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AN INTEGRATIVE INVESTIGATION OF THE MECHANISMS SHAPING HOST-ASSOCIATED MICROBIAL COMMUNITY ASSEMBLAGE

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AN INTEGRATIVE INVESTIGATION OF THE MECHANISMS SHAPING HOST-ASSOCIATED MICROBIAL COMMUNITY ASSEMBLAGE

A DISSERTATION APPROVED FOR THE

SCHOOL OF BIOLOGICAL SCIENCES

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"*To my family*, *Brad*, *Tiffany*, *and Nick Smith*, *and my partner*, *Hunter Read*: *your constant love and support made this possible*. *Thank you eternally*."

Abstract

A critical goal of biologists is to describe the diversity of organisms on the planet and to illuminate the evolutionary processes that have shaped the global biodiversity we observe today. This is often accomplished by exploring diversity at a single scale such as the diversity of microorganisms within a soil sample or the number of plant species within an area of grassland. However, integrating across biological scales can generate a more comprehensive understanding of our planet's biodiversity. My research employs a holistic approach to investigate how differences at the organismal, community, and landscape levels drive variation at the microscopic level. This is made possible through the use of genomic approaches. Genomics is the study of an organism's genes (i.e., DNA) and, using genomic methods, I isolated the genetic material of microorganisms found within different areas of the focal vertebrate host organisms, specifically reptiles and amphibians. These communities of microorganisms are referred to as microbiomes and they serve fundamental roles in numerous processes that benefit their hosts, including digestion, metabolism, and immunity. Given the critical roles that microbiomes play in modulating host health and persistence, understanding the environmental and evolutionary processes that shape host-associated microbial communities remains critical as the planet's biodiversity continues to face threats posed by rapid environmental change, habitat modification, and emerging infectious diseases. Through three chapters, my dissertation research investigates if factors such as host evolutionary history, host ecology, pathogen presence, geographic location, and island size contribute to variation in vertebrate microbiome diversity, applying fundamental theories in ecology, evolutionary biology, and biogeography to further explore the mechanisms that shape host-associated microbiomes. To date, most vertebrate microbiome research has focused on humans and other mammals; however, increased availability and utilization of genomic techniques has expanded our knowledge of the diversity, structure, and functional capabilities of these microbial communities and has increased the taxonomic scope of microbiome studies to include more vertebrate taxa such as birds, fishes, amphibians, and reptiles. Despite these advances, large gaps remain in our understanding of the non-mammalian vertebrate microbiome, specifically among wild reptiles and amphibians.

Amphibians (anurans, salamanders, caecilians) and reptiles (lizards, snakes, crocodilians, turtles, tuataras) represent two of the five major clades of terrestrial vertebrates on the planet, and they display an astonishing diversity of life history traits. Their reproductive diversity and specialized body form adaptations have enabled them to diversify across a near complete spectrum of environments on the planet, including subterranean, ground-level, above-ground, and aquatic habitats. Amphibians and reptiles have been instrumental in addressing many questions in ecology and evolutionary biology such as phenotypic evolution, reproductive diversity, and adaptive radiation. Despite such a rich history of foundational research on these groups, we continue to have a limited understanding of the mechanisms that drive the assemblage of symbiotic microbial communities associated with amphibian and reptilian hosts. To address this shortfall, my dissertation research utilizes genomic methods to understand the environmental and evolutionary processes that shape amphibian and reptile microbiomes.

Chapter one investigates how host ecology and host species differences correlate with distinct oral and gut microbiomes among three venomous snake species from the Philippines. The focal species occupy three disparate habitat types: marine, semi-arboreal, and arboreal, and our results suggest that the diversity of snake mouth and gut microbial communities correlate with differences in both host ecology and phylogeny. Most research on snake mouth and gut

microbiota are limited to studies of a single species or captive-bred individuals; therefore, results from this comparative study contribute profoundly to our understanding of reptilian microbiomes, providing a foundation from which researchers can begin addressing broader questions concerning the evolution of host-microbe interactions. ***Chapter two*** combines existing frog disease data with skin microbiome data to elucidate the roles that geographic location, host evolutionary history, host ecology, and pathogen presence play in the microbial community assemblage of five co-distributed frog host species in Oklahoma. These focal species possess distinct ecological preferences: aquatic, semi-aquatic, and arboreal, and our results indicate that host ecology is the primary driver of frog skin microbial community structure. Results from this integrative study contribute to our growing understanding of the environmental and host-associated drivers of skin microbial community assemblage and represents one of the first studies on landscape-level variation in skin microbial communities among North American frogs. ***Chapter three*** provides the first assessment of variation in gut microbiomes among vertebrate species spanning true islands, applying theories of island biogeography to further investigate the mechanisms that shape the vertebrate gut microbiome. We sampled the microbial communities of ecologically diverse reptilian hosts from four islands in the Philippines to investigate the roles that host evolutionary history, host ecology, host microhabitat, geographic location, and island size play in reptilian gut microbiome assembly. Our results indicated that island size and host ecology were the primary contributors to gut microbial community differences observed among our focal samples. Results from this integrative study contribute to our growing understanding of the environmental, host-associated, and biogeographic processes that drive patterns of vertebrate gut microbiome diversity and assemblage among host communities inhabiting true islands.

My dissertation research integrates across numerous biological scales—microscopic, organismal, community, landscape—to understand how variation at any of these levels drives differences within another in order to address fundamental questions in evolutionary biology and ecology. Given that microbiomes are critical for promoting host health and persistence by aiding in digestion, nutrient acquisition, pathogen defense, and immune system function, investigations of the processes that shape host-associated microbiome community composition and assembly remain critical as the planet's biodiversity continues to face threats posed by rapid environmental change, habitat modification, and emerging infectious diseases.

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Chapter 1

Venomous snakes reveal ecological and phylogenetic factors influencing variation in gut and oral microbiomes

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1.1 Abstract

The gastrointestinal tract (GIT) of vertebrates contains a series of organs beginning with the mouth and ending with the anus or cloacal opening. Each organ represents a unique environment for resident microorganisms. Due to their simple digestive anatomy, snakes are good models for studying microbiome variation along the GIT. Cloacal sampling captures the majority of the microbial diversity found in the GIT of snakes—yet little is known about the oral microbiota of snakes. Most research on the snake mouth and gut microbiota are limited to studies of a single species or captive-bred individuals. It therefore remains unclear how a host's life history, diet, or evolutionary history correlate with differences in the microbial composition within the mouths and guts of wild snakes. We sampled the mouth and gut microbial communities from three species of Asian venomous snakes and utilized 16S rRNA microbial inventories to test if host phylogenetic and ecological differences correlate with distinct microbial compositions within the two body sites. These species occupy three disparate habitat types: marine, semi-arboreal, and arboreal, our results suggest that the diversity of snake mouth and gut microbial communities correlate with differences in both host ecology and phylogeny.

1.2 Introduction

The organs within the gastrointestinal tract (GIT) of vertebrates (mouth, stomach, colon, cloaca, etc.) harbor microbiomes that serve fundamental roles in a variety of processes that benefit their animal hosts, including digestion, immunity, and nutrient acquisition (Colston and Jackson, 2016; Varela et al., 2018; Arizza et al., 2019; Qin et al., 2019). As such, studies of microbiomes are essential to understanding host health, and can also be utilized to address interesting questions across broad fields in evolutionary biology, from processes of coevolution and adaptation to the evolution of antibiotic resistance (Hird, 2017; Ul-Hasan et al., 2019). To date, most vertebrate microbiome research has focused on humans and other mammals (Colston and Jackson, 2016; Hird, 2017; Qin et al., 2019); however, increased availability and utilization of culture-independent techniques, such as next-generation sequencing technologies and 16S ribosomal RNA (rRNA) microbial inventories, has expanded our knowledge of the diversity, structure, and potential functional capabilities of these symbiotic bacterial communities (Bletz et

al., 2017; Medina et al., 2017). Furthermore, such techniques have allowed for microbiome studies across an increasingly wide taxonomic diversity of vertebrate organisms, including birds (Hird et al., 2015; Waite and Taylor, 2015), fishes (Clements et al., 2014; Givens et al., 2015; Sullam et al., 2015; Gajardo et al., 2016), amphibians (Bletz et al., 2016, 2017; Bird et al., 2018; Varela et al., 2018; Rebollar and Harris, 2019), and reptiles (Hong et al., 2011; Keenan et al., 2013; Colston et al., 2015; Hyde et al., 2016; Ren et al., 2016; Allender et al., 2018; Arizza et al., 2019; Tang et al., 2019). Despite these advances, large gaps remain in our understanding of the non-mammalian vertebrate microbiome, especially among wild reptiles (Colston and Jackson, 2016; Hird, 2017; Krishnankutty et al., 2018; Qin et al., 2019; Tang et al., 2019).

Squamate reptiles (snakes, lizards, and amphisbaenids) possess the ability to persist in a wide variety of habitats, occurring on every continent but Antarctica (Vitt et al., 2003; Vitt and Caldwell, 2013). These reptiles display a vast diversity of life history traits, particularly in dietary habits and reproductive modes (Shine and Bonnet, 2000; Vitt and Caldwell, 2013). They have also played an important role in addressing many higher-level questions in ecology and evolutionary biology across numerous fields of study that include using venom to study evolutionary key innovations (Casewell et al., 2013; Sunagar et al., 2016; Calvete, 2017) and studies of phenotypic evolution (Wagner et al., 2018; Watanabe et al., 2019), reproductive mode (Blackburn, 2006; Pyron and Burbrink, 2014), and adaptive radiation (Losos and Miles, 2002). Despite such a rich history of foundational research on the group, microbiome evolution among squamate reptiles remains poorly understood. Previous work has determined that different reptilian body sites along the GIT possess distinct microbiomes (Keenan et al., 2013; Colston et al., 2015; Hyde et al., 2016; Tang et al., 2019); however, few studies to date have investigated the diversity and composition of the squamate reptile mouth microbiome (Shek et al., 2009; Goldstein et al., 2013; Hyde et al., 2016; Krishnankutty et al., 2018). Additionally, most studies of the reptilian gut (endogenous, cloacal) microbiomes are limited to comparisons between individuals of the same species (Keenan et al., 2013; Colston et al., 2015; McLaughlin et al., 2015; Hyde et al., 2016; Allender et al., 2018; Arizza et al., 2019; Tang et al., 2019), with few studies investigating how the gut microbiome differs between taxa (Hong et al., 2011; Ren et al., 2016; Qin et al., 2019; Zhang et al., 2019). By comparing the microbiomes of reptiles that have different habitat preferences, diets, and ecologies, we can begin to understand the relationship between evolutionary factors and the diversity and composition of host-associated microbial communities.

By far one of the most successful and charismatic radiations of squamates are snakes (Squamata: Serpentes), with more than 3,840 species recognized currently (Uetz et al., 2020) across all major biomes on the planet, including freshwater and marine environments, and even southern regions of the tundra (Vitt and Caldwell, 2013). Represented by this global vertebrate radiation is an incredible diversity of ecologies and life histories, including the evolution of a wide spectrum of dietary preferences and adaptations (Vitt et al., 2003; Colston et al., 2010; Vitt and Caldwell, 2013; Tang et al., 2019). Interestingly, such variation in diet preferences and the degree of specialization has evolved despite the presence of a rather simple digestive anatomy, and for this reason snakes are widely used as ideal systems for studying digestive physiology (Secor and Diamond, 1998; Castoe et al., 2013). Unfortunately, we continue to have a limited understanding of the composition, diversity, and functional capabilities of snake microbiomes, particularly gut and mouth microbial communities (Krishnankutty et al., 2018)—a critical component to understanding the important roles host-specific microbiomes may have played in snake adaptive evolution. Currently, the culture-independent snake gut microbiome literature

consists of a few studies that describe the diversity present at different segments of the snake gastrointestinal tract (excluding mouth; Hill et al., 2008; Colston et al., 2015; McLaughlin et al., 2015; Tang et al., 2019), one study investigating the composition of gut microbiota in captive pythons (again, excluding mouth; Costello et al., 2010), one study comparing small and large intestinal bacteria among three species of snakes (Qin et al., 2019), and one comparative study of bacterial communities sequenced from fecal samples from four species of farmed snakes in China (Zhang et al., 2019). Such a general paucity of data on snake host-associated microbiomes extends to the burgeoning studies of the venom-microbiome, which aims to describe the presence and diversity of venom-associated microbial communities to address questions of how microorganisms colonize and inhabit venom glands (McFall-Ngai, 2014; Ul-Hasan et al., 2019). For example, the oral cavity of snakes can harbor an extensive diversity of bacteria, including potentially pathogenic groups that may cause post-bite infection (Shek et al., 2009; Lam et al., 2011; Krishnankutty et al., 2018). Such host-microbe interactions that occur in the venom microenvironment and the oral cavity of snakes also remain largely understudied (Ul-Hasan et al., 2019). Until more comparative and baseline data on host-specific microbiomes among snakes becomes available, our understanding of how host ecology, venom system dynamics, and evolutionary history correlate with, and are impacted by, these microbial communities remains incomplete.

In this study, we present novel comparisons of host microbiome diversity and composition among three venomous snake species from the Philippines in Southeast Asia, each of which possesses a distinct ecology: (1) the blue-lipped sea krait (Family Elapidae: *Laticauda laticaudata*) is a neurotoxic, marine species (Leviton et al., 2014); (2) the Philippine pit viper (Family Viperidae: *Trimeresurus flavomaculatus*) is a hemotoxic, strictly arboreal, terrestrial species (Sugihara et al., 1983; Nikai et al., 1985; Debono et al., 2019); and (3) the mangrove snake (Family Colubridae: *Boiga dendrophila*) is a semi-arboreal, terrestrial species that possesses a bird-specific toxin in its venom referred to as denmotoxin (Pawlak et al., 2006; Pawlak and Kini, 2008). These key differences, and the general availability of these three species in the field, made them ideal for investigating the influence and interaction of host ecology and evolutionary history (phylogenetic relatedness) on gut and mouth microbiome structure and diversity. *Laticauda laticaudata* is an amphibious marine snake in the family Elapidae that is widely distributed across Southeast Asia and comes to shore only to rest and lay eggs (Leviton et al., 2014). This species can be observed hiding in crevices on coral reefs and they feed primarily on eels (Dabruzzi et al., 2012). The two terrestrial species, *T. flavomaculatus* and *B. dendrophila*, are common snakes found throughout large regions of the Philippines (Siler et al., 2011; Brown et al., 2013). *Trimeresurus flavomaculatus* is an ovoviviparous pit viper in the family Viperidae that prefers more arboreal microhabitats, often preying on frogs and small mammals (Leviton, 1962; Brown et al., 2013; Leviton et al., 2014). In comparison, *Boiga dendrophila* is an oviparous mangrove snake in the family Colubridae that prefers more shrub- and ground-level microhabitats (i.e., semi-arboreal) and feeds primarily on birds (Brown et al., 2013).

Herein, we provide an assessment and summary of microbial diversity and composition of the mouth and gut microbiomes among these three species of wild venomous snakes to identify patterns associated with host ecology (e.g., habitat preferences and specializations, etc.) and host species differences. The results contribute to a nascent body of literature on wild reptile endogenous microbiomes and specifically expands our knowledge on snake mouth and gut microbiomes, with which researchers can begin to address broader questions in evolutionary biology and digestive physiology as they apply to host-microbe interactions.

1.3 Materials and Methods

Sample collection

Fieldwork was conducted from May 27 to June 4, 2018 on two islands of the Babuyan Island Group—Calayan and Camiguin Norte. Cloacal swabs are an effective proxy for sampling gut microbial diversity in snakes (Colston et al., 2015), therefore we used cloacal swabs to sample gut microbial diversity in this study. Gut microbiome samples were collected by inserting a sterile swab (MWE, Corsham, United Kingdom) into the cloaca of the individual for approximately 3–4 s, twirling the swab 2– 4 times. We collected mouth microbiome samples by swabbing the tongue, teeth, and roof of the snake's mouth 4–5 times with sterile swabs (Puritan Medical Products, Guilford, ME, United States). We swabbed 22 adult individuals representing the three focal species: *Laticauda laticaudata* (*N* = 7 [four males, three individuals not sexed], SVL 680–855 mm), *Trimeresurus flavomaculatus* (*N* = 7 [four females, three individuals not sexed], SVL 308–900 mm), and *Boiga dendrophila* (*N* = 8 [four males, four females], SVL 814–1,395 mm) (Appendix A, Table S1). All swabs were preserved and stored in DNA/RNA Shield (Zymo Research Products, Irvine, CA, United States) at the time of collection at ambient temperature in field conditions until returned to the United States (10–14 days) where they were stored in a –20°C freezer until DNA extraction. All samples were collected in strict accordance with the regulations established by the University of Oklahoma's Institutional Animal Care and Use Committee (IACUC Permit Nos: R17–019). Field collection and export permits were provided by the Biodiversity Management Bureau (BMB) of the Philippine Department of Environment and Natural Resources (DENR) Nos. 260 (Renewal) and 273 (Renewal).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from all 44 swabs using XpeditionTM Soil/Fecal DNA MiniPrep kits (Zymo Research Products). DNA concentration for each extracted sample was determined using a QuantusTM Fluorometer (Promega, Madison, WI, United States). Ten sterile swabs were extracted alongside the 44 samples to be used as negative controls and three of the 10 negative controls were amplified and sequenced using the following methods. Using a one-step Polymerase Chain Reaction (PCR) method, we amplified the V4 hypervariable region of the 16S rRNA gene using primers described in Kozich et al. (2013). Two microliters of PCR product from each sample was visualized using gel electrophoresis and the remaining 18 µL was cleaned with KAPA Pure Beads (Roche Sequencing Solutions, Pleasanton, CA, United States). After quantification, all samples were normalized to 10 nM of DNA before pooling samples into a sterile, 1.5 mL microcentrifuge tube. If the DNA quantity of a sample was above 10 nM, 5 µL of the sample was added to a calculated amount of sterile, laboratory grade water to dilute the sample to 10 nM. After dilution, 4 µL of the diluted PCR product was added to the final pool. In contrast, if the DNA quantity of a sample was below 10 nM, no water was added to the sample and 2 µL of the PCR product was added to the final pool. Sequencing was performed at the University of Oklahoma Consolidated Core Lab using the 2 × 250 bp paired-end sequencing on a single run of an Illumina MiSeq.

