed significantly in membership but not alpha diversity. This is in contrast to other studies that document a loss of microbial diversity as animals enter captivity (Kohl & Dearing 2014; Kohl *et al.* 2014b). The herbivorous species, *P. williamsi*, exhibited a larger shift in bacterial community membership and structure in captivity compared to the omnivorous species. These differences could be due to species-specific changes in diet and/or physiology in captivity, such as stress or immune function. Captivity resulted in the introduction of *Enterobacter* and *Salmonella* to the gut bacterial communities of lizards, both of which are potentially pathogenic to reptiles and zoonotic to humans (Schumacher 2006). Additionally, SourceTracker revealed that ~32% of the bacterial community of captive lizards came from 'unknown' sources. This result could be due to stochasticity in sequencing, such that these 'unknown' microbes were also present in the wild but not detected. Alternatively, these 'unknown' microbes may have been introduced into the gut from other environmental sources that we did not inventory, such as animal care staff or air. Overall, these changes in gut microbial communities upon entrance into captivity could have implications for animal health in captivity and potentially the success of conservation efforts involving captive breeding and release of threatened animals (Redford *et al.* 2012).

The various gut chambers of the vertebrate gastrointestinal tract vary in their pH, nutrient composition and other physiological characteristics (Stevens & Hume 2004), which may impact microbial community structure. Two genera, *Oscillospira* and *Ruminococcus*, exhibited highest abundances in the hindgut region, especially in herbivorous *P. williamsi*. These genera are associated with feeding on plant-rich diets and may aid in fibre digestion (Mackie *et al.* 2003). Additionally, relative abundances of the genus *Desulfovibrio* also exhibited significant differences across gut regions, largely driven by high abundances in the small intestines of the herbivorous species, *P. williamsi*. A controlled feeding trial using *L. ruibali* demonstrated that the abundance of *Desulfovibrio* in the small intestine was correlated with whole-animal fibre digestibility (Kohl *et al.* 2016). Further, the presence of *Desulfovibrio* may be important for reducing the H_2 by-products associated with anaerobic fermentation in herbivorous iguanas (Hong *et al.* 2011). Thus, this genus may be important for herbivory in lizards.

We also compared bacterial communities across gut regions to assess the validity of using faecal samples as representatives of the gut communities. Understanding these relationships is essential, as rodent studies have demonstrated that faecal samples are not always representative of other gut regions (Kohl *et al.* 2014a; Wirth *et al.* 2014). Here, we found that faecal communities of

lizards were very similar to hindgut microbial communities, especially in terms of community membership, and thus may be an acceptable indicator for microbial diversity in this gut region. Other studies have demonstrated the utility of using cloacal swabs for nondestructive sampling of the reptilian gut microbiota (Colston *et al.* 2015). However, the communities of cloacal swabs have distinct microbial community signatures from large intestinal communities (Colston *et al.* 2015). A comparison of faeces and cloacal swabs as representatives for gut communities is warranted. Moreover, the use of faeces has some additional caveats. Collection of faecal samples in our study was conducted daily due to the fact that reptiles often only defecate once per day. Thus, some faecal samples had the potential to be ~24 h old when collected. A recent controlled study documented that the microbial community structure of faeces can change over a 24-h period under field conditions (Hale *et al.* 2016). However, this study only used a single pooled faecal sample and so it is unclear whether the effects of time are larger or smaller than variation across individuals. Another controlled study with multiple individuals of wood rats found that individual microbial signatures were highly retained between fresh faeces and those collected from the floor of Sherman traps after the rodent hosts spent a night in the trap with cotton batting, apple slices and oatmeal bait for ~10 h (Kohl *et al.* 2015). Thus, further investigations into the different types of microbial samples and sources of variation are needed. Currently, researchers should hesitate to extrapolate the results of faecal inventories to other gut regions. However, it should be noted that faecal inventories might still be useful for repeated sampling or when researchers must collect nonlethal samples.