Sequence analysis

Adapter sequences were trimmed from the paired-end assembled raw sequencing reads using AdapterRemoval v2 (Schubert et al., 2016). Sequence data was analyzed using the QIIME 2 software package (Bolyen et al., 2019), and the sequences were clustered into operational taxonomic units (OTUs) with a closed-reference OTU database at 97% sequencing similarity

using VSEARCH (Rognes et al., 2016) against the Silva 132 database (Quast et al., 2012). A total of 9,190 OTUs were found among the 737,795 sequences obtained. The OTU table was rarified to a sequence count of 500 for downstream analyses, which removed 10 samples from the dataset ($N = 5$ each for both mouth and gut samples; Appendix A, Table S1 and Figure S1). Our decision to rarefy samples to a sequence count of 500 was based on our specific dataset and the associated rarefaction curves (Appendix A, Figure S1; Hughes and Hellmann, 2005; Aguirre de Cárcer et al., 2011; Weiss et al., 2017). As a result, the 10 removed samples were excluded from analysis due to low sequence counts and low post-amplification DNA quantities compared to the other samples that amplified well above a sequence count of 500. Raw sequence data generated in this study can be found in the Sequence Read Archive (SRA) under accession no. PRJNA702542. Analysis workflow can be found on Github (https://github.com/sierra-smith/16S_Microbiome_pipeline.git).

Statistical analysis

To evaluate the effect of host ecology and phylogenetic relatedness on gut and mouth diversity, alpha diversity (within sample) and beta diversity (among samples) analyses were performed using QIIME 2. Alpha and beta diversity analyses were performed on both body sites separately and a value of $p < 0.05$ was considered a statistically significant difference. Alpha diversity analyses (Shannon Diversity, Faith's Phylogenetic Diversity, and Observed OTUs) were performed using QIIME 2 (Bolyen et al., 2019). To test whether the gut microbiomes of these three snake species differed based on host ecology (marine, semi-arboreal, arboreal), we analyzed beta diversity of the gut samples using the phylogeny-based distance matrices Unweighted- and Weighted-Unifrac (Lozupone et al., 2007, 2011) in addition to the Bray-Curtis dissimilarity matrix (Beals, 1984). We performed these three analyses on mouth microbiome samples to test if the microbiomes differed among the three species. Additionally, we separated samples based on the host's ecology (arboreal, semi-arboreal, marine) and performed the same three analyses on the separated datasets to test for differences in microbial diversity between the two body sites (mouth vs. gut). All beta diversity analyses were visualized by principal coordinate analysis (PCoA) using Qiime 2R (Bisanz, 2018) and Tidyverse (Wickham, 2017) packages in R version 3.6.1 (R Core Team, 2019). Diversity comparisons were conducted using the alpha-group-significance and beta-group-significance plugins in QIIME 2 which performs Pairwise Kruskal-Wallis and PERMANOVAs, respectively, to test group significance (Anderson, 2001).

1.4 Results

Our initial microbiome dataset consisted of 22 mouth and 22 gut samples taken from three different venomous snake species (*L. laticaudata*, *T. flavomaculatus*, and *B. dendrophila*) which occupy three distinct habitat types—14 marine samples ($N = 7$ mouth, $N = 7$ gut), 16 semi-arboreal samples ($N = 8$ mouth, $N = 8$ gut), and 14 arboreal samples ($N = 7$ mouth, $N = 7$ gut; Appendix A, Table S1). In total, 737,795 sequences were obtained from these 44 samples with a total of 9,190 unique OTUs classified using a 97% sequence similarity threshold against the Silva database (v.132, Quast et al., 2012). After we rarefied the 44 samples to a sequencing depth of 500 for all downstream statistical analyses, 10 out of the 44 samples were removed (mainly *B. dendrophila* samples; Appendix A, Table S1). The 34 remaining samples used in subsequent analyses ($N = 17$ mouth, $N = 17$ gut samples; $N = 13$ marine, $N = 7$ semi-arboreal, $N = 14$

arboreal) consisted of 734,366 sequences with 9,033 unique OTUs classified based on 97% similarity using the Silva database (Appendix A, Table S1; Quast et al., 2012).

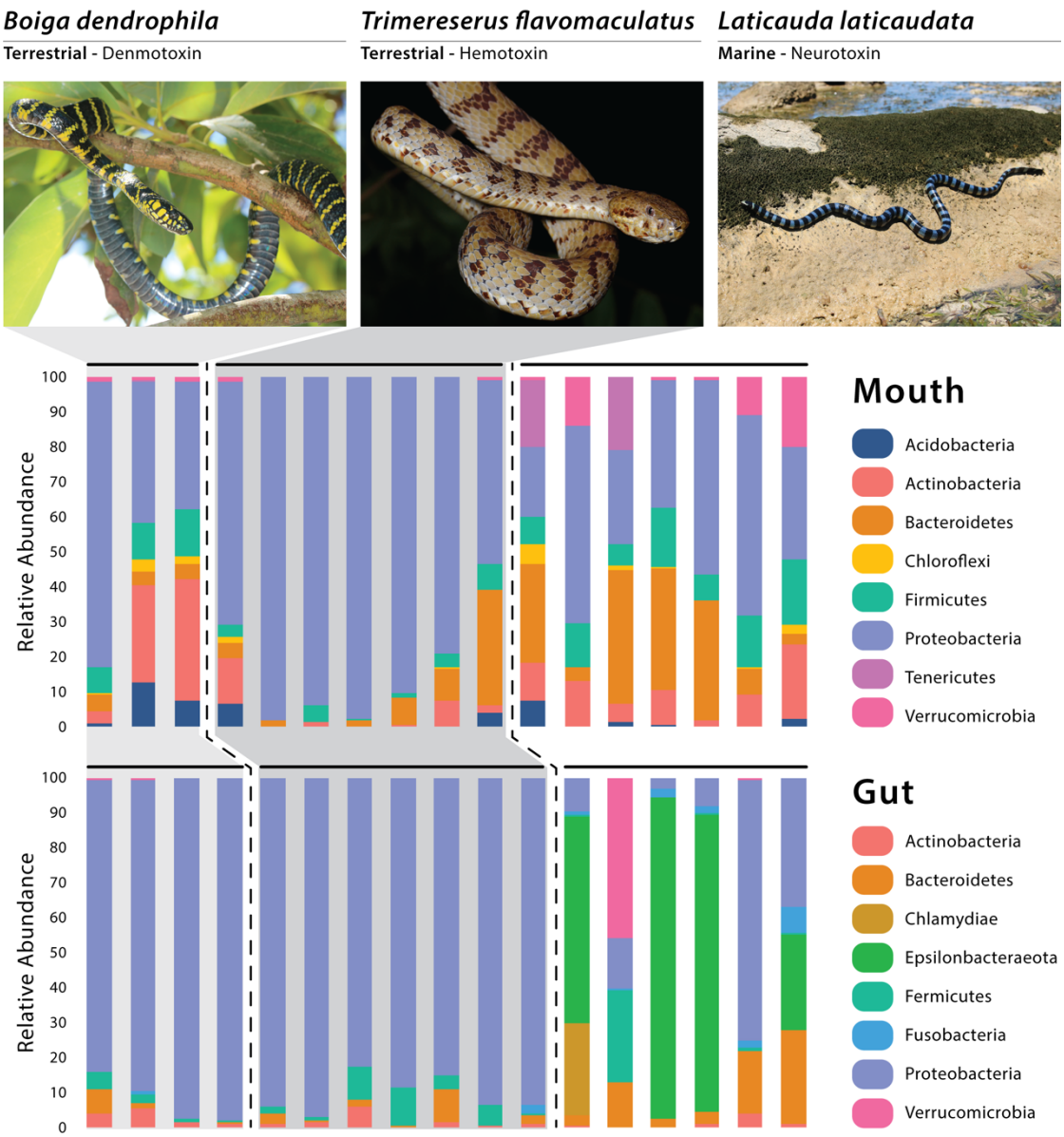

Figure 1.1 | Relative abundance of the dominant bacterial phyla recovered through 16S rRNA amplicon sequencing, with each vertical bar representing an individual swab. Photos of *Boiga* and *Laticauda* by Joseph Brown; Photo of *Trimereserus* by Kai Wang.

Taxonomic composition of GIT microbial communities across host species and ecologies

A total of four dominant phyla (average relative abundance >1.0%; Suenami et al., 2019) were observed among all gut samples—Proteobacteria (64.87%), Bacteroidetes (5.73%), Firmicutes (4.14%), and Actinobacteria (1.87%; Figure 1.1). Four bacterial phyla had dominant abundances

(avg >1.0%) in the marine gut samples only—Epsilonbacteraeota (43.56%), Verrucomicrobia (7.46%), Chlamydiae (4.40%), and Fusobacteria (2.56%; Figure 1.1). Among arboreal (*T. flavomaculatus*) gut samples only, we found that the most dominant phylum was Proteobacteria (90.15%; Figure 1.1). Additionally, all seven gut samples collected from this species contained Firmicutes (4.83%), Bacteroidetes (2.47%), and Actinobacteria (1.81%; Figure 1.1). All other phyla had an average relative abundance below 1%. The same dominant phyla were found among gut samples collected from the semi-arboreal species (*B. dendrophila*). Proteobacteria was also the most dominant phylum within gut samples collected from *B. dendrophila* (92.03%; Figure 1.1). Additionally, all gut samples collected from this species contained Actinobacteria (3.05%), Bacteroidetes (2.32%), and Firmicutes (2.12%; Figure 1.1). All other phyla had an average relative abundance below 1%. All six gut samples from the marine species (*L. laticaudata*) contained Proteobacteria (24.07%), Bacteroidetes (10.94%), Firmicutes (4.61%), Fusobacteria (2.56%), and Actinobacteria (1.25%; Figure 1.1). Four out of the six gut samples from *L. laticaudata* had high abundances of Epsilonbacteraeota (average for four samples = 65.34%, total average = 43.56%) and two phyla had a high abundance in only one marine gut sample each: Verrucomicrobia (average for one sample = 44.07%) and Chlamydiae (average for one sample = 26.38%; Figure 1.1).

When evaluating mouth microbiomes only, five dominant phyla were present in samples collected from the three species in varying abundances: Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, and Acidobacteria (Appendix A, Table S2). Two phyla had especially high relative abundances in two of the seven *L. laticauda*ta mouth samples only: Patescibacteria (average for the two samples = 5.06%, total average = 1.59%) and Tenericutes (average for the two samples = 18.5%, total average = 5.33%; Figure 1.1). Planctomycetes was a dominant phylum in the *B. dendrophila* mouth samples only (1.61%; Figure 1.1). Whereas Verrucomicrobia (*L. laticaudata*: 6.60% and *B. dendrophila*: 1.19%) and Chloroflexi (*L. laticaudata*: 1.45% and *B. dendrophila*: 1.97%) were dominant phyla among *L. laticaudata* and *B. dendrophila* mouth samples but were absent from the *T. flavomaculatus* samples (Figure 1.1).

Alpha and beta diversity analyses across host ecologies, species, and body site

Significant differences were observed among species-specific mouth and gut microbiomes for various alpha diversity metrics evaluated. For mouth microbiome sample comparisons, Shannon Diversity analyses showed significant differences among all three species: *B. dendrophila* vs. *T. flavomaculatus* ($H = 5.73$; p-value = 0.02); *B. dendrophila* vs. *L. laticaudata* ($H = 4.69$; p-value = 0.03); *L. laticaudata* vs. *T. flavomaculatus* ($H = 3.92$; p-value = 0.05; Figure 1.2). Additionally, Faith's Phylogenetic diversity analyses supported a significant difference between the mouth microbiome of *T. flavomaculatus* and both *L. laticaudata* ($H = 5.59$; p-value = 0.02) and *B. dendrophila* ($H = 3.75$; p-value = 0.05), while analysis of observed OTUs showed significant differences between *B. dendrophila* and *T. flavomaculatus* ($H = 3.75$; p-value = 0.05; Figure 1.2). In comparison, analyses of gut samples supported a significant difference between *L. laticaudata* and *T. flavomaculatus* ($H = 7.43$; p-value = 0.006) in observed OTUs, and between *B. dendrophila* and *L. laticaudata* ($H = 4.55$; p-value = 0.03) for Faith's Phylogenetic Diversity analyses (Figure 1.2). We used Unweighted- and Weighted-Unifrac distance matrices in addition to the Bray-Curtis dissimilarity matrix to analyze beta diversity among mouth and gut microbiome samples. Unweighted-Unifrac analysis resulted in a significant difference when comparing *T. flavomaculatus* mouth samples to those collected from *L. laticaudata* (pseudo-F = 2.13; p-value = 0.011), but no significant difference was found between *B. dendrophila* and *T. flavomaculatus* (pseudo-F = 1.76; p-value = 0.062) and *B. dendrophila* and *L. laticaudata* mouth

samples (pseudo-F = 1.33; p-value = 0.131; Appendix A, Table S3). Additionally, this analysis found significant differences among gut samples from the semi-arboreal and marine species (pseudo-F = 3.68; p-value = 0.004), the semi-arboreal and arboreal species (pseudo-F = 2.09; p-value = 0.004), and the arboreal and marine species (pseudo-F = 3.08; p-value = 0.001; Appendix A, Table S3). When analyzing the habitat types separately, we found significant differences between the body sites of the marine (pseudo-F = 3.39; p-value = 0.002) and semi-arboreal species (pseudo-F = 2.89; p-value = 0.032), but no significant difference was found between the mouth and gut samples collected from the arboreal species (pseudo-F = 1.39; p-value = 0.111; Appendix A, Table S3).

Weighted-Unifrac beta diversity analysis of mouth samples revealed significant differences between all three species (*B. dendrophila* vs. *L. laticaudata*: pseudo-F = 2.47; p-value = 0.048; *B. dendrophila* vs. *T. flavomaculatus*: pseudo-F = 3.92; p-value = 0.028; *L. laticaudata* vs. *T. flavomaculatus*: pseudo-F = 6.60; p-value = 0.002; Appendix A, Table S3). We found significant differences among gut samples collected from the semi-arboreal and marine species (pseudo-F = 3.83; p-value = 0.039) and the arboreal and marine species (pseudo-F = 6.14; p-value = 0.003), but not among the semi-arboreal and arboreal species (pseudo-F = 1.11; p-value = 0.352; Appendix A, Table S3). Among marine samples only, microbiome compositions at the two GIT segments (mouth vs. gut) were significantly different (pseudo-F = 4.41; p-value = 0.01), but not among semi-arboreal (pseudo-F = 3.27; p-value = 0.122) or arboreal samples (pseudo-F = 0.694; p-value = 0.765; Appendix A, Table S3).

Analysis of beta diversity using Bray-Curtis dissimilarity matrix yielded significant differences between *B. dendrophila* and *L. laticaudata* (pseudo-F = 2.18; p-value = 0.02), *B. dendrophila* and *T. flavomaculatus* (pseudo-F = 1.78; p-value = 0.034), and *L. laticaudata* and *T. flavomaculatus* (pseudo-F = 3.71; p-value = 0.003; Appendix A, Table S3) mouth samples. Additionally, significant differences were found among gut samples collected from the semi-arboreal and marine species (pseudo-F = 5.40; p-value = 0.003) and the arboreal and marine species (pseudo-F = 4.88; p-value = 0.001), but not among the semi-arboreal and arboreal species (pseudo-F = 1.16; p-value = 0.295; Appendix A, Table S3). Samples collected from the two body sites (mouth vs. gut) were significantly different among marine samples only (pseudo-F = 3.67; p-value = 0.001), but no significant difference between body sites was found among semi-arboreal (pseudo-F = 2.35; p-value = 0.169) or arboreal samples (pseudo-F = 0.857; p-value = 1.0; Appendix A, Table S3).

1.5 Discussion

In this study, we collected samples from 22 snakes representing three venomous species from the Philippines to investigate whether host ecology and species differences were correlated with potential differences in microbial community structure and diversity within the gut and mouth of the host organisms. The results of this study broaden our understanding of the compositional variation of venomous snake microbiomes at different GIT regions (mouth vs. gut). We found significant differences between the bacterial communities of these GIT body sites in the arboreal, semi-arboreal, and marine species, with these differences being more pronounced in the marine species across all beta diversity analyses (Appendix A, Table S3). Additionally, when

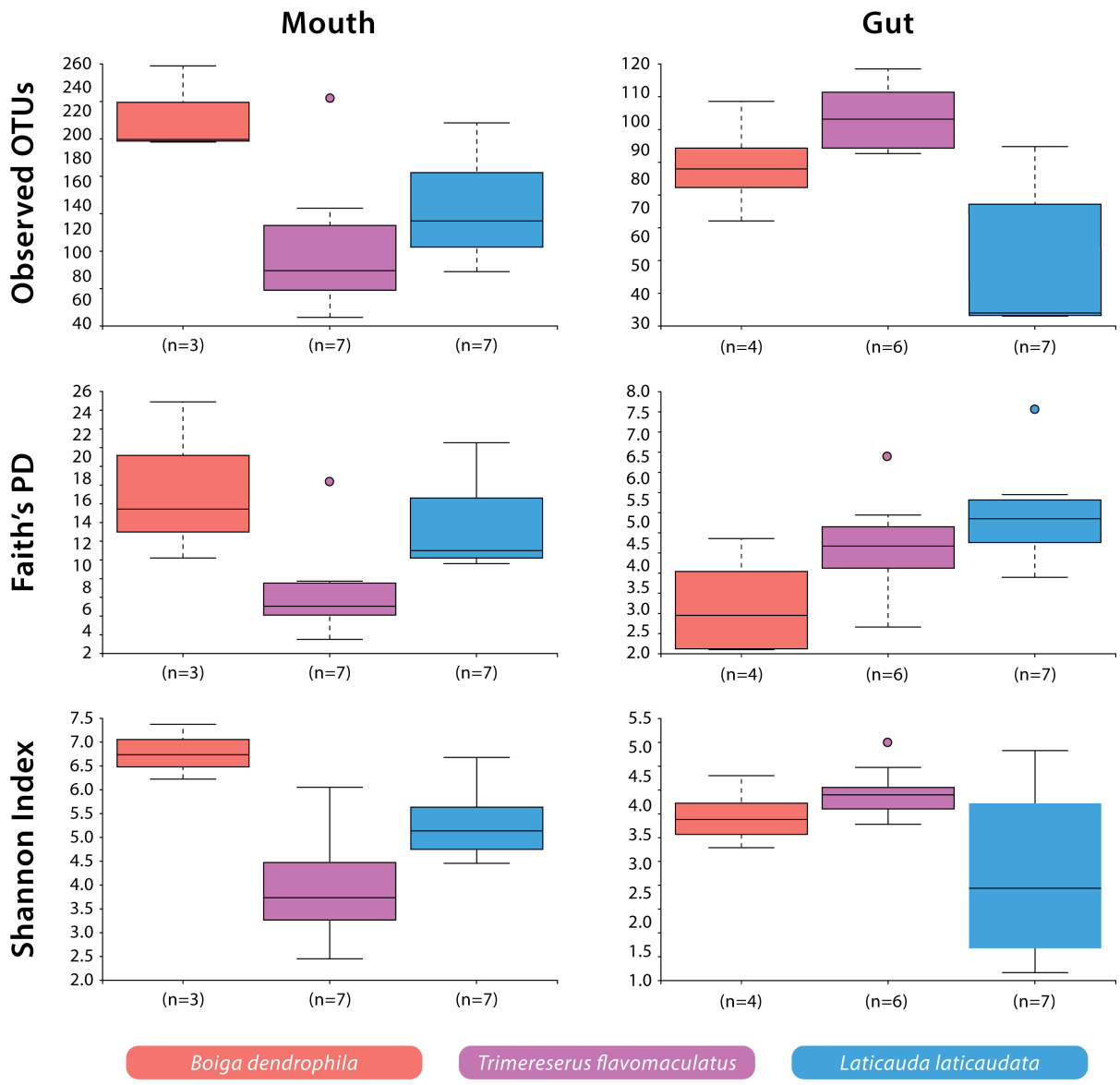

Figure 1.2 | Alpha diversity (Observed OTUs, Faith's Phylogenetic Diversity, and Shannon Diversity) of bacterial OTUs by host species and body site.

analyzing both GIT body sites separately, we found mouth microbiome composition and diversity differed between the three host species, revealing that each of the focal species harbored unique mouth microbiomes (Figure 1.3; Appendix A, Figure S2). Host microhabitat preference (arboreal, semi-arboreal, and marine) was correlated with distinct gut microbial compositions among the three species (Figure 1.3; Appendix A, Figure S2). The results of this work add to our understanding of regionalized microbiome diversity along the host GIT and establish a foundation for future research to explore the relationship between host-specific microbiomes and snake adaptive evolution.

Microbial community composition across host species and ecologies

The host species investigated in this study are recognized as members of three distinct snake families (Colubridae: *B. dendrophila*; Elapidae: *L. laticaudata*; Viperidae: *T. flavomaculatus*), providing a unique opportunity to explore interspecific structural differences in the mouth microbiomes of three distantly related venomous snakes (Figure 1.3; Appendix A, Figure S2). More exciting is that the three species collectively represent two ends of an important ecological spectrum among vertebrates on the planet—marine vs. terrestrial species—with the two terrestrial species occupying distinct microhabitats (arboreal and semi-arboreal). Furthermore, the host species possess distinct dietary preferences that match their broad ecological grouping (marine vs. terrestrial), with the marine species, *Laticauda laticaudata*, recognized as an eel specialist (Tabata et al., 2017), and the two terrestrial snakes, *Trimeresurus flavomaculatus* and *Boiga dendrophila*, are dietary generalists (Creer et al., 2002; Davies and Arbuckle, 2019). Although the current datasets cannot determine whether differences in gut and mouth microbiome composition are due to broader ecological (marine vs. terrestrial), dietary (specialist vs. generalist), taxonomic (phylogenetic relatedness), or other yet unidentified factors, we observed significant host-specific compositional differences in bacterial communities (Figure 1.3; Appendix A, Figure S2). Additionally, our results reveal important connections to findings in several recent microbiome studies in other vertebrate systems.

Interestingly, gut microbial communities in marine *L. laticaudata* share compositional similarities to microbiomes of loggerhead sea turtles (*Caretta caretta*; Arizza et al., 2019) and American alligators (*Alligator mississippiensis*; Keenan et al., 2013). First, the phylum Epsilonbacteraeota observed recently in gut microbiome samples of loggerhead sea turtles was found in the *L. laticaudata* gut samples only (Figure 1.1). To date, Epsilonbacteraeota has not been observed in any other terrestrial reptile gut microbiome study (Keenan et al., 2013; Colston et al., 2015; Hyde et al., 2016; Allender et al., 2018; Tang et al., 2019; Zhang et al., 2019). Second, Fusobacteria has been observed as a dominant phylum in the gut microbiomes of American alligators (Keenan et al., 2013) and was observed in *L. laticaudata* gut samples only (Figure 1.1). Fusobacteria are known to play a role in plaque formation within the oral cavity of mammals (Mira et al., 2004). However, it has been proposed that the phylum may be assisting with digestive organ development and nutrient acquisition in the primarily aquatic American alligators (Keenan et al., 2013).

In comparison, two phyla were observed in mouth samples of *L. laticaudata* only: Patescibacteria and Tenericutes (Figure 1.1). We have not encountered a study that includes Patescibacteria as a dominant phylum in the reptilian mouth or gut microbiomes, but Tenericutes has been observed in the upper GIT (stomach and esophagus) of the red-necked keelback, *Rhabdophis subminiatus*, a semi-aquatic snake (Tang et al., 2019), the stomach of the giant African snail (*Achatina fulica*; Pawar et al., 2012), the oral and fecal microbiota of a passerine bird (*Parus major*; Kropáčková et al., 2017), and in the gut microbiomes of numerous species of fish (Sullam et al., 2015; Llewellyn et al., 2016). Tenericutes are also dominant members of the coral microbiome (Kellogg et al., 2009; Gray et al., 2011), which adds support for the phylum's connection to aquatic, and particularly marine, organisms (Colston and Jackson, 2016).

In addition to the observed similarities in microbial community composition among aquatic reptiles, both mouth and gut samples of the terrestrial snakes (*T. flavomaculatus*, *B. dendrophila*) showed interesting compositional patterns, with both species dominated by Proteobacteria (Figure 1.1), a phylum observed at much lower relative abundance in most *L. laticaudata* marine samples, particularly gut samples (Figure 1.1). Additionally, one dominant phylum,

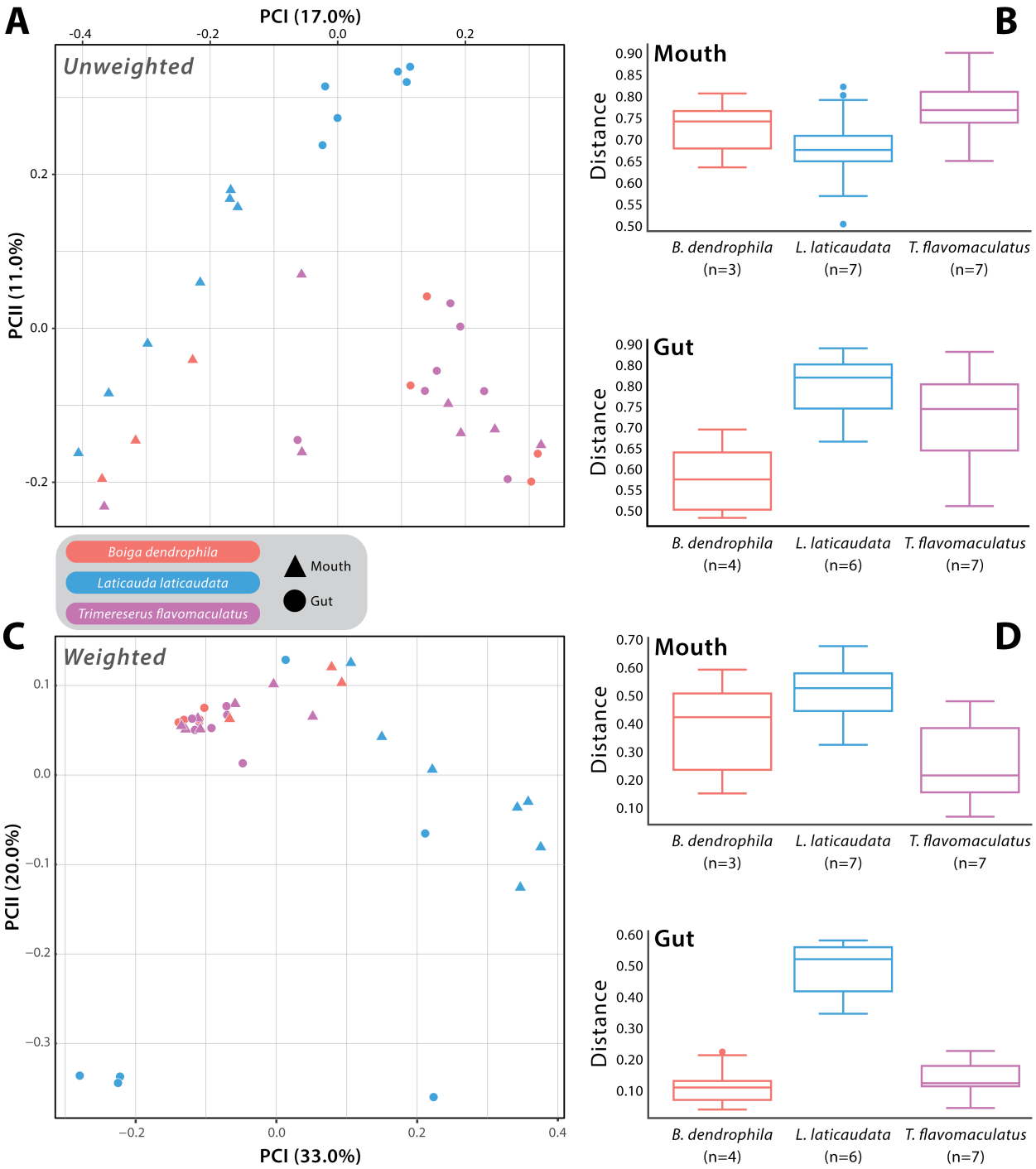

Figure 1.3 | Beta diversity comparisons based on Unweighted- and Weighted-Unifrac distances. **(A)** Principal Coordinates Analysis (PCoA) of all samples with point shape indicating different body sites: triangle = mouth sample and circle = gut sample, and point color indicating the different host species: red = *Boiga dendrophila*, blue = *Laticauda laticaudata*, and purple = *Trimeresurus flavomaculatus*. **(B)** Boxplots generated by the PERMANOVA comparing mouth and gut microbiome samples from each host species. **(C)** PCoA of all samples based on Weighted-Unifrac distances. **(D)** The associated mouth and gut boxplots generated by the PERMANOVA. Each point represents a single swab.

Planctomycetes, was observed exclusively in the mouth samples of *B. dendrophila* (Figure 1.1) but has also been observed previously in small relative abundances associated with the GIT of red-necked keelback snakes (*Rhabdophis subminiatus*; Tang et al., 2019), Galápagos land iguanas (*Conolophus subscristatus*; Hong et al., 2011), and Santa Fe land iguanas (*C. pallidus*; Hong et al., 2011). Together these findings reveal new insights into bacterial phyla that may be associated with reptiles that occupy marine vs. terrestrial habitats differentially, presenting exciting directions for future research. Finally, with the three focal snakes each possessing distinct toxins in their venom (neurotoxin, hemotoxin, and denmotoxin; Appendix A, Table S1), we were interested in how our findings might relate to microbiome work in other venomous taxa. Interestingly, members of the phylum Chloroflexi were present only in the *L. laticaudata* and *B. dendrophila* mouth samples and have been found in the oral cavity of several other snake species, including the Indian cobra and king cobra, which like *L. laticaudata*, are both members of the snake family Elapidae (Krishnankutty et al., 2018). Given the growing interest in venomics and improving our understanding of venom evolution and antimicrobial resistance, it is critical that baseline datasets be established for the identification and understanding of host-specific microbiome composition present in venom microenvironments (Conlin et al., 2014; Adnani et al., 2017; Esmaeilishirazifard et al., 2018; Krishnankutty et al., 2018). Our findings add to this small but growing body of literature with the hope that future studies will be capable of testing for evidence of coevolutionary processes between mouth and venom gland microbiomes and the evolution of diverse venom systems in snakes.

Comparisons to the culture-dependent snake microbiome literature

Although we utilized high-throughput sequencing methods for our study, similarities can be found with several wild snake microbiome studies previously conducted using culture-dependent techniques (Shek et al., 2009; Lam et al., 2011; Dehghani et al., 2016; Lukač et al., 2017). Lukač et al. (2017) sampled the oral cavity and cloaca of wild four-lined snakes (*Elaphe quatuorlineata*) and reported several genera of pathogenic bacteria that were also observed in mouth and/or gut samples in this study. First, *T. flavomaculatus* gut samples revealed prominent relative abundances of *Bacillus*, *Escherichia coli*/*Shigella* spp., *Pseudomonas*, *Serratia*, and *Stenotrophomonas*. Additionally, among mouth samples analyzed in our study, the presence and relative abundances of two potentially pathogenic bacterial genera stood out: (1) *Escherichia coli*/*Shigella* spp. were found in high relative abundances among mouth samples from all three host species we surveyed (average relative abundances = *B. dendrophila*: 10.46%; *L. laticaudata*: 2.81%; *T. flavomaculatus*: 25.73%); (2) One *B. dendrophila* mouth sample had a high relative abundance of *Aeromonas* (56.94%), which is a bacterium known to cause snakebite wound infections (Lam et al., 2011).

Two other culture-dependent studies sampled the oral cavity of snakes collected from the same location in Hong Kong (Shek et al., 2009; Lam et al., 2011), also belonging to the same families as the snakes sampled here (Colubridae, Elapidae, and Viperidae; Lam et al., 2011). Snakes sampled by Shek et al. (2009) and Lam et al. (2011) had *Providencia rettgeri* within their oral cavities, but the genus was absent from snakes sampled by Lukač et al. (2017). Comparatively, the bacterial genus *Providencia* was observed in high relative abundances (i.e., average relative abundance 79.69% in *T. flavomaculatus*) among mouth samples from each focal host species in our study. Finally, two species of the bacterial genus *Chryseobacterium* have been observed in the oral cavities of the Chinese cobra (*Naja atra*) and bamboo pit viper (*Trimeresurus albolabris*; Shek et al., 2009), and the genus was also found among *T. flavomaculatus* mouth samples from our study (average relative abundance = 6.15%). The

presence of *Chryseobacterium* and other potentially pathogenic bacteria observed in our study may indicate that certain host individuals of the three focal snakes may harbor disease-causing bacteria within their mouths and guts. This is particularly true for *T. flavomaculatus*, which tended to have higher relative abundances for bacterial genera shown to be potentially pathogenic within culture-dependent studies conducted to date (Shek et al., 2009; Lam et al., 2011; Lukač et al., 2017). The implications of this extend to wildlife trade, where unmanaged and/or illegal trade of wildlife can have negative impacts on human health, including zoonotic disease transmission, at times through the consumption of wild meats (i.e., Covid-19 and HIV-1; Roe, 2008).

1.6 Conclusion

Despite gradual improvements to our understanding of microbial differences between distinct body sites across the reptilian GIT (Keenan et al., 2013; Colston et al., 2015; Hyde et al., 2016; Tang et al., 2019), there remains a fundamental paucity of information on the composition, diversity, and functional capabilities of snake microbiomes (Krishnankutty et al., 2018). However, developing a baseline understanding of venomous snake microbiomes with regional associations in the body (e.g., GIT vs. venom gland) will be vital in order to address the important roles host-specific microbiomes may have played in venomous snake adaptive evolution. Additionally, although the use of 16S rRNA gene amplicon sequencing is often the first step to elucidating important patterns on compositional differences among host-associated bacterial communities, other genomic sequencing technologies (i.e., shotgun metagenomic sequencing) present exciting opportunities to begin identifying potential functional roles of these microbiomes and how they interact with their hosts (Rebollar et al., 2018). With the results of this comparative study of three different host species, each representing a unique snake family (Colubridae, Elapidae, Viperidae), we can continue to address this information gap for one of the most ecologically diverse and speciose vertebrate groups on the planet.