It has been demonstrated that internal or external transmission of microbes from mother to offspring is common across animals (Funkhouser & Bordenstein 2013). In mammals, microbial transmission occurs during the birthing process via exposure to vaginal and faecal microbes (Dominguez-Bello *et al.* 2010). Given that lizards have single urogenital opening, the cloaca, we predicted that maternal transmission of the gut microbiota should also occur in viviparous lizard species, which give live birth through this opening. We found that offspring had less diverse bacterial communities than their mothers, and these gut communities were more similar to their food sources. However, we did see some evidence of maternal or environmental transmission of the gut bacterial microbiota. Offspring clustered within their species when communities were visualized using the second and third principal coordinates (see Fig. 6). Also, the two omnivorous species exhibited distinct microbial community membership, which could be indicative of inoculation with a species-specific microbiota at birth. Last, SourceTracker estimated that ~35% of the gut microbiota of juvenile lizards overlapped with their mothers. Across animals, the microbial communities of juveniles are distinct from that of adults and largely resemble environmental sources (Dominguez-Bello *et al.* 2010; Burns *et al.* 2015). These microbial communities then mature as the hosts further develops over time (Trosvik *et al.* 2010; Burns *et al.* 2015). Thus, we hypothesize that the microbial 'seeding' from lizard mothers may further mature over time.

While lizards do not exhibit extensive parental care (Shine 1988), there is evidence for some maternal protection of offspring in *Liolaemus* species (Halloy *et al.* 2007). Additionally, coprophagy, or the consumption of adult faeces, is important for transmission of the gut microbiota in green iguanas (*Iguana iguana*; Troyer 1982, 1984), and this behaviour has been observed in young individuals of *Phymaturus palluma* (Vicenzi 2015) and *P. williamsi* (Laspiur, personal observation). In this study, we were primarily interested in microbial transmission associated with the birth process. By removing animals within 24 h, we are unable to investigate transmission associated with coprophagy or other interactions with adult animals. Further investigations into the roles that these behaviours play in facilitating transmission of the gut microbiota across generations would be interesting.

Collectively, this work represents a foundation for understanding the gut microbial ecology of lizards and constitutes a detailed account of the diversity and dynamics of the microbiota inhabiting the guts of Andean lizards. Overall, lizards exhibit many similarities to other host taxa, such as species-specific microbial communities, variation across gut regions and maternal transmission or environmental sharing of these communities to some extent. Two key findings pave the way for further studies into the gut microbial ecology of lizards: that lizards maintain host species-specific microbial communities for at least 8 weeks in captivity and the validity of using faecal samples for microbial inventories. Future investigations will continue to reveal how gut microbial communities may be impacting the ecology and evolution of lizard and reptile hosts.

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Conflict of interest

The authors declare no conflict of interest.

References

Alberts AC (2007) Behavioral considerations of headstarting as a conservation strategy for endangered Caribbean rock iguanas. *Applied Animal Behaviour Science*, **102**, 380–391.

Archie EA, Theis KR (2011) Animal behavior meets microbial ecology. *Animal Behaviour*, **82**, 425–436.

Ardeshir A, Narayan NR, Méndez-Lagares G *et al.* (2014) Breast-fed and bottle-fed infant rhesus macaques develop distinct gut microbiotas and immune systems. *Science Translational Medicine*, **6**, 252ra120.

Beasley DE, Koltz AM, Lambert J, Fierer N, Dunn RR (2015) The evolution of stomach acidity and its relevance to the human microbiome. *PLoS One*, **10**, e0134116.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, **57**, 289–300.

Brucker RM, Bordenstein SR (2013) The hologenomic basis of speciation: gut bacteria cause hybrid lethality in the genus *Nasonia*. *Science*, **341**, 667–669.

Burns AR, Stephens WZ, Stagaman K *et al.* (2015) Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. *ISME Journal*, **10**, 655–664.

Caporaso JG, Kuczynski J, Stombaugh J *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, **7**, 335–336.

Caporaso JG, Lauber CL, Walters WA *et al.* (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME Journal*, **6**, 1621–1624.