1.7 References

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Chapter 2

Host ecology drives frog skin microbiome diversity across ecotone in South-Central North America

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2.1 Abstract

Anurans (frogs and toads) are an ecologically diverse group of vertebrate organisms that display a myriad of reproductive modes and life history traits. To persist in such an expansive array of habitats, these organisms have evolved specialized skin that is used for respiration while also protecting against moisture loss, pathogens, and environmental contaminants. Anuran skin is also colonized by communities of symbiotic microorganisms, and these skin microbiota serve critical roles in numerous processes associated with anuran host health and persistence such as pathogen resistance and immunity. However, gaps remain in our understanding of the environmental and evolutionary processes that shape frog skin microbial communities. Here, we combined existing anuran disease data with 16S rRNA skin microbial inventories to elucidate the roles that geographic location, host evolutionary history, host ecology, and pathogen presence play in the microbial community assemblage of five co-distributed frog host species in Oklahoma. These focal species possess distinct ecological preferences: aquatic, semi-aquatic, and arboreal, and our results indicate that host ecology is the primary driver of frog skin microbial community structure. Additionally, compositional differences were observed among select host species based on geographic location, but this was not consistent among all five frog species. We did not find evidence of phylogenetic signal among our samples and results from the Classification and Regression Tree Analysis revealed that the presence of the amphibian pathogen *Batrachochytrium dendrobatidis* and the severity of infection were not drivers of skin microbiome differences among our focal host species. Results from this comparative study contribute to our growing understanding of the environmental and host-associated drivers of skin microbial community assemblage and represents one of the first studies on landscape-level variation in skin microbial communities among North American frogs.

2.2 Introduction

Representing one of the five major clades of terrestrial vertebrates on the planet, amphibians (anurans, salamanders, and caecilians) display an astonishing diversity of life history traits (Vitt and Caldwell, 2013; Crump, 2015; Bardua et al., 2021). Their reproductive diversity (e.g., biphasic tadpole metamorphosis, direct development, etc.) and specialized ecomorphological

adaptations (e.g., webbed toes, enlarged toe pads, etc.) have enabled amphibians to diversify across a near complete spectrum of environments on the planet, including fossorial, terrestrial, arboreal, aquatic, and riparian habitats (Afonso and Eterovick, 2007; Gómez and Lires, 2019; Engelkes et al., 2020; Bardua et al., 2021). To persist in such an expansive array of environments, amphibians have evolved specialized skin that serves as an essential respiratory organ and performs numerous physiological functions, including ion transport and osmoregulation, while also acting as a defensive barrier against harmful environmental and pathogenic agents (Duellman and Trueb, 1994; Lillywhite, 2006; Campbell et al., 2012; Kueneman et al., 2014; Wake and Koo, 2018; Varga et al., 2019). However, this same unique morphological feature also makes amphibians highly sensitive to instability in local environments, such as habitat modification and degradation, which has led to their recognition as bioindicators of ecosystem health (Wake, 1991; Blaustein et al., 1994; Houlahan et al., 2000; Ethier et al., 2021) and has contributed to alarming patterns of population declines on a global scale (Ford et al., 2020).

Currently, a staggering 41% of IUCN-assessed amphibian species are threatened with extinction (IUCN, 2023), with global population declines linked to a variety of factors, from pollution and invasive species to climate change and human-mediated habitat modification and destruction (Allentoft and O'Brien, 2010; Campbell Grant et al., 2020; Ford et al., 2020; Green et al., 2020). Furthermore, global declines have been exacerbated by growing threats posed by amphibian infectious diseases, including fungal and viral pathogens (Carey et al., 1999; Daszak et al., 1999; Greenberg and Palen, 2019; Scheele et al., 2019). In particular, *Batrachochytrium dendrobatidis* (*Bd*), a fungal pathogen that causes chytridiomycosis (often referenced as chytrid), has been especially devastating to anurans (frogs and toads), the most diverse of the three extant orders of amphibians with more than 7,500 species making up 88% of all amphibian diversity (AmphibiaWeb, 2023). To date, *Bd* infection has contributed to large-scale population declines of numerous species, as well as the confirmed or presumed extinction of more than 80 lineages to date (Scheele et al., 2019). Given that frogs and toads are essential members of both aquatic and terrestrial ecosystems (Whiles et al., 2006; Hocking and Babbitt, 2014), an improved understanding of the factors impacting anuran health, persistence, and immunity is needed for effective identification and mitigation of threats posed by rapid environmental change, habitat modification, and emerging pathogens.

Recently, a growing focus on the symbiotic microbial communities of host organisms, referred to as microbiomes, has revealed their fundamental roles in numerous processes associated with vertebrate host health, including digestion, nutrient acquisition, metabolism, immunity, development, and behavior (Ley et al., 2008; McFall-Ngai et al., 2013; McFall-Ngai, 2014; Alberdi et al., 2016; Colston and Jackson, 2016; Arizza et al., 2019; Rollins-Smith, 2020; Sehnal et al., 2021). Certain gut microbiota been shown to improve host metabolic activity in response to harsh environmental conditions (Li et al., 2019), with others often altering their composition and gene-expression patterns in response to physiological changes imposed by the host organism, underscoring the important role microbiomes may play in promoting host adaptation (Alberdi et al., 2016). Anuran skin microbiomes also serve a critical role as a protective barrier against pathogens (Harris et al., 2006; Harris et al., 2009; Becker et al., 2011; Kueneman et al., 2016; Ross et al., 2019; Rebollar et al., 2020; Jani et al., 2021). For example, metabolites produced by members of the frog skin microbiome have been shown to inhibit *Bd* zoospore development (Brucker et al., 2008; Loudon et al., 2014a; Walke and Belden, 2016). Furthermore, previous research has found that the presence of anti-fungal microbiota, such as

Janthinobacterium lividum, on the skin of uninfected frogs reduced morbidity and mortality when the organisms were exposed to *Bd* (Harris et al., 2009; Walke and Belden, 2016). Unfortunately, despite the recognition that skin microbiome composition is influenced by many host-associated and environmental factors (Kueneman et al., 2014; Bletz et al., 2017; Carda-Diéguez et al., 2017; Prado-Irwin et al., 2017; Chiarello et al., 2018; Sehnal et al., 2021), little remains known about the patterns and the degree with which skin microbial communities change in response to environmental variation, particularly among diverse anuran hosts.

Studies to date indicate that anuran skin microbiomes are likely shaped by a number of factors such as host evolutionary history (McKenzie et al., 2012; Kueneman et al., 2014; Walke et al., 2014), host ecology (Bletz et al., 2017), and developmental stage (Kueneman et al., 2014; Jiménez et al., 2019). Additionally, local environment (Varela et al., 2018; Ellison et al., 2019; Rebollar and Harris, 2019), geographic location (Kueneman et al., 2014; Belden et al., 2015; Rebollar et al., 2016; Bletz et al., 2017), and the prevalence of emerging infectious disease (i.e. *Bd* infection; Rebollar et al., 2016; Familiar-López et al., 2017; Bell et al., 2018; Knutie et al., 2018; Rebollar and Harris, 2019; Jani et al., 2021) have been shown to influence anuran skin microbiome diversity. Although previous studies have compared the skin microbiomes of host communities from different sites (Kueneman et al., 2014; Belden et al., 2015; Rebollar et al., 2016; Bletz et al., 2017; Medina et al., 2017), few have systematically sampled frog skin microbiomes across environmental gradients (Bletz et al., 2017; Medina et al., 2017). Yet, climatic variation across ecotones is known to impact anuran community assembly through processes such as environmental filtering(Cortés-Gómez et al., 2013; Díaz-García et al., 2017; Álvarez-Grzybowska et al., 2020), implying that there are major gaps in our understanding of how large-scale climatic and ecological variation may impact anuran skin microbiomes, and in turn, host survival and persistence. Additionally, many anuran skin microbiome studies compare only a few (i.e., 1–3) host species (McKenzie et al., 2012; Kueneman et al., 2014; Walke et al., 2014; Varela et al., 2018). As such, there exists a need for studies that investigate communities of co-distributed anuran species representing a variety of host ecologies across complex environmental landscapes to better understand, and predict, expected future shifts in anuran skin microbiomes as climatic and habitat changes progress.

In this study, we evaluate the skin microbial communities of five widely distributed species of frogs across four major Oklahoma ecoregions to elucidate the roles that geographic location, host evolutionary history, host ecology, and pathogen presence play in anuran skin microbiome assembly. Ecoregions are areas of general ecosystem similarity characterized by abiotic and biotic variables such as climate, geology, soil, and vegetation (Omernik, 1995; Bryce et al., 1999; McMahon et al., 2001). Oklahoma is one of only four states in the United States that possesses more than 10 distinct ecoregions (U.S. Environmental Protection Agency, 2013; Figure 2.1), with 29 native species of frogs distributed across broad regions of this complex landscape (Sievert and Sievert, 2021). Furthermore, recent efforts to determine and monitor the distribution and prevalence of *Bd* infection among amphibian communities across the state has resulted in robust pathogen datasets that are now publicly available (Marhanka et al., 2017; Watters et al., 2018; Watters et al., 2019; Watters et al., 2021). Therefore, the state represents an ideal spatial framework for investigating the evolutionary and ecological processes involved in anuran skin microbiome assembly dynamics across changing environments (McMahon et al., 2001; Omernik, 2004) and in the presence of growing threats from emerging infectious diseases (Marhanka et al., 2017; Watters et al., 2018; Watters et al., 2019; Watters et al., 2021).

We perform both intra- and interpopulation comparisons of frog skin microbiomes across four ecoregions in Oklahoma for populations of Blanchard's cricket frog (*Acris blanchardi*), the American green tree frog (*Hyla cinerea*), members of the morphologically indistinguishable gray treefrog species complex comprised of Gray's (*H. chrysoscelis*) and Cope's gray (*H. versicolor*) treefrogs (Jaslow and Vogt, 1977), which we refer to as *H. chrysoscelis*/*versicolor*, the American bullfrog (*Rana catesbeiana*), and the coastal plains leopard frog (*R. sphenocephala*) (Figure 2.1; Appendix B, Table S1). Through these comparisons, our study aims to address the roles that (1) geographic location, (2) host ecology, (3) host evolutionary history, and (4) pathogen presence play in anuran skin microbiome assemblage. If geographic location has a dominant role in shaping frog skin microbial communities, we expect regional populations of each host species to possess distinct skin microbiomes. Conversely, if skin microbiome assemblage is impacted largely by host ecology, we expect frog species with similar life histories (aquatic, semi-aquatic, arboreal) to possess similar microbial community structure and diversity, regardless of their evolutionary history or geographic location. Furthermore, by comparing populations of closely related taxa within two distinct anuran families, we test whether host evolutionary history plays a role in microbiome assemblage by examining whether closely related species harbor greater microbial similarities than can be explained by geographic location or host ecology. Finally, if pathogen presence drives differences in skin microbiome diversity, we predict that anurans infected with *Bd* will possess distinct skin microbiomes when compared to frogs that tested negative for the fungus. Results from this comparative study contribute to our understanding of the environmental and host-associated processes that drive vertebrate skin microbiome assemblage. Furthermore, we provide the first assessment of variation in skin microbiomes among frog species spanning an ecological gradient in North America, allowing for future studies to investigate the impact environmental stressors, disease, and urbanization will have on population and species persistence.

2.3 Materials and Methods

Sample collection

Fieldwork for this study was conducted from 2015–2021 (excluding 2020 due to the onset of the global pandemic) across four distinct ecoregions in Oklahoma: Arkansas Valley, Central Great Plains, Crosstimbers, and South Central Plains (Figure 2.1). These data were collected during a multi-year effort to sample amphibian microbiomes statewide. Frog communities from each ecoregion were sampled across a minimum of two years, while others were sampled over numerous years (Appendix B, Table S1). These four focal ecoregions can be distinguished based on their geology, vegetation, and general location within the state, with the Arkansas Valley and South Central Plains ecoregions located in the eastern part of the state, and the Central Great Plains and Crosstimbers ecoregions spanning the central portion of Oklahoma (Figure 2.1; Woods et al., 2005). The Arkansas Valley is home to the richest fish fauna in Oklahoma and its landscape includes plains, hills, floodplains, wooded areas, and scattered mountains (Woods et al., 2005). The Central Great Plains ecoregion is characterized by red sedimentary rocks, scattered hills, salt plains, low mountains, and sandy flats (Woods et al., 2005). The natural vegetation of this ecoregion is mostly mixed grass prairie; therefore, there is almost no forest coverage and much of the land is used for rangeland, cropland, and oil extraction. A mixture of woodland, savanna, and prairie characterize the Crosstimbers ecoregion which serves as the border between the moister, more forested eastern ecoregions from the more arid, prairie-dominated western areas within the state (Woods et al., 2005). Finally, the humid South Central

Plains ecoregion is comprised of floodplains, wetlands, forests, savannas, and some pastureland (Woods et al., 2005).

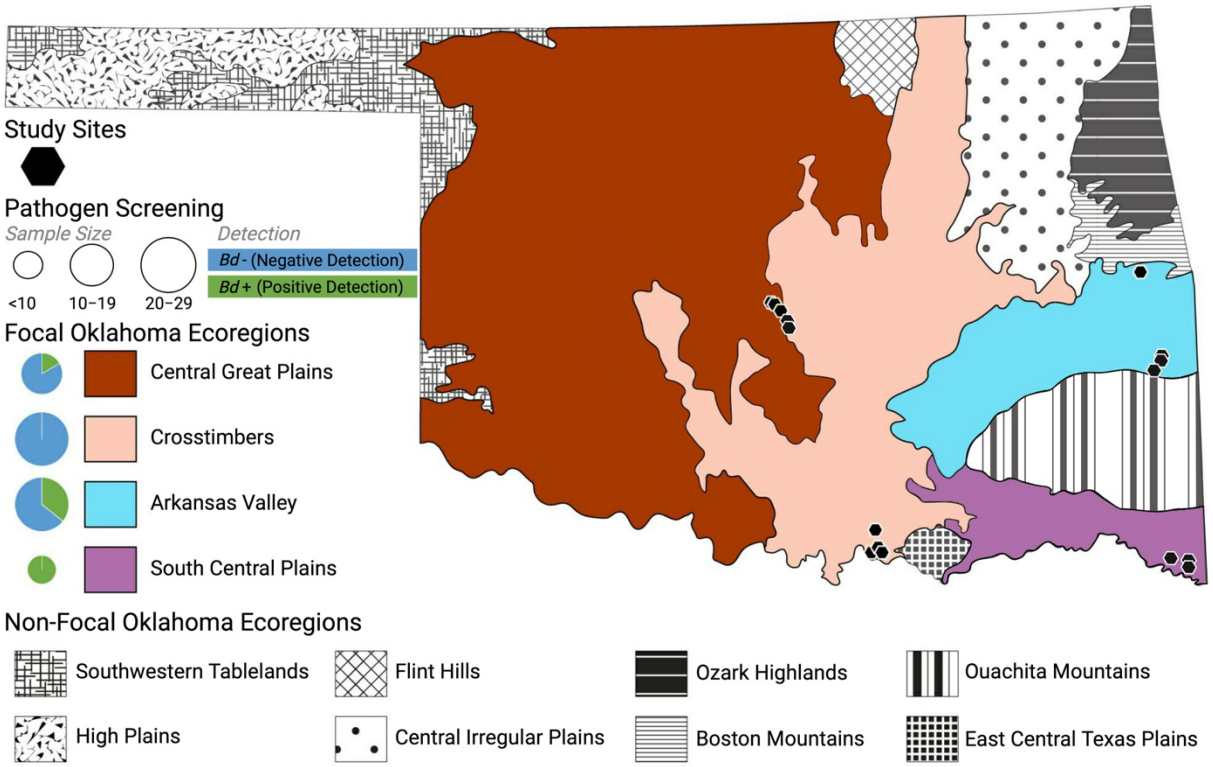

Figure 2.1 | Map of Oklahoma depicting the 12 distinct ecoregions found in the state. The four focal ecoregions are distinguished based on color: burnt red/orange = Central Great Plains, peach = Crosstimbers, light blue = Arkansas Valley, purple = South Central Plains. All other (non-focal) ecoregions are defined by distinct patterns in grayscale. Sites surveyed for microbiome and disease samples are shown with black hexagons on the map. Pathogen screening results are summarized for each focal ecoregion with circular pie chart showing the percentage of screened samples positive (green) or negative (blue) for *Bd*. Each pie chart is size corrected to be proportional to sample size.

Four of our focal host species (*Acris blanchardi*, *Hyla chrysoscelis*/*versicolor*, *Rana catesbeiana*, and *R. sphenocephala*) are distributed across all four ecoregions, excluding *H. cinerea*, which has a range that spans the southeastern portion of the state only (Sievert and Sievert, 2021). Conversely, the range of *R. catesbeiana* encompasses the entire state and *A. blanchardi* is found in all portions of the state, excluding the northwestern "panhandle" of Oklahoma. Lastly, *Hyla chrysoscelis*/*versicolor* and *R. sphenocephala* are found only in the central and eastern parts of the state (Sievert and Sievert, 2021). Based on these distributions, we sampled the skin microbial communities of *Rana catesbeiana* and *R. sphenocephala* individuals from the Central Great Plains and South Central Plains ecoregions and *A. blanchardi*, *Hyla cinerea*, and *H. chrysoscelis*/*versicolor* populations in the Arkansas Valley and Crosstimbers ecoregions (Figure 2.1). These five species can also be distinguished based on their evolutionary histories, as *A. blanchardi*, *H. cinerea*, and *H. chrysoscelis*/*versicolor* belong to the family Hylidae (superfamily Hyloidea), whereas both *Rana* species are members of the Ranidae family

(superfamily Ranoidea; Hime et al., 2021). Additionally, the focal species have different ecological preferences. For example, *A. blanchardi* and *R. sphenocephala* are semi-aquatic and found most often on the banks of waterbodies (Lehtinen and Skinner, 2006; Meade, 2008), while *R. catesbeiana* is fully aquatic and these frogs spend most of their time submerged in the shallow portions of ponds, lakes, swamps, and streams (Bruening, 2002). In contrast, both *Hyla* species prefer arboreal habitats (Martof et al., 1980; Harding, 1997); however, *H. cinerea* often rests on the shorter green vegetation that surrounds the banks of waterbodies. Conversely, *H. chrysoscelis*/*versicolor* is most often found higher in trees that are sometimes further away from water sources (Sievert and Sievert, 2021).

Skin microbiome samples were collected by rubbing a large, sterile rayon swab (Puritan Medical Products, Guilford, ME, USA) across the epidermis of an individual's back, stomach, legs, and the webbing of both feet five times each (each down and back stroke was considered a single pass). We swabbed 179 individuals representing the five focal species: *Acris blanchardi* (N = 38), *Hyla chrysoscelis*/*versicolor* (N = 37), *H. cinerea* (N = 37), *Rana catesbeiana* (N = 42), and *R. sphenocephala* (N = 25; Appendix B, Table S1). All swabs were preserved in liquid nitrogen or placed on ice immediately after collection until they were transferred to a -20°C freezer. Samples stored on ice temporarily were transferred to a -20°C freezer within 2–4 hours of collection. All samples were collected in strict accordance with the regulations established by the University of Oklahoma's Institutional Animal Care and Use Committee (IACUC Permit Nos: R17-031, R21-005, T15-002, and T21-001). Each author secured Scientific Collectors Permits annually through the Oklahoma Department of Wildlife Conservation (ODWC).

DNA extraction, polymerase chain reaction (PCR) amplification and sequencing

Genomic DNA was extracted from all 179 swabs using ZymoBIOMICS DNA Miniprep kits (Zymo Research Products, Irvine, CA, USA) in the Shared Genomics Core facilities of the Sam Noble Museum. The DNA concentration for a random subset of 50 extracted samples was determined using a Qubit 4 Fluorometer (Thermofisher Scientific, Waltham, Massachusetts, USA). Ten negative controls (i.e., ZymoBIOMICS reagents only, without a swab sample) were extracted alongside the 179 focal samples, nine of which were amplified and sequenced. Additionally, 75 mL of the ZymoBIOMICS Microbial Community Standard (i.e., a mock microbial community of known concentration; Zymo Research Products, Irvine, CA, USA) was extracted with the skin microbiome samples. We amplified and sequenced the 179 focal skin microbiome samples, nine extraction negative controls, two sets of ZymoBIOMICS Microbial Community Standards (Cat. No. D6300 and D6305, Zymo Research Products, Irvine, CA, USA), and two PCR-negative controls (one on each 96-well plate) using the methods outlined in Smith et al. (2021) and explained here in brief. We amplified the V4 hypervariable region of the 16S rRNA gene using primers and adapter sequences described in Kozich et al. (2013). After gel electrophoresis and bead clean-up (KAPA Pure Beads; Roche Sequencing Solutions, Pleasanton, CA, USA), we quantified each sample and normalized to 6 nM of DNA before pooling the samples into a single sterile, 1.5 mL microcentrifuge tube. Sequencing was performed at the University of Oklahoma Consolidated Core Lab using the 2x250 bp paired-end sequencing on a single run of an Illumina MiSeq.

Sequence analysis

Adapter sequences described in Kozich et al. (2013) were trimmed from the paired-end raw sequencing reads using AdapterRemoval v2 and the following parameters—minquality: 30, trimqualities, maxns: 0, trimns, threads: 18 (Schubert et al., 2016). The sequence data was then

imported into QIIME 2 (Bolyen et al., 2019) where *de novo* chimera checking and removal was performed using VSEARCH (Rognes et al., 2016) and UCHIME (Edgar et al., 2011). Then, the nonchimeric sequences were clustered into operational taxonomic units (OTUs) with a closed-reference OTU database at 97% sequencing similarity using VSEARCH (Rognes et al., 2016) against the Silva 138 database (Quast et al., 2012). Among all samples, including positive and negative controls, 4,050,580 sequences were clustered into 22,641 OTUs. Once we removed the positive and negative controls for downstream analyses, 20,542 OTUs and 3,469,844 sequences remained. We rarefied the OTU table to a sequencing depth of 1,000 for analysis based on our specific dataset and the associated rarefaction curves (Appendix B, Table S1), which removed 66 samples (Appendix B, Table S2). These 66 samples were excluded from analysis due to low sequence counts when compared to the other samples (N = 113; Appendix B, Table S2) which amplified well above a sequence count of 1,000. Raw sequence data generated in this study can be found in the Sequence Read Archive (SRA) under BioProject PRJNA1024480.

Statistical analysis

To test how host species, family, ecology, and ecoregion correlated with differences in frog skin microbial communities, alpha diversity (within groups) and beta diversity (between groups) analyses were performed using the QIIME 2 software package. Diversity analyses were also performed on each species separately to determine if skin microbiomes differed among host communities occupying distinct ecoregions within the state, and a p-value or q-value < 0.05 was considered a statistically significant difference. Alpha and beta diversity comparisons were conducted using the alpha-group-significance and beta-group-significance plugins in QIIME 2, which performs Kruskal-Wallis tests (alpha diversity) and Pairwise PERMANOVAs (beta diversity) to test group significance (Anderson, 2001). When multiple pairwise comparisons were conducted, we reported q-values instead of p-values to correct for multiple tests (Storey, 2002; Storey, 2003; Storey and Tibshirani, 2003). Alpha diversity analyses were performed on three metrics of diversity Shannon Diversity, Faith's Phylogenetic Diversity, and Observed OTUs using QIIME 2 (Bolyen et al., 2019). To evaluate how the skin microbiomes differed based on host species, family, and ecology (aquatic, semi-aquatic, arboreal), we analyzed beta diversity of skin samples using the phylogeny-based distance matrices Unweighted- and Weighted-Unifrac (Lozupone et al., 2007; Lozupone et al., 2011). Additionally, we performed these two analyses on the data from each focal frog species separately to determine if skin microbiomes differed between individuals of a single species occupying two distinct ecoregions. All beta diversity analyses were visualized by principal coordinate analysis (PCoA) using Qiime 2R (Bisanz, 2018) and Tidyverse (Wickham, 2017) packages in R version 4.2.1 (R Core Team, 2022). To understand the impact that host evolutionary history had on frog skin microbial diversity, we calculated phylogenetic signal using the three alpha diversity estimates (Shannon Diversity, Faith's Phylogenetic Diversity, and Observed OTUs) and the R packages ape (Paradis and Schliep, 2019) and brms (Bürkner, 2017). The host phylogeny was obtained through VertLife (Jetz and Pyron, 2018). Lastly, we utilized the package PARTY in R (Hothorn et al., 2006; R Core Team, 2022) to perform Classification and Regression Tree (CART) analyses on a subset of skin microbiome samples that also had *Bd* disease results for the host organisms (N = 71) to determine which variable (host species, host family, ecoregion, ecology, infection status, or infection load) accounted for most of the variance observed in each of our three alpha diversity metrics.

2.4 Results

Among the 179 skin microbiome swabs we collected from our five focal frog species, we obtained 4,050,580 sequences which were clustered into 22,641 OTUs using a 97% sequence similarity threshold against the Silva database (version 138, Quast et al., 2012). After rarefying the 179 samples to a sequencing depth of 1,000 sequences and removing positive and negative control samples, 113 samples and 7,694 OTUs remained and were used in subsequent analyses (*A. blanchardi* [N = 17], *H. chrysoscelis*/ *versicolor* [N = 27], *H. cinerea* [N = 24], *R. catesbeiana* [N = 30], and *R. sphenocephala* [N = 15]; Appendix B, Table S2). Visualization of the data obtained from the two positive control samples indicated that the community compositions of the Extraction-Stage and PCR-Stage ZymoBIOMICS Microbial Community Standards were similar, suggesting that our extraction-to-analysis workflow performed consistently (Appendix B, Figure S2).

Taxonomic composition of skin microbial communities across host species, ecoregion, and ecology

Proteobacteria was the most abundant bacterial phylum found among the 113 skin microbiome samples (relative abundance of 75.48%; Figure 2.2). Three other dominant phyla (average relative abundance > 1.0% excluding values equal to zero; Suenami et al., 2019) were found in the majority of the samples (N = 111)—Bacteroidetes (9.71%), Firmicutes (6.82%), and Actinobacteria (4.30%; Figure 2.2). Among all aquatic (*R. catesbeiana*) samples, the most dominant phyla was Proteobacteria (62.67%) followed by Firmicutes (16.49%), Bacteroidetes (12.11%), Actinobacteria (3.23%), Cyanobacteria (1.98%), Fusobacteria (1.36%), and Acidobacteria (1.07%; Figure 2.2). Chloroflexi (3.54%; N = 1), Desulfobacterota (1.25%–3.45%; N = 3), Gemmatimonadota (1.84%; N = 1), Myxococcota (1.34%–1.82%; N = 2), Planctomycetota (1.20%–2.55%; N = 2), and Verrucomicrobia (1.09%–3.40%; N = 8) were found in varying abundances among *R. catesbeiana* skin samples (Figure 2.2).

The most dominant phylum among semi-aquatic (*A. blanchardi* and *R. sphenocephala*) samples was Proteobacteria with an average relative abundance of 73.60%. Bacteroidetes was the second most abundant (11.70%) followed by Actinobacteria (6.29%), then Firmicutes (3.27%; Figure 2.2). Additionally, Acidobacteria (1.07%–7.54%; N = 6), Chloroflexi (1.35%–9.57%; N = 6), Cyanobacteria (1.15%–18.65%; N = 7), Myxococcota (1.32%–1.37%; N = 2), Planctomycetes (2.07%–4.11%; N = 4), and Verrucomicrobia (1.19%–2.35%; N = 6) were found among samples from both semi-aquatic species (Figure 2.2). Interestingly, the Archaea phylum Crenarchaeota was present within four *R. sphenocephala* samples (1.51%–5.07%) and one *A. blanchardi* sample (2.4%; Figure 2.2). With the exception of one *R. catesbeiana* sample (2.36%), Crenarchaeota was not found in abundances greater than 1% in any of the other samples. Additionally, four phyla, Actinobacteria, Bacteroidetes, Cyanobacteria, and Verrucomicrobia were present in abundances greater than 1% (1.64%–32.38%, 3.48%–29.52%, 1.70%–6.65%, and 1.43%–2.35%, respectively) among *A. blanchardi* skin samples from the Arkansas Valley only with the exception of two samples from the Crosstimbers ecoregion that had relative abundances greater than 1% for these four phyla (Figure 2.2). Conversely, all but one of the Crosstimbers *A. blanchardi* skin samples were dominated by Proteobacteria (95.40%–99.66%; Figure 2.2). Lastly, two phyla, Desulfobacteria and Fusobacteria were found among samples from *A. blanchardi* and *R. catesbeiana* only, in abundances ranging from 1.25%–4.66% (Desulfobacteria) and 1.35%– 7.80% (Fusobacteria).

Among skin samples collected from the two arboreal frog species (*H. chrysoscelis*/*versicolor* and *H. cinerea*), the same four dominant phyla were found—Proteobacteria (84.2%), Bacteroidetes (7.03%), Actinobacteria (3.70%), and Firmicutes (3.22%; Figure 2.2). However,

the phyla Acidobacteriota, Chloroflexi, Cyanobacteria, Myxococcota, and Planctomycetes were represented in abundances greater than 1% (1.05%–7.5%) in 4–5 *H. chrysoscelis/versicolor* samples only with the exception of Acidobacteriota which had a relative abundance of 1.07% in a single sample of *H. cinerea* (Figure 2.2).

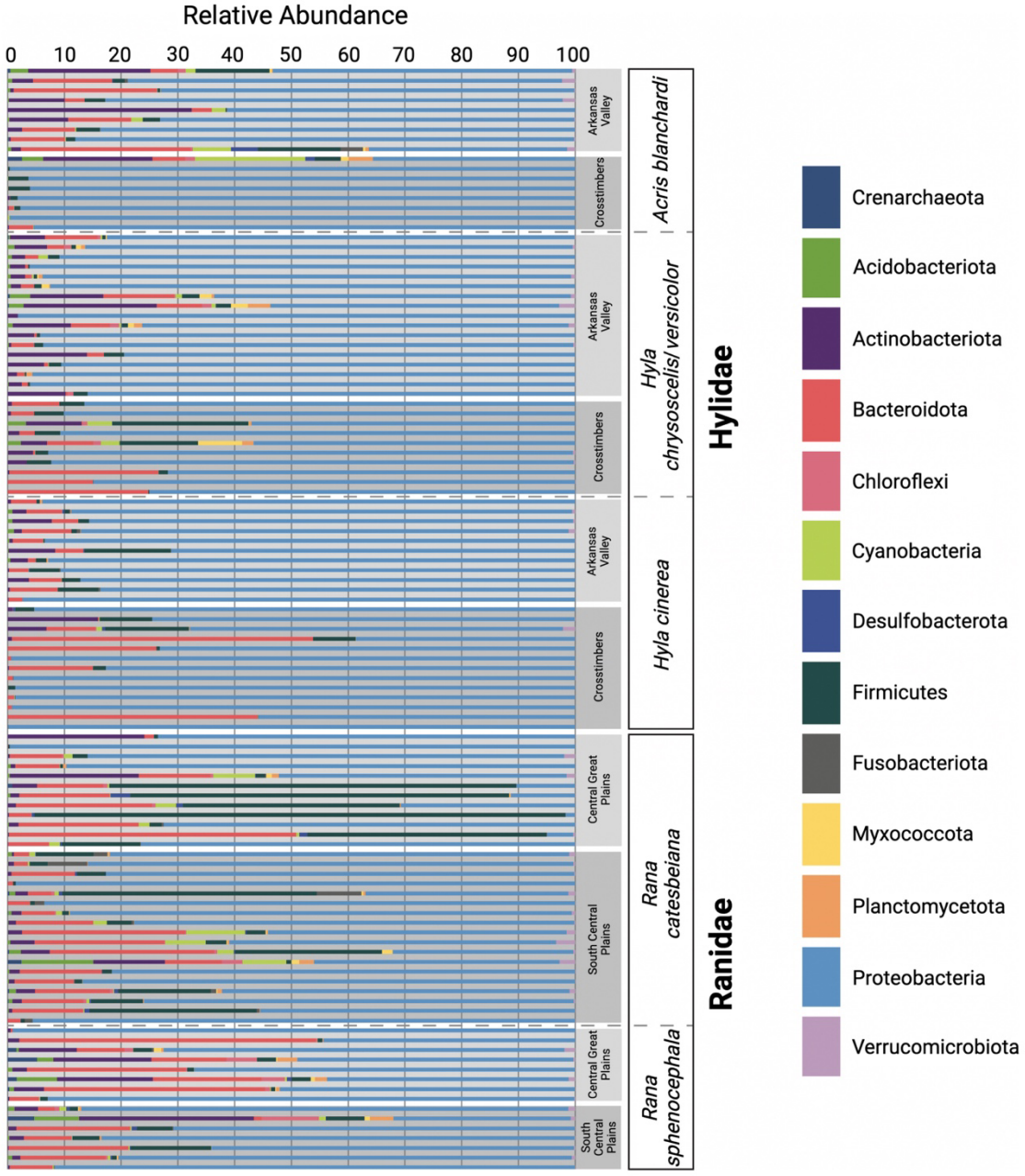

Figure 2.2 | Relative abundances of the dominant microbial phyla acquired through 16S rRNA amplicon sequencing. Each horizontal bar represents an individual swab. Samples are grouped by host family, host species, and ecoregion.