Cartes FC, Boretto JM, Acosta JC *et al.* (2010) Reproductive biology of *Phymaturus* cf. *palluma*: a vulnerable lizard from the highlands of the Andes, San Juan, Argentina. *Herpetological Conservation and Biology*, **5**, 430–440.

Castro SA (2013) *Comparación de la dieta de las especies sintópicas Liolaemus cf. ruibali y Phymaturus cf. palluma: variación intrapoblacional y estacional en los Andes central de San Juan, Argentina*, Universidad Nacional de San Juan.

Castro SA, Laspiur A, Acosta JC (2013) Variación anual e intrapoblacional de la dieta de *Phymaturus* cf. *palluma* (Iguania: Liolaemidae) de los Andes centrales en Argentina. *Revista Mexicana de Biodiversidad*, **84**, 1258–1265.

Clarke KR (1993) Non-parametric multivariate analysis of changes in community structure. *Australian Journal of Ecology*, **18**, 117–143.

Clements KD, Angert ER, Montgomery WL, Choat JH (2014) Intestinal microbiota in fishes: what's known and what's not. *Molecular Ecology*, **23**, 1891–1898.

Colston TJ, Jackson CR (2016) Microbiome evolution along divergent branches of the vertebrate tree of life: what is known and unknown. *Molecular Ecology*, **25**, 3776–3800.

Colston TJ, Noonan BP, Jackson CR (2015) Phylogenetic analysis of bacterial communities in different regions of the gastrointestinal tract of *Agkistrodon piscivorus*, the cottonmouth snake. *PLoS One*, **10**, e0128793.

Connolly JD, Cree A (2008) Risks of a late start to captive management for conservation: phenotypic differences between wild and captive individuals of a viviparous endangered skink (*Oligosoma otagense*). *Biological Conservation*, **141**, 1283–1292.

Costello EK, Gordon JI, Secor SM, Knight R (2010) Postprandial remodeling of the gut microbiota in Burmese pythons. *ISME Journal*, **4**, 1375–1385.

David LA, Maurice CF, Carmody RN *et al.* (2014) Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, **505**, 559–563.

Dill-McFarland KA, Neil KL, Zeng A *et al.* (2014) Hibernation alters the diversity and composition of mucosa-associated bacteria while enhancing antimicrobial defence in the gut of 13-lined ground squirrels. *Molecular Ecology*, **23**, 4658–4669.

Dominguez-Bello MG, Costello EK, Contreras M *et al.* (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences*, **107**, 11971–11975.

Dudley JP, Hang'Ombe BM, Leendertz FH *et al.* (2016) Carnivory in the common hippopotamus *Hippopotamus amphibius*: implications for the ecology and epidemiology of anthrax in African landscapes. *Mammal Review*, **46**, 191–203.

Espinoza RE, Wiens JJ, Tracy CR (2004) Recurrent evolution of herbivory in small, cold-climate lizards: breaking the ecophysiological rules of reptilian herbivory. *Proceedings of the National Academy of Sciences*, **101**, 16819–16824.

Ezenwa V, Gerardo NM, Inouye DW, Medina M, Xavier JB (2012) Animal behavior and the microbiome. *Science*, **338**, 198–199.

Faith DP (1992) Conservation evaluation and phylogenetic diversity. *Biological Conservation*, **61**, 1–10.

Funkhouser LJ, Bordenstein SR (2013) Mom knows best: the universality of maternal microbial transmission. *PLoS Biology*, **11**, e1001631.

Godon J-J, Arulazhagan P, Steyer J-P, Hamelin J (2016) Vertebrate bacterial gut diversity: size also matters. *BMC Ecology*, **16**, 12.

Hale VL, Tan CL, Niu K *et al.* (2016) Effects of field conditions on fecal microbiota. *Journal of Microbiol Methods*, **130**, 180–188.

Halloy M, Boretto JM, Ibargüengoytía N (2007) Signs of parental behavior in *Liolaemus elongatus* (Sauria: Liolaemidae) of Neuquén, Argentina. *South American Journal of Herpetology*, **2**, 141–147.