Analyses of mechanistic processes influencing host microbial diversity

We utilized several alpha diversity metrics to test for significant differences among the skin microbiome samples using the Kruskal-Wallis test. First, Faith's Phylogenetic Diversity analysis found significant differences among *A. blanchardi* and both *Rana* species (*A. blanchardi* vs. *R. catesbeiana*: H = 5.61; q-value = 0.03; *A. blanchardi* vs. *R. sphenocephala*: H = 7.50; q-value = 0.016; Appendix B, Figure S3). Additionally, this analysis found that the skin microbial diversity of both *Hyla* species were significantly different from both *Rana* species (*H. chrysoscelis*/*versicolor* vs. *R. catesbeiana*: H = 7.04; q-value = 0.016; *H. chrysoscelis*/*versicolor* vs. *R. sphenocephala*: H = 7.10; q-value = 0.016; *H. cinerea* vs. *R. catesbeiana*: H = 18.79; q-value = 0.0001; *H. cinerea* vs. *R. sphenocephala*: H = 17.04; q-value = 0.0002; Appendix B, Figure S3). However, no significant differences were found when comparing the skin microbiomes of the three species within the Hylidae family (*A. blanchardi* vs. *H. chrysoscelis*/*versicolor*: H = 0.106; q-value = 0.745; *A. blanchardi* vs. *H. cinerea*: H = 1.89; q-value = 0.211; *H. chrysoscelis*/*versicolor* vs. *H. cinerea*: H = 3.28; q-value = 0.100; Appendix B, Figure S3). Additionally, we found no significant difference when comparing the skin microbiomes of the two Ranidae species (*R. catesbeiana* vs. *R. sphenocephala*: H = 1.17; q-value = 0.31; Appendix B, Figure S3). In comparison, the Observed OTU analysis indicated significant differences between *R. sphenocephala* and both *Hyla* species only (*H. chrysoscelis*/ *versicolor*: H = 8.88; q-value = 0.014; *H. cinerea*: H = 12.82; q-value = 0.003; Appendix B, Figure S3). Results from the Shannon Diversity analysis yielded significant differences between *A. blanchardi* and *H. cinerea* skin microbiome samples (H = 5.67; q-value = 0.04; Appendix B, Figure S3). Additionally, the skin microbial communities of both *Hyla* species were significantly different from the two *Rana* species with the exception of *H. chrysoscelis*/*versicolor* vs. *R. catesbeiana*: H = 4.79; q-value = 0.057 (*H. chrysoscelis*/*versicolor* vs. *R. sphenocephala*: H = 7.52; q-value = 0.03; *H. cinerea* vs. *R. catesbeiana*: H = 6.82; q-value = 0.03; *H. cinerea* vs. *R. sphenocephala*: H = 9.19; q-value = 0.024; Appendix B, Figure S3).

Subsequently, we incorporated the alpha diversity data into two additional analyses: (1) a Phylogenetic Generalized Linear Mixed Model to test for phylogenetic signal, and (2) a CART analysis to determine which variable (host species, host family, ecoregion, ecology, infection status, or infection load) accounted for most of the variation within skin microbial communities associated with the subset of frogs that had corresponding *Bd* disease data (N = 71). We did not detect phylogenetic signal among the skin microbiomes of our five focal host species using the three alpha diversity metrics: Shannon (h^2 = 0.22, Estimated Error = 0.19), Faith's Phylogenetic Diversity (h^2 = 0.22, Estimated Error = 0.18), and Observed OTUs (h^2 = 0.18, Estimated Error = 0.16). Additionally, the results of the CART analysis indicated that, when analyzing Shannon Diversity, ecology was the only variable (χ^2 = 16.626; p-value = 0.006) that explained the variance in skin microbiome diversity, with aquatic and semi-aquatic frogs having significantly higher Shannon Diversity when compared to arboreal frogs (Appendix B, Figure S4). The Observed OTU CART analysis found that ecoregion explained the majority of the variance, with samples from the Crosstimbers ecoregion having significantly fewer OTUs when compared to samples from the Arkansas Valley, Central Great Plains, and South Central Plains ecoregions (χ^2 = 21.852, p-value < 0.001; Appendix B, Figure S5). Among samples from the Arkansas Valley, Central Great Plains, and South Central Plains ecoregions, we found that ecology explained the remaining variance in skin microbiome diversity, with samples from semi-aquatic frogs having more OTUs compared to aquatic and arboreal frogs (χ^2 = 18.951, p-value = 0.002; Appendix B, Figure S5). Lastly, results from the CART analysis with Faith's Phylogenetic Diversity indicated that ecoregion explained all of the variance within our focal skin microbiome samples with

samples from the Crosstimbers ecoregion being significantly less diverse than samples from the other three tested ecoregions ($\chi^2 = 27.449$, p-value < 0.001; Appendix B, Figure S6). Additionally, samples from the Arkansas Valley, Central Great Plains, and South Central Plains ecoregions were further split, with samples from the Central Great Plains and South Central Plains being more diverse than samples from the Arkansas Valley ecoregion ($\chi^2 = 13.936$, p-value = 0.012; Appendix B, Figure S6). As such, results from our CART analysis suggest that host ecology and geographic location are the primary variables that explain the variance in our focal skin microbiome samples. In contrast, host species, host family, *Bd* status, and *Bd* infection intensity did not explain any of the variance in our microbiome samples.

Unweighted- and Weighted-Unifrac distance matrices were used to analyze beta diversity among skin microbiome samples. Unifrac is a phylogenetic distance metric that measures the difference between microbial communities based on the degree of divergence between different sequences (Lozupone and Knight, 2005). There are two types of Unifrac distances, Unweighted and Weighted, with Weighted-Unifrac also accounting for differences in the relative abundances of microbial taxa within samples (Lozupone et al., 2007). We report findings from both tests here as they produce different but complementary findings (Figure 2.3; Appendix B, Figure S7). In summary, both analyses indicated that there were significant differences in the skin microbiomes of the focal host species, with the exception of *A. blanchardi* vs. both *Hyla* species (Unweighted) and *A. blanchardi* vs. *H. cinerea* (Weighted; Supplementary Materials). Additionally, both analyses found significant differences between host families and host ecologies (Figure 2.3; Appendix B, Figure S7). Further, intra-specific comparisons of skin microbiomes between ecoregions yielded significant differences within most host species (with the exception of *H. chrysoscelis/versicolor* and *R. sphenocephala*; Supplementary Materials). For more detailed comparisons, please refer to the Supplementary Materials.

2.5 Discussion

Our study sampled the skin microbial communities of anuran hosts across distinct ecoregions in Oklahoma to determine if geographic location, host ecology, pathogen infection, or host evolutionary history correlated with differences in frog skin microbiomes. Results from the CART analysis indicated that host ecology and geographic location were the primary drivers of the skin microbiome diversity differences we observed among the five focal frog species sampled (Appendix B, Figures S4–S6). Although we observed some compositional differences among skin microbiome samples based on the geographic locations of our hosts, such patterns were not shared across all five host species (Figure 2.2). Interestingly, the most prominent differences were observed among *A. blanchardi* skin microbiomes, which differed profoundly between individuals from the two unique ecoregions we sampled (Figure 2.2). Four phyla, Actinobacteria, Bacteroidetes, Cyanobacteria, and Verrucomicrobia, were present in abundances greater than 1% among samples from Arkansas Valley only, with the exception of two samples from the Crosstimbers ecoregion. This is likely because most of the *A. blanchardi* samples from the Crosstimbers were dominated largely by Proteobacteria (Figure 2.2). For the geographic location hypothesis to be fully supported, we would expect to see more ecoregion-specific compositional differences among the skin microbiomes of our other focal anuran species. Additionally, *Bd* infection status and severity did not emerge as primary drivers of anuran skin microbiome diversity among our focal samples (Appendix B, Figures S4–S6).

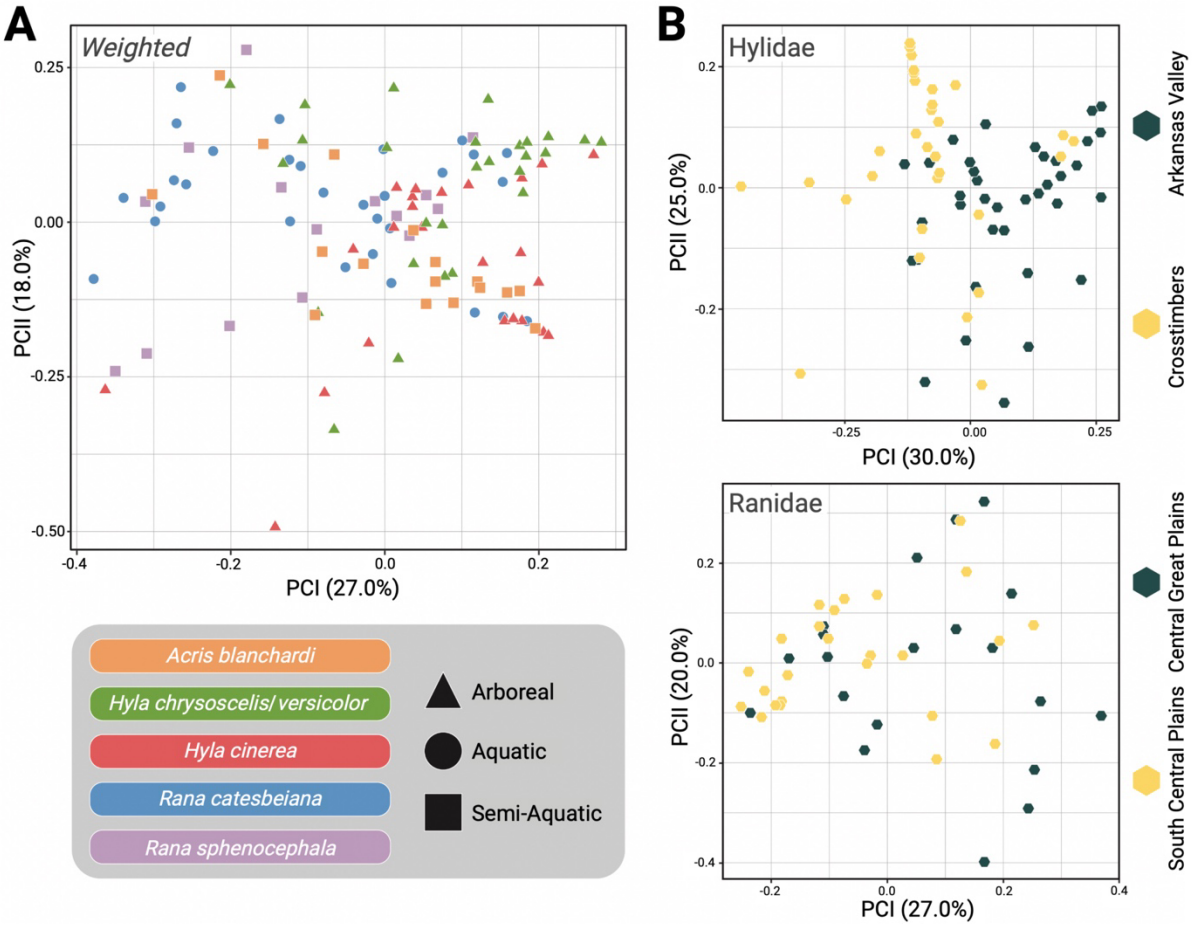

Figure 2.3 | Beta diversity comparisons using Weighted-Unifrac distance. **(A)** Principal Coordinates Analysis (PCoA) of all samples with host species represented by distinct colors and point shape indicating if the sample was from an arboreal (triangle), aquatic (circle), or semi-aquatic (square) frog. **(B)** Samples were separated into their respective host families with Hylidae samples plotted in the PCoA at the top and Ranidae samples on the bottom. Point color indicates the ecoregion that the sample was collected from (yellow = Crosstimbers and South Central Plains, gray = Arkansas Valley and Central Great Plains).

However, previous studies have found that *Bd* infection impacts the composition of anuran skin microbiomes (Rebollar et al., 2016; Familiar-López et al., 2017; Rebollar and Harris, 2019; Jani et al., 2021), and disease data was only available for a subset of samples in our study (N = 71 out of 113 samples). Further, while both *Hyla* species had associated disease data for 8–13 individuals per ecoregion, *R. catesbeiana* and *R. sphenocephala* only had disease information for 2–6 individuals per ecoregion, and no *R. catesbeiana* individuals from the South Central Plains ecoregion had corresponding *Bd* disease data (Figure 2.1; Appendix B, Table S1). As such, it may be difficult to detect a correlation between *Bd* disease status/severity and microbiome compositional differences when sample sizes are small. Therefore, increased sampling is needed to further investigate how *Bd* disease status and severity alters anuran skin microbiome diversity. We did not find evidence of phylogenetic signal among the skin samples from our focal hosts,

indicating that anuran skin microbiomes are influenced by host ecology and geographic location, whereas host evolutionary history and pathogen infection did not emerge as primary drivers.

Four dominant bacterial phyla emerged within nearly all samples (N = 111/113), Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, a pattern consistent with findings from other amphibian skin microbiome studies to date (Kueneman et al., 2014; Walke et al., 2014; Walke and Belden, 2016; Bletz et al., 2017). Additionally, previous research has indicated that Chloroflexi (Walke et al., 2014; Ellison et al., 2019), Cyanobacteria (McKenzie et al., 2012; Walke et al., 2014; Ellison et al., 2019), and Planctomycetes (Jiménez and Sommer, 2017; Prado-Irwin et al., 2017; Muletz Wolz et al., 2018; Albecker et al., 2019; Ellison et al., 2019) are also dominant members of the amphibian skin microbiome. Therefore, it is not surprising that we found these phyla in abundances greater than 1% among samples from four of our focal host anurans (excluding *H. cinerea*). Although Firmicutes was a dominant phylum among skin microbiome samples from all of our focal host species, some *R. catesbeiana* samples had abundances of Firmicutes as high as 93.54% (Figure 2.2). This was surprising given that previous research, and results from our study, indicate that Proteobacteria dominates the amphibian skin microbiome (Walke et al., 2014; Walke et al., 2015; Bataille et al., 2016; Medina et al., 2017; Muletz Wolz et al., 2018; Figure 2.2). However, studies have also found that skin microbial communities dominated by Firmicutes are associated with the presence of skin lesions caused by psoriasis (Gao et al., 2008; Yan et al., 2017) and atopic dermatitis (Zheng et al., 2019) in humans, and papillomatous digital dermatitis in cows (Santos et al., 2012). Therefore, it is possible that the *R. catesbeiana* individuals with high abundances of Firmicutes in their microbiomes could have experienced prior skin infections or were infected at the time of sampling. However, only a small subset (N = 6) of the focal *R. catesbeiana* hosts had associated *Bd* infection data. As such, we cannot confirm if *Bd* presence is correlated with an abundance of Firmicutes in the skin microbiomes of this species at this time, but future studies should investigate if amphibian skin lesions are also colonized by Firmicutes, as observed in the mammalian skin microbiome literature (Santos et al., 2012; Zheng et al., 2019).

We found species-specific compositional differences among the five anuran species sampled. Notably, the Archaea phylum Crenarchaeota was found among aquatic and semi-aquatic species only (*A. blanchardi*, *R. catesbeiana*, and *R. sphenocephala*; Figure 2.2). All cultured Crenarchaeota are extreme thermophiles (Ochsenreiter et al., 2003); however, with increased use of culture-independent sequencing methods, studies have indicated that there are members of the Crenarchaeota phylum that are associated with freshwater sediments and other soil environments (Ochsenreiter et al., 2003; Brochier-Armanet et al., 2008). *Acris blanchardi*, *R. catesbeiana*, and *R. sphenocephala* are commonly encountered within or around water and marshy habitats, which could explain why Crenarchaeota was found on the skin of these species and not the two arboreal host species (*H. cinerea* and *H. chrysoscelis*/*versicolor*). Additionally, we found Myxococcota among skin microbiome samples from four of our host species (excluding *H. cinerea*). Members of this phylum colonize soil and rotting plant material, including decaying wood and tree bark, but also freshwater environments (Reichenbach, 1999). *Hyla cinerea* is an arboreal frog which is found most often on green, weedy vegetation on the margin of swamps and ponds (Sievert and Sievert, 2021), possibly limiting their exposure to bacteria that inhabit soil and decaying plant material, such as Myxococcota. In contrast, *H. chrysoscelis*/*versicolor* inhabits woody vegetation such as shrubs and trees (Sievert and Sievert, 2021), which could explain why Myxococcota was found on the skin of *H. chrysoscelis*/ *versicolor* and not *H. cinerea*.

After a recent splitting of the bacterial class Deltaproteobacteria, Myxococcota and Desulfobacterota emerged as two distinct phyla (Waite et al., 2020; Langwig et al., 2022). Desulfobacterota was detected among a few *A*. *blandchardi* and *R*. *catesbeiana* samples. This phylum has been found in Oklahoma wetland sediment previously (Murphy et al., 2021), which may be why we detected members of this phylum on the skin of *A*. *blandchardi* and *R*. *catesbeiana*, two host species that are most often found within or around waterbodies, and emphasizes the importance of collecting environmental samples alongside host microbiome samples. To our knowledge, no studies have reported Crenarchaeota, Desulfobacterota, or Myxococcota as dominant members of the amphibian skin microbiome. However, the recent reclassification of Deltaproteobacteria could explain why Desulfobacterota and Myxococcota have not yet been detected on the skin of amphibians. Deltaproteobacteria was found on the skin of frogs from Madagascar in small relative abundances (1.6%; Bletz et al., 2017), and among the different environmental samples collected by Bletz et al. (2017). Therefore, more studies are needed to determine if Crenarchaeota, Desulfobacterota, and Myxococcota are prominent members of the amphibian skin microbiome or if our findings are an example of host frogs obtaining some of their skin microbiota from their surrounding environment, a concept which has been discussed in numerous other studies (Belden and Harris, 2007; Becker et al., 2014; Loudon et al., 2014b; Bletz et al., 2017; Jiménez and Sommer, 2017; Kueneman et al., 2019; Rebollar and Harris, 2019; Hernández-Gómez et al., 2020; Barnes et al., 2021). For example, soil and water can act as reservoirs for microbiota (Loudon et al., 2014b) which can then colonize the skin of amphibians (Belden and Harris, 2007; Becker et al., 2014; Hernández-Gómez et al., 2020; Barnes et al., 2021). It is thought that these reservoirs support the presence of rare bacterial taxa within the amphibian skin microbiome (Loudon et al., 2014b) and changes to microbial communities in the environment may impact amphibian skin microbiome composition and diversity (Belden and Harris, 2007). Given that the skin microbiome is critical for promoting amphibian immune system function and pathogen defense (Bernardo-Cravo et al., 2020; Rebollar et al., 2020), altering reservoir microbiota with captive breeding or head-start initiatives could impact host health (Belden and Harris, 2007; Becker et al., 2014; Loudon et al., 2014b). As such, future investigations which explore the exchange of rare microbes between the environmental and amphibian skin microbiomes are essential for understanding this complex dynamic and may have critical implications for conservation initiatives. To accomplish this, future studies should consider sampling, and subsequently sequencing, the environmental microbiome present at the exact location where the host organism is collected, a method which has been employed in several other studies to date (Walke et al., 2014; Bletz et al., 2017; Jiménez and Sommer, 2017). Implementing this methodology in future studies may improve our understanding of the mechanisms shaping the composition and diversity of anuran skin microbiomes while also providing a way to differentiate skin microbiome samples that have been potentially contaminated with environmental microbes from those collected from host organisms that have rare microbes within their skin microbiomes. Previous findings indicate that the same dominant microbial phyla comprise both environmental and amphibian skin microbiome samples (e.g., Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria) but in different relative abundances (Walke et al., 2014; Bletz et al., 2017; Hernández-Gómez et al., 2020; Barnes et al., 2021). Therefore, analyzing environmental microbiome samples alongside skin microbiome samples may help identify samples that have been contaminated with environmental microbiota because the compositions of the contaminated samples will be more similar to the environmental samples than to the focal skin microbiome samples. In contrast, we would expect

that samples collected from hosts with rare microbial taxa in their skin microbiomes would have compositions that are more similar to the other hosts when compared to the environmental samples but with the addition of the rare microbial taxa.

Another consideration for future work is to expand the taxonomic scope of amphibian skin microbiome studies, particularly in a state like Oklahoma which is home to an impressive diversity of amphibian species distributed across a diverse landscape. For the purposes of our study, we aimed to sample co-distributed anuran species that are found commonly in the wild to allow for sufficiently large sample sizes and more robust comparisons of skin microbiomes among taxa. However, continued field surveys across central and eastern Oklahoma would enable expanded comparisons, both taxonomically (across orders, families, genera, and species) and geographically (across more ecoregions) in Oklahoma (Figure 2.1). For example, Oklahoma is home to several species of true toads (family Bufonidae), narrow-mouthed toads (family Microhylidae), and spadefoot toads (family Scaphiopodidae), along with other members of the focal families Hylidae and Ranidae from this study that lacked sufficient sample sizes to include in our dataset, such as *Pseudacris clarkii*, *P. crucifer*, *P. fouquettei*, and *P. streckeri* in the family Hylidae and *Rana areolata*, *R. blairi*, *R. clamitans*, and *R. palustris* in the family Ranidae (Sievert and Sievert, 2021). There is also an incredible diversity of salamanders (order Caudata) distributed across the eastern portion of the state which presents exciting opportunities for future research to compare and contrast the findings of this study with the skin microbiome diversity of this divergent clade of amphibians. Further, given the variable nature in which we sampled frog communities from the four focal ecoregions—frogs from certain ecoregions were sampled across multiple years, other sampled in only two years—future research should investigate to what degree anuran skin microbiomes vary across seasons or years (Douglas et al., 2021).

Symbiotic microbial communities serve fundamental roles in a variety of processes that promote host health. As such, investigating the environmental and evolutionary mechanisms that drive the assemblage of frog skin microbiomes remains essential as we begin to form a more comprehensive understanding of the factors impacting anuran health, persistence, and immunity. Results from this study suggest that multiple factors influence the structure of anuran skin microbial communities; however, host ecology plays the largest role in the assembly of skin microbiome diversity among our sampled Oklahoma frog communities. Comparative studies of skin microbiomes among co-distributed anuran species spanning complex landscapes allow us to better understand, and predict, impending shifts in anuran skin microbial communities in response to continued climate and habitat changes. The investigation of amphibian skin microbiomes remains critical as the vertebrate group continues to face population declines imposed by disease, habitat loss, pollution, and climatic changes.

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Chapter 3

Bending the rules of island biogeography: Microbiome diversity decreases with increased island size among insular reptilian hosts in the Philippines.

3.1 Abstract

Island biogeography theory has long served as a paradigm for identifying the mechanisms that shape the distribution of insular plant and animal diversity; however, a burgeoning body of literature has applied the theory to studies of microbial biogeography within island-like systems. Despite this expanded application, few studies have utilized the theory to explain patterns of microbial diversity spanning true islands, especially among communities of microorganisms that colonize vertebrate hosts, referred to as microbiomes. Here, we use 16S rRNA microbial inventories to elucidate the roles that host evolutionary history, host ecology, host microhabitat, geographic location, and island size play in the assemblage of gut microbiomes among reptilian hosts spanning multiple islands in the Philippines. Host ecology and island size explained most of the variation in gut microbiome diversity observed among our focal hosts. However, we report a negative correlation between island size and microbial diversity, deviating from fundamental expectations of island biogeography theory. We did not find evidence of phylogenetic signal or cophylogeny, rejecting the host evolutionary history hypothesis. Further, no significant differences were found among intra-island site comparisons of microbiomes, and while some inter-island site comparisons were significant, these results were not consistent across all measures of microbial diversity, leading us to reject the geographic location hypothesis. As more studies begin to apply fundamental theories in island biogeography, ecology, and evolutionary biology to describe patterns of host-associated microbial diversity, we may find that our previous expectations for plant and animal biodiversity may not always apply to microbiomes.

3.2 Introduction

Islands have long been recognized as ideal, replicative ‘natural laboratories’ with their well-defined land-positive boundaries, diversity of ecological and geographic characteristics (e.g., land area, degree of isolation, and topographic complexity), and impressive levels of floral and faunal diversity. It is for these reasons that islands and island systems (i.e., archipelagos) have provided the template for the development and expansion of the field of biogeography (MacArthur and Wilson, 1963; Brown and Kodric-Brown, 1977; Heaney, 2000; Whittaker et al., 2008), as well as processes of adaptive radiation (Grant and Grant, 2008; Losos and Ricklefs, 2009; Setiadi et al., 2011; Lapoint et al., 2013) and community assembly (Mittelbach and Schemske, 2015; Warren et al., 2015; Rominger et al., 2016). Indeed, the study of island systems laid the foundation for the development of the Equilibrium Theory of Island Biogeography (ETIB; MacArthur and Wilson, 1963), which is grounded on three fundamental principles: (1) Island size is correlated positively with terrestrial species richness, referred to as the Species-Area Relationship; (2) Insular species richness decreases with increased isolation from the continental mainland; and (3) Species turnover occurs continuously over time (Figure 3.1;

MacArthur and Wilson, 1963; Lomolino et al., 2010; Wang, 2021). Since its conception, the ETIB has served as a paradigm to describe patterns of diversity within a myriad of plant and animal taxa spanning true, oceanic islands such as *Anolis* lizards in the Caribbean (Losos and Ricklefs, 2009), Drosophilid flies and honeycreeper birds in the Hawaiian islands (Lapoint et al., 2013), finches in the Galapagos islands (Grant and Grant, 2008; Setiadi et al., 2011), flora in Macaronesia (Kim et al., 2008), and amphibians and reptiles on the Indonesian island of Sulawesi (Setiadi et al., 2011; Mcguire et al., 2023). Shortly after its original description, MacArthur and Wilson (1967) noted that the ETIB could be applied to many other kinds of isolated habitats as well, not just 'true' oceanic islands. These 'island-like' systems (ILS) are similar to true oceanic islands as they are spatially fragmented, separated by barriers, and limited in their connectivity (Itescu, 2019). However, ILS may take on a terrestrial, freshwater, marine, or biotic form, while true islands are, by definition, isolated fragments of land that are surrounded completely by water in the ocean or a freshwater body (Itescu, 2019). Although the majority of island biogeography studies to date have focused on true islands and island archipelago systems (Warren et al., 2015; Meiri, 2017; Itescu, 2019), there exists a growing body of literature employing the ETIB to explore biogeographic processes across ILS such as high elevation islands (i.e., 'sky islands', Warshall, 1995; Dawson et al., 2016), inselbergs (Porembski and Barthlott, 2012), caves (White and Culver, 2012), lakes (Arnott, 2009; Dawson et al., 2016), and even hydrothermal vents (Sibuet and Olu, 1998; Van Dover et al., 2002). Despite the expanded application of the ETIB to island-like systems, many of these studies have explored these patterns among floral and faunal taxa exclusively; however, an increasing number of studies have incorporated the theory within biogeographic studies of microbial communities.

Applied traditionally to groups of oceanic islands, the ETIB has also been utilized to explore microbial biogeography across various terrestrial (i.e., leaves, trees) and aquatic ILS (i.e., lakes, pools, hot springs, wastewater tanks; Andrews et al., 1987; Kinkel et al., 1987; Papke et al., 2003; Whitaker et al., 2003; Bell et al., 2005; Reche et al., 2005; Van Der Gast et al., 2005, 2006; Peay et al., 2007; Lepère et al., 2013; Glassman et al., 2017; Spiesman et al., 2018). Results from several of these studies support foundational expectations of the ETIB. For example, microbial diversity tends to increase with 'island' size (i.e., the Species-Area Relationship; Bell et al., 2005; Chlebicki and Olejniczak, 2007; Dinnage et al., 2019), and microbial community similarity and diversity often decline with increased distance from 'mainland' populations (Whitaker et al., 2003; Bahram et al., 2013; Lepère et al., 2013; Glassman et al., 2017). Due to the general paucity of studies which investigate patterns of microbial diversity across true islands, it remains unclear if ETIB expectations apply to microbial communities spanning true island systems. Indeed, only one study to date has employed theories of island biogeography to help explain patterns of host-associated microbial diversity among insular floral communities (Davison et al., 2018), underscoring the need for studies which utilize ETIB expectations to investigate patterns of microbial diversity spanning true, oceanic islands, especially among symbiotic microbial communities that colonize vertebrate host organisms, referred to as microbiomes (Dickey et al., 2021).

Increased research interest in the microbiomes associated with vertebrate host organisms have illuminated their fundamental roles in numerous processes associated with host health (e.g., digestion, nutrient acquisition, metabolism, immunity; Ley et al., 2008; Tremaroli and Bäckhed, 2012; McFall-Ngai et al., 2013; Alberdi et al., 2016; Colston and Jackson, 2016; Sehnal et al., 2021). This is particularly true for microbial communities that reside in the gut, as they play instrumental roles in mitigating the development and severity of disease (Martin et al., 2018),

improving host metabolic activity in response to harsh environmental conditions (Li et al., 2019), and regulating thermal homeostasis (Chevalier et al., 2015; Alberdi et al., 2016). Despite the recognition that gut microbiomes play a critical role in modulating host health, persistence, and adaptation, many studies of non-mammalian gut microbiomes include intraspecific comparisons only (Keenan et al., 2013; Colston et al., 2015; McLaughlin et al., 2015; Colston and Jackson, 2016; Arizza et al., 2019; Qin et al., 2019; Tang et al., 2019), limiting our ability to identify environmental and host-associated processes that contribute to microbiome differences observed among distinct populations and communities of vertebrate taxa. As such, there exists a need for integrative investigations of microbiome variation among diverse host organisms, and a growing number of studies have applied fundamental theories in ecology and evolutionary biology to help illuminate the mechanisms that shape the assemblage and diversity of non-mammalian vertebrate microbiomes (Sullam et al., 2012; Sanders et al., 2014; Bletz et al., 2017; Hird, 2017; Sherrill-Mix et al., 2018; Youngblut et al., 2019; Song et al., 2020; Smith et al., 2021, 2023; Bodawatta et al., 2022; Eliades et al., 2022; Kirsch et al., 2024). However, no studies to date have incorporated theories of island biogeography to explore patterns of microbial diversity among vertebrate hosts spanning true islands. Therefore, we provide the first assessment of variation in gut microbiomes among vertebrate species distributed across a true island archipelago system in Southeast Asia, the Philippines, employing theories in island biogeography to further investigate the mechanisms that shape the vertebrate gut microbiome.

Situated at the interface of the Asian and Australasian faunal zones, the Philippine archipelago has emerged as a natural laboratory for studying various evolutionary and ecological phenomena from adaptive radiation (Brown et al., 2008, 2013a; Heaney et al., 2011; Linkem et al., 2011; Setiadi et al., 2011; Siler and Brown, 2011; Blackburn et al., 2013), diversification (Gillespie, 2007; Losos and Ricklefs, 2009; Vences et al., 2009; Brown et al., 2013a) and long-distance dispersal (Linkem et al., 2013; Brown and Siler, 2014; Welton et al., 2014), to patterns of community assembly (Esselstyn et al., 2009, 2011; Heaney et al., 2011; Siler and Brown, 2011; Brown et al., 2013a) and continental and island biogeography (Siler et al., 2012). Further, three decades of taxonomic revisions, novel species discoveries, extensive and repeated biodiversity surveys, and comprehensive reviews of the resident biodiversity have contributed to our growing understanding of the boundaries, distributions, and phylogenetic relationships of many vertebrate taxa residing within the island system (Brown et al., 2002, 2008, 2011, 2013b; Posa et al., 2008; Heaney et al., 2011; Siler et al., 2010, 2011), but a particularly expansive body of work has focused on describing the diversity of reptiles found within the island nation (Brown et al., 2002, 2008, 2011, 2013b; Siler et al., 2010, 2011).

Reptiles (amphisbaenians, crocodilians, lizards, snakes, tuataras, turtles) are an ecologically diverse group of vertebrate organisms that inhabit a wide array of habitats, occurring on all continents except Antarctica (Vitt et al., 2003; Vitt and Caldwell, 2013). The vertebrate group comprises over 12,000 species (Uetz et al., 2023) which display a myriad of life history traits (i.e., dietary habits, parental care, behavioral thermoregulation; Shine, 2005), reproductive modes, and morphological adaptations (Shine and Bonnet, 2000; Vitt and Caldwell, 2013). Despite a rich history of foundational research on the group, and their continual incorporation into studies of high-level concepts in ecology and evolutionary biology (e.g., venom evolution, adaptive radiation, development, dispersal, diversification; Crews et al., 1994; Losos and Miles, 2002; Rocha et al., 2006; Ricklefs et al., 2007; Evans and Jones, 2010; Losos, 2011; Townsend et al., 2011; Siler et al., 2012; Pincheira-Donoso et al., 2015; Kissling et al., 2016; Bars-Closel et al., 2017; Griffing et al., 2019; Oaks et al., 2019; Schield et al., 2019; Bergmann et al., 2020;

Perry et al., 2022; Rothier et al., 2022; Simões et al., 2022), the reptile gut microbiome literature comprises few comparative studies between diverse host organisms, and there exists a general paucity of information regarding the processes that shape gut microbiome diversity and assemblage (Hoffbeck et al., 2023; Vasconcelos et al., 2023), especially among reptilian host species that inhabit true oceanic island systems. Further, although it has been recognized that host evolutionary history influences reptile gut microbiome diversity (Ren et al., 2016; Zhang et al., 2019; Smith et al., 2021; Hoffbeck et al., 2023; Vasconcelos et al., 2023), no published studies to date have tested for signatures of phylogenetic signal or cophylogeny between reptilian hosts and their gut microbiomes, leaving gaps in our understanding of the role host evolutionary history may play in shaping reptile gut microbiome diversity and evolution.

Here, we compare the gut microbiomes of reptilian hosts spanning four islands in the Philippines, applying island biogeography theory to explore patterns of insular host-associated microbiome diversity. Surprisingly, our findings indicate an inverse relationship between island size and reptilian gut microbiome diversity, underscoring the need for future studies which investigate patterns of host-associated microbial diversity across true island systems. We also provide the first assessments of cophylogeny and phylogenetic signal between reptilian hosts and their associated gut microbial communities, sampling reptiles from two orders, 11 families, and 50 species to further examine if host evolutionary history contributes to variation in reptile gut microbiome diversity. Results from our comparative study contribute to our growing understanding of the environmental, host-associated, and biogeographic processes that shape vertebrate gut microbiome diversity and assemblage among host communities inhabiting true island systems.

3.3 Materials and Methods

Sample collection

Fieldwork for this study was conducted from 2016–2018 on four islands in the Philippines—Calayan, Camiguin Norte, Luzon, and Negros (Figure 3.1). These islands are located within three distinct faunal regions (Brown et al., 2013a). Calayan and Camiguin Norte islands occur within the Babuyan Island Group faunal region, Negros is found within the Negros-Panay faunal region, and Luzon Island is situated within the Luzon faunal region. In total, we sampled reptile communities from nine sites across the four islands: five sites on Luzon Island (North: Gattaran and Quezon, South: Albay, Labo, and Tabaco), two sites on Negros Island (Hinoba-an and Valencia), one site on Calayan Island, and one on Camiguin Norte Island. All samples collected from the Gattaran site were removed from the dataset due to poor sequencing data, resulting in eight focal sites being included in downstream analyses (Figure 3.1; Appendix C, Table S1). To explore the geographic location hypothesis, we conducted robust analyses of microbiome diversity across various geographic scales. This included intra-island and inter-island site comparisons, total island comparisons, and faunal region comparisons. We also grouped the three southern Luzon sites into their own ‘sub-faunal region’, referred to as the Bicol, which we compared to the northern Luzon site of Quezon (Figure 3.1; Appendix C, Table S1).