He Y, Caporaso JG, Jiang X-T *et al.* (2015) Stability of operational taxonomic units: an important but neglected property for analyzing microbial diversity. *Microbiome*, **3**, 20.

Hird SM, Sánchez C, Carstens BC, Brumfield RT (2015) Comparative gut microbiota of 59 Neotropical bird species. *Frontiers in Microbiology*, **6**, 1403.

Hong P-Y, Wheeler E, Cann IKO, Mackie RI (2011) Phylogenetic analysis of the fecal microbial community in herbivorous land and marine iguanas of the Galápagos Islands

using 16S rRNA-based pyrosequencing. *ISME Journal*, **5**, 1461–1470.

Hong P-Y, Mao Y, Ortiz-Kofoed S *et al.* (2015) Metagenomic-based study of the phylogenetic and functional gene diversity in Galápagos land and marine iguanas. *Microbial Ecology*, **69**, 444–456.

Hooper LV, Wong MH, Thelin A *et al.* (2001) Molecular analysis of commensal host-microbial relationships in the host. *Science*, **291**, 881–884.

Knights D, Kuczynski K, Charlson ES *et al.* (2011) Bayesian community-wide culture-independent microbial source tracking. *Nature Methods*, **8**, 761–763.

Kohl KD, Dearing MD (2014) Wild-caught rodents retain a majority of their natural gut microbiota upon entrance into captivity. *Environmental Microbiology Reports*, **6**, 191–195.

Kohl KD, Miller AW, Marvin JE, Mackie RI, Dearing MD (2014a) Herbivorous rodents (*Neotoma* spp.) harbour abundant and active foregut microbiota. *Environmental Microbiology*, **16**, 2869–2878.

Kohl KD, Skopec MM, Dearing MD (2014b) Captivity results in disparate loss of gut microbial diversity in closely related hosts. *Conservation Physiology*, **2**, cou009.

Kohl KD, Luong K, Dearing MD (2015) Validating the use of trap-collected feces for studying the gut microbiota of a small mammal (Neotoma lepida). *Journal of Mammalogy*, **96**, 90–93.

Kohl KD, Brun A, Magallanes M *et al.* (2016) Physiological and microbial adjustments to diet quality permit facultative herbivory in an omnivorous lizard. *Journal of Experimental Biology*, **219**, 1903–1912.

Kudo H, Cheng K-J, Costerton JW (1987) Interactions between *Treponema bryantii* and cellulolytic bacteria in the in vitro degradation of straw cellulose. *Canadian Journal of Microbiology*, **33**, 244–248.

Kumar PS, Mason MR, Brooker MR, O'Brien K (2012) Pyrosequencing reveals unique microbial signatures associated with healthy and failing dental implants. *Journal of Clinical Periodontology*, **39**, 425–433.

Lankau EW, Hong P-Y, Mackie RI (2012) Ecological drift and local exposures drive enteric bacterial community differences within species of Galápagos iguanas. *Molecular Ecology*, **21**, 1779–1788.

Ley RE, Hamady M, Lozupone C *et al.* (2008) Evolution of mammals and their gut microbes. *Science*, **320**, 1647–1651.

Lobo F, Laspiur A, Acosta JC (2013) Description of new Andean species of the genus *Phymaturus* (Iguania: Liolaemidae) from Northwestern Argentina. *Zootaxa*, **3683**, 117–132.

Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environment Microbiology*, **71**, 8228–8235.

Mackie RI (2002) Mutualistic fermentative digestion in the gastrointestinal tract: diversity and evolution. *Integrative and Comparative Biology*, **42**, 319–326.

Mackie RI, Aminov RI, Hu W *et al.* (2003) Ecology of uncultivated *Oscillospira* species in the rumen of cattle, sheep, and reindeer as assessed by microscopy and molecular approaches. *Applied and Environment Microbiology*, **69**, 6808–6815.

Martin MO, Gilman FR, Weiss SL (2010) Sex-specific asymmetry within the cloacal microbiota of the striped plateau lizard, *Sceloporus virgatus*. *Symbiosis*, **51**, 97–105.

Maurice CF, Knowles SC, Ladau J *et al.* (2015) Marked seasonal variation in the wild mouse gut microbiota. *ISME Journal*, **9**, 2423–2434.

McFall-Ngai M, Hadfield MG, Bosch TCG *et al.* (2013) Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences*, **110**, 3229–3236.

McKenney EA, Williamson L, Yoder AD *et al.* (2015) Alteration of the rat cecal microbiome during colonization with the helminth *Hymenolepis diminuta*. *Gut Microbes*, **6**, 182–193.

McMurdie PJ, Holmes S (2014) Waste not, want not: why rarefying microbiome data is inadmissible. *PLoS Biology*, **10**, e1003531.

Moeller AH, Li Y, Ngole EM *et al.* (2014) Rapid changes in the gut microbiome during human evolution. *Proceedings of the National Academy of Sciences*, **111**, 16431–16435.

Nelson TM, Rogers TL, Carlini AR, Brown MV (2013) Diet and phylogeny shape the gut microbiota of Antarctic seals: a comparison of wild and captive animals. *Environmental Microbiology*, **15**, 1132–1145.

Paster BJ, Canale-Parola E (1985) *Treponema saccharophilum* sp. nov., a large pectinolytic spirochete from the bovine rumen. *Applied and Environment Microbiology*, **50**, 212–219.

Pérez Mecado MG (2016) *Ecología trófica en dos especies de lagartos de la Puna (Liolaemus ruibali y Liolaemus parvus) en la Reserva de Usos Múltiples "Don Carmelo", Ullum, San Juan: Variación interespecífica, intrapoblacional y temporal*., Universidad Nacional de San Juan.

Pianka ER, Vitt LJ (2003) *Lizards: Windows to the Evolution of Diversity*. University of California Press, Los Angeles, CA, USA.

Piknova M, Guczynsa W, Miltko R *et al.* (2008) *Treponema zioleckii* sp. nov., a novel fructan-utilizing species of rumen treponemes. *FEMS Microbiology Letters*, **289**, 166–172.

Pope PB, Denman SE, Jones M *et al.* (2010) Adaptation to herbivory by the Tammar wallaby includes bacterial and glycoside hydrolase profiles different from other herbivores. *Proceedings of the National Academy of Sciences*, **107**, 14793–14798.

Pough FH (1973) Lizard energetics and diet. *Ecology*, **54**, 837–844.

Price SA, Hopkins SSB, Smith KK, Roth VL (2012) Tempo of trophic evolution and its impact on mammalian diversification. *Proceedings of the National Academy of Sciences*, **109**, 7008–7012.

Redford KH, Segre JA, Salafsky N, Martinez del Rio C, McAloose D (2012) Conservation and the microbiome. *Conservation Biology*, **26**, 195–197.

Ren T, Kahrl AF, Wu M, Cox RM (2016) Does adaptive radiation of a host lineage promote ecological diversity of its bacterial communities? A test using gut microbiota of Anolis lizards *Molecular Ecology*, **25**, 4793–4804.

Salter SJ, Cox MJ, Turek EM *et al.* (2014) Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology*, **12**, 87.

Schumacher J (2006) Selected infectious diseases of wild reptiles and amphibians. *Journal of Exotic Pet Medicine*, **15**, 18–24.

Scupham AJ, Patton TG, Bent E, Bayles DO (2008) Comparison of the cecal microbiota of domestic and wild turkeys. *Microbial Ecology*, **56**, 322–331.

Shchipkova AY, Nagaraja HN, Kumar PS (2010) Subgingival microbial profiles of smokers with periodontitis. *Journal of Dental Research*, **89**, 1247–1253.

Shine R (1988) Parental care in reptiles. In: *Biology of the Reptilia*(eds Gans C, Huey RB), pp. 275–379. John Wiley & Sons, New York, NY, USA.

Shropshire JD, Bordenstein SR (2016) Speciation by symbiosis: the microbiome and behavior. *mBio*, **7**, e01785-15.

Sinervo B, Méndez-de-la-Cruz F, Miles DB *et al.* (2010) Erosion of lizard diversity by climate change and altered thermal niches. *Science*, **328**, 894–899.

Stevens CE, Hume ID (2004) *Comparative Physiology of the Vertebrate Digestive System*, 2nd edn. Cambridge University Press, Cambridge, UK.

Sullam KE, Essinger SD, Lozupone CA *et al.* (2012) Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. *Molecular Ecology*, **21**, 3363–3378.

Trosvik P, Stenseth NC, Rudi K (2010) Convergent temporal dynamics of the human infant gut microbiota. *ISME Journal*, **4**, 151–158.

Troyer K (1982) Transfer of fermentative microbes between generations in a herbivorous lizard. *Science*, **216**, 540–542.

Troyer K (1984) Behavioral acquisition of the hindgut fermentation system by hatchling *Iguana iguana*. *Behavioral Ecology and Sociobiology*, **14**, 189–193.

Uenishi G, Fujita S, Ohashi G *et al.* (2007) Molecular analysis of the intestinal microbiota of chimpanzees in the wild and in captivity. *American Journal of Primatology*, **69**, 367–376.

Uetz P, Hošek J (2016) *The Reptile Database*.

Vences M, Lyra ML, Kueneman JG *et al.* (2016) Gut bacterial communities across tadpole ecomorphs in two diverse tropical anuran faunas. *Science of Nature*, **103**, 1–14.

Vicenzi N (2015) *Phymaturus palluma* (high mountain lizard) coprophagy. *Herpetological Review*, **46**, 93.

Villavicencio HJ, Acosta JC, Cánovas MG (2005) Dieta de *Liolaemus ruibali* Donoso Barros (Iguania: Liolaeminae) en la reserva de usos múltiples Don Carmelo, San Juan, Argentina. *Multequina*, **14**, 47–52.

Villers LM, Jang SS, Lent CL, Lewin-Koh S-C, Norosoarinalvo JA (2008) Survey and comparison of major intestinal flora in captive and wild ring-tailed lemur (*Lemur catta*) populations. *American Journal of Primatology*, **70**, 175–184.

Wienemann T, Schmitt-Wagner D, Meuser K *et al.* (2011) The bacterial microbiota in the ceca of capercaillie (*Tetrao urogallus*) differs between wild and captive birds. *Systematic and Applied Microbiology*, **34**, 542–551.

Wirth R, Bódi N, Maróti G *et al.* (2014) Regionally distinct alterations in the composition of the gut microbiota in rats with streptozotocin-induced diabetes. *PLoS One*, **9**, e110440.

Xenoulis PG, Gray PL, Brightsmith D *et al.* (2010) Molecular characterization of the cloacal microbiota of wild and captive parrots. *Veterinary Microbiology*, **146**, 320–325.

Yokoyama H, Wagner ID, Wiegel J (2010) *Caldicoprobacter oshimai* gen. nov., sp. nov., an anaerobic, xylanolytic, extremely thermophilic bacterium isolated from sheep faeces, and proposal of Caldicoprobacteraceae fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, **60**, 67–71.

Zimmerman LM, Vogel LA, Bowden RM (2010) Understanding the vertebrate immune system: insights from the reptilian perspective. *Journal of Experimental Biology*, **213**, 661–671.

K.D.K., A.B., M.M. and J.B. performed the experiment. A.L. and J.C.A. provided assistance with collecting lizards and samples in the wild. E.C.V. and S.R.B. oversaw the study and offered insight into data analysis and interpretation. K.D.K. wrote the manuscript, and all other authors provided comments and approved the final version.

Data accessibility

All 16S rRNA sequences have been deposited in the Sequence Read Archive (SRA) under Accessions nos PRJNA293117 and PRJNA312520.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1. Rarefaction curves of microbial diversity in various samples.

Fig. S2. Diversity indices across gut regions in three lizard species.

Fig. S3. Diversity indices between mothers and captive born offspring.

Appendix S1. Relative abundances of microbial taxa and statistics for differences across groups.