We swabbed 299 individuals representing two reptilian orders, 11 families, and 50 species. A complete species list is provided in Appendix C, Table S1. Reptiles were categorized into four broad ecological groups, aquatic (N = 20), arboreal (N = 162), burrowing (N = 57), and ground-dwelling (N = 60), based on personal observations and literature reviews. Then, we divided the aquatic and arboreal groups into freshwater/marine and saxicolous/lower-level arboreal/upper-

level arboreal, respectively, to conduct a more fine-scale evaluation of microbiome variation across host microhabitat groups (Appendix C, Table S1).

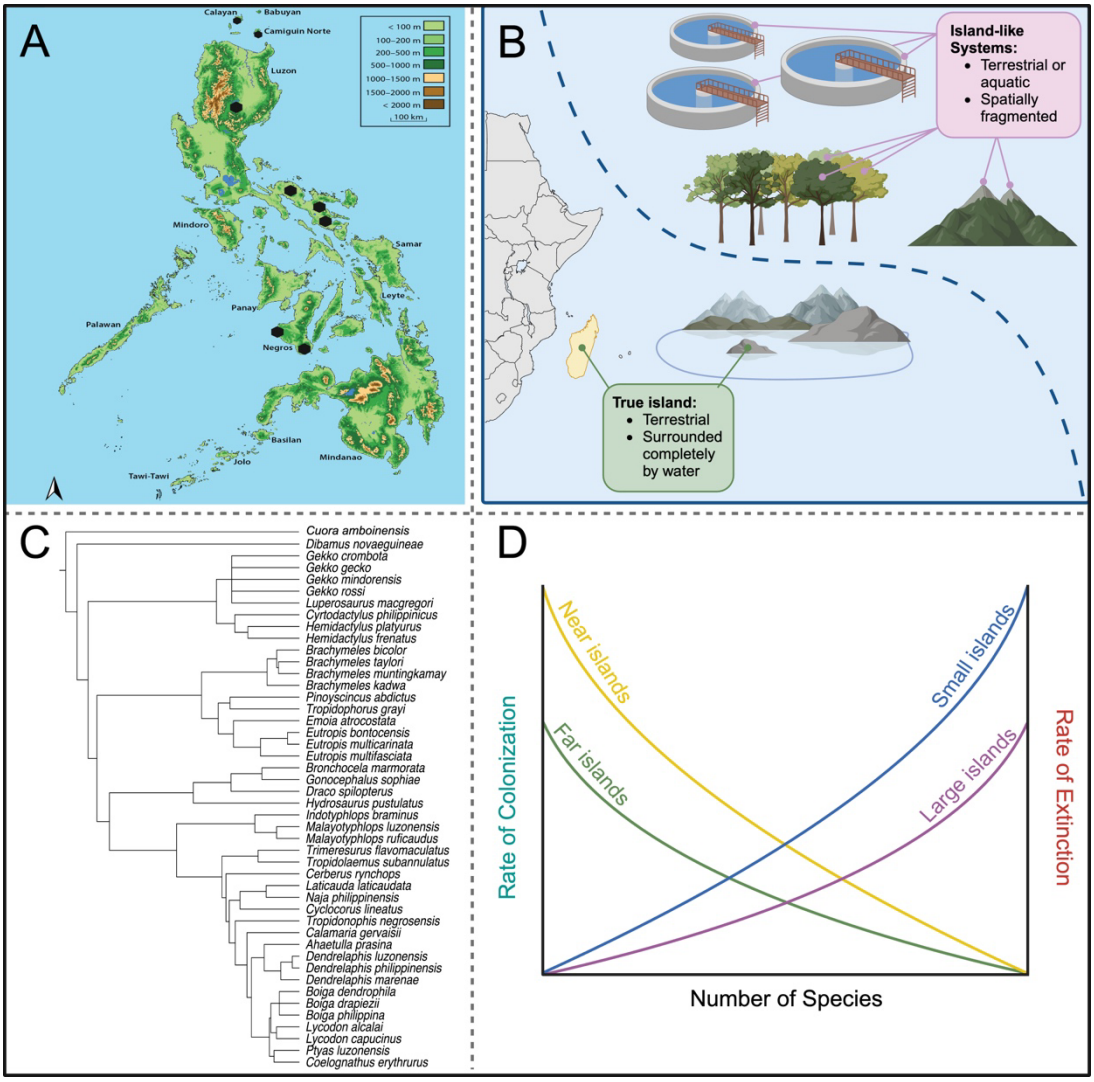

Figure 3.1 | **(A)** Map of the Philippines with black hexagons depicting the eight focal sites sampled. **(B)** Graphical representation of the characteristics that distinguish a true island from an island-like system. Examples of island-like systems include wastewater tanks, trees, and high elevation 'sky' islands. **(C)** Phylogenetic relationships between the focal host taxa depicted using a cladogram obtained through VertLife (Tonini et al., 2016). **(D)** Expectations for MacArthur and Wilson's Equilibrium Theory of Island Biogeography. Small islands will experience a greater rate of extinction when compared to large islands, and islands that are more distant from the mainland will have a slower rate of colonization than islands that are closer to the mainland.

Gut microbiome samples were collected by inserting a sterile swab (MWE, Corsham, England) into the cloaca of the individual for approximately 3–4 seconds, twirling the swab 5–7 times. All swabs were preserved and stored in DNA/RNA Shield (Zymo Research Products, Irvine, CA, USA) at the time of collection at ambient temperature in field conditions until returned to the United States (approximately 10–21 days) where they were stored in a –20°C freezer until DNA extraction. All samples were collected in accordance with the University of Oklahoma's Institutional Animal Care and Use Committee (IACUC Permit Nos: R17–019). Field collection and export permits were provided by the Biodiversity Management Bureau (BMB) of the Philippine Department of Environment and Natural Resources (DENR) Nos. 260 (Renewal) and 273 (Renewal).

DNA extraction and sequencing

Genomic DNA was extracted from 299 swabs using Xpedition™ Soil/Fecal DNA MiniPrep kits (Zymo Research Products, Irvine, CA, USA) in the Shared Genomics Core facilities of the Sam Noble Museum. Ten negative controls (i.e., Xpedition™ Soil/Fecal DNA MiniPrep reagents only without a swab sample) were extracted alongside our 299 focal samples. We amplified and sequenced the 299 gut microbiome samples, 10 extraction negative controls, and two PCR-negative controls using methods outlined in Smith et al. (2021) and described here in brief. We used a one-step Polymerase Chain Reaction (PCR) method to amplify the V4 hypervariable region of the 16S rRNA gene using primers described in Kozich et al. (2013). After bead clean-up (KAPA Pure Beads; Roche Sequencing Solutions, Pleasanton, CA, USA), we quantified each sample and normalized to 10 nM of DNA before pooling the samples into a single sterile, 1.5 mL microcentrifuge tube. Sequencing was performed at the University of Oklahoma Consolidated Core Lab using the 2x250 bp paired-end sequencing on two runs of an Illumina MiSeq.

Sequence analysis

Adapter sequences described in Kozich et al. (2013) were trimmed from the paired-end raw sequencing reads using AdapterRemoval v2 and the following parameters—minquality: 30, trimqualities, maxns: 0, trimns, threads: 18 (Schubert et al., 2016). The sequences were then imported into QIIME 2 (Bolyen et al., 2019) where *de novo* chimera checking, and removal, was performed on 6,616,892 raw sequences using UCHIME (Edgar et al., 2011). This process removed around 47,000 reads, and 5,070,909 nonchimeric sequences were grouped into 50,124 operational taxonomic units (OTUs) with a closed-reference OTU database at 97% sequencing similarity using VSEARCH (Rognes et al., 2016) against the Silva database (version 138; Quast et al., 2012), indicating that nearly 80% of the raw sequences were grouped successfully into OTUs. There were 37 samples from the Quezon and Gattaran sites that were removed due to poor sequencing data (Appendix C, Table S1). Once removed, 262 samples, 38,496 OTUs, and 4,369,039 sequences remained. We rarefied the remaining 262 samples to a sequencing depth of 1,000 sequences for downstream analyses which removed 45 additional samples from the dataset due to low sequence counts, leaving 217 samples and 13,288 OTUs to be used in the focal analyses (Appendix C, Figure S1). Raw sequence data generated in this study can be found in the Sequence Read Archive (SRA) under BioProject PRJNA1071189.

Statistical Analysis

The final dataset consisted of 217 post-rarefaction samples that were incorporated into downstream analyses. We applied a suite of analytical techniques to determine the variables that contributed to gut microbial differences observed among our 47 reptile host species. Alpha

(within sample) diversity was obtained via QIIME 2 (Bolyen et al., 2019) and measured using three metrics: Observed OTUs, Shannon Diversity, and Faith's Phylogenetic Diversity. Alpha diversity was compared initially using univariate analyses and the alpha-group-significance plugin in QIIME 2, which performed pairwise Kruskal-Wallis tests to determine significant differences between groups. A p-value or q-value < 0.05 was considered statistically significant (Kennedy-Shaffer, 2019), and when multiple pairwise comparisons were conducted, we reported q-values instead of p-values to correct for multiple tests (Storey, 2002, 2003; Storey and Tibshirani, 2003). Further, we used a data mining approach via the R package *PARTY* (Hothorn et al., 2006; R Core Team, 2023) to perform a Classification and Regression Tree Analysis (CART), and determine which variable (host species, family, suborder and order, island, county, faunal region, sub-faunal region, ecology, microhabitat, and island size) accounted for most of the variance observed in the alpha diversity of our 47 focal host species.

In addition to these exploratory analyses, we also used a suite of analyses in R to explicitly test the relationship between island size and gut microbial diversity among our focal hosts. We tested for the species-area relationship (SAR) using the package *sars* (Matthews et al., 2019; R Core Team, 2023) which log-transformed the island size and two of the diversity variables: Observed OTUs and Shannon Diversity (i.e., log-log power model). We also tested the SAR on log-transformed island size and non-transformed microbial diversity using a regression analysis within a Phylogenetic Generalised Linear Mixed Model (PGLMM) framework using the R packages *brms* (Bürkner, 2017) and *ape* (Paradis and Schliep, 2019) with the following parameters: chain = 4, iter = 20,000, thin = 50, warmup = 7,000, adapt_delta = 0.98. Log-transformed host body mass was included as a covariate while host species and host phylogeny were treated as random effects. An additional PGLMM was used to test the relationship between log-transformed host body mass and non-transformed Faith's Phylogenetic Diversity, with host species and host phylogeny treated as random effects, as this diversity metric was excluded from the SAR analyses. The Highest Density Interval was calculated using the *bayestestR* package in R (Makowski et al., 2019).

To determine the impact that host evolutionary history had on reptilian gut microbial diversity, we used a PGLMM to calculate phylogenetic signal (h^2). We tested for phylogenetic signal using the three alpha diversity metrics and a reptile host phylogeny obtained through Open Tree of Life (Redelings and Holder, 2017). We used default PGLMM parameters, and our random effects were host species and host phylogeny. Further, we tested for signatures of co-diversification between our focal host species and their associated gut microbial phyla using the Procrustes Approach to Cophylogeny (PACo; Hutchinson et al., 2017) and ParaFit (Legendre et al., 2002) which both utilized distance matrices of our reptile host phylogeny (obtained through Open Tree of Life; Redelings and Holder, 2017) and a published, phylum-level microbial phylogeny from the Web of Life (Zhu et al., 2019) in addition to a binary host-microbe association matrix. These analyses were performed using the R packages *ape* (Paradis and Schliep, 2019), *paco* (Hutchinson et al., 2017), *reshape2* (Wickham, 2007), and *ggtree* (Yu et al., 2017; R Core Team, 2023).

Beta diversity (among samples) analyses were conducted using the beta-group-significance plugin in QIIME 2 and a q-value < 0.05 was considered statistically significant (Kennedy-Shaffer, 2019). We used two phylogenetic measures of microbial community beta diversity, Unweighted- and Weighted-Unifrac, and group significance was tested using pairwise permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001). We reported

q-values instead of p-values to correct for multiple tests (Storey, 2002, 2003; Storey and Tibshirani, 2003).

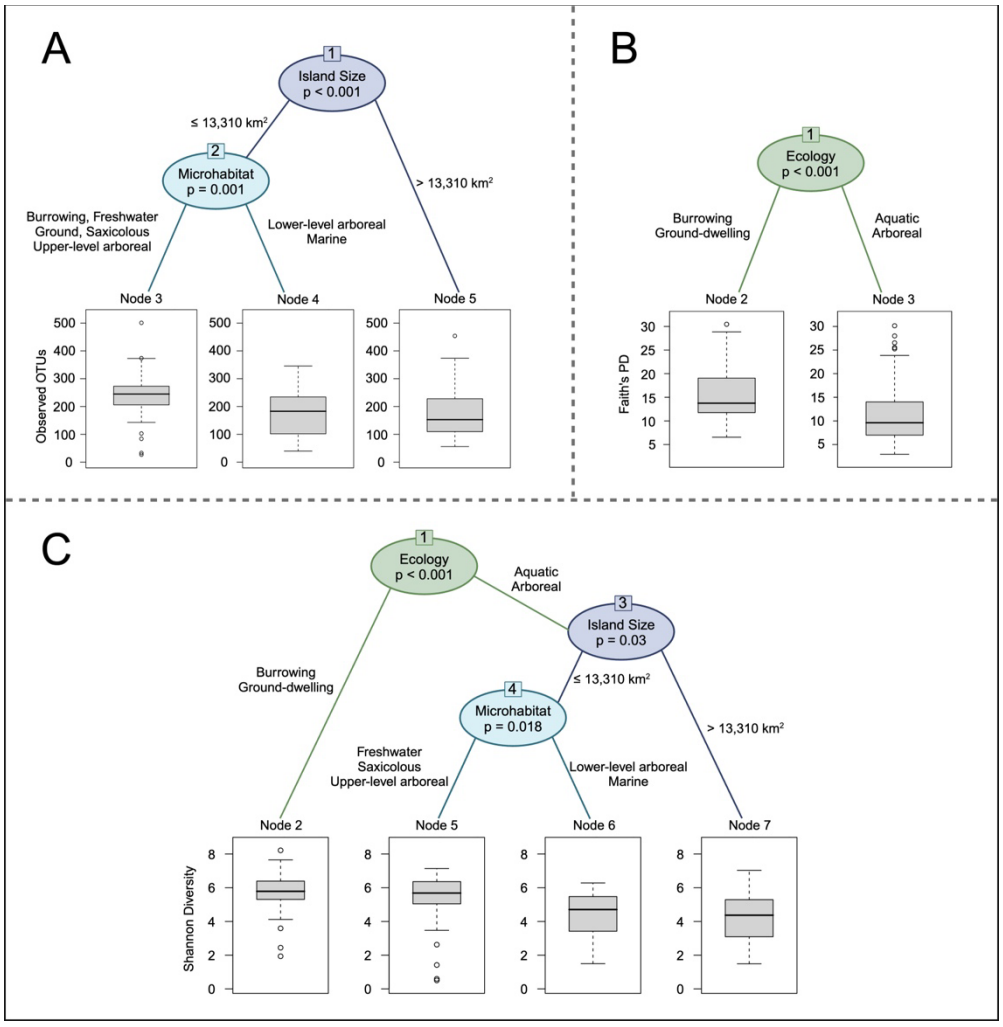

Figure 3.2 | Results of the Classification and Regression Tree analysis using three alpha diversity metrics: **(A)** Observed OTUs, **(B)** Faith's Phylogenetic Diversity (PD), and **(C)** Shannon Diversity. Host ecology was the primary contributor to gut microbiome differences according to the Faith's PD and Shannon Diversity analyses. The Observed OTUs analysis indicated that island size explained most of the variation in microbiome diversity, with individuals from smaller islands (i.e., ≤ 13,310 km^2) having more diverse microbiomes when compared to reptiles from the largest island of Luzon. A similar pattern was observed in the Shannon Diversity analysis, which found that host ecology and island size explained most of the variance in microbiome diversity, followed by microhabitat. Microhabitat was also determined to be the secondary contributor to microbiome differences within the Observed OTUs analysis. This figure was created with BioRender.com.

3.4 Results

Our focal dataset consisted of 217 gut microbiome samples representing two host orders, 10 host families, and 47 host species spanning eight sites across four islands in the Philippines (Appendix C, Table S1). Results from our CART analyses revealed that host ecology, island size, and host microhabitat were key contributors to gut microbiome alpha diversity differences among our focal reptile species (Figure 3.2). For Observed OTUs, island size explained most of the variance ($\chi^2 = 80.778$, $p < 0.001$), with microbiomes from hosts on the largest island, Luzon, being less diverse than those from hosts on the smaller islands ($\leq 13{,}310$ km^2; Figure 3.2). Host microhabitat explained the remaining variance, with the microbiomes of burrowing, freshwater, ground, saxicolous, and upper-level arboreal reptiles being significantly more diverse than the microbiomes of lower-level arboreal and marine species ($\chi^2 = 65.863$, $p = 0.001$; Figure 3.2). These differences were confirmed by many of our beta diversity analyses and univariate Kruskal-Wallis tests (Appendix C, Figure S2, Table S2).

When analyzing Shannon Diversity, host ecology explained most of the variance, with microbiomes of burrowing and ground-dwelling hosts being more diverse than those of aquatic and arboreal species ($\chi^2 = 73.874$, $p < 0.001$; Figure 3.2). This was confirmed by our univariate Kruskal-Wallis test, which determined that the microbiomes of aquatic individuals had significantly lower Shannon Diversity when compared to burrowing ($H = 13.969$, $q = 0.0006$) and ground-dwelling reptilian microbiomes ($H = 15.476$, $q = 0.0005$). Similarly, microbiomes of arboreal hosts had significantly lower Shannon Diversity when compared to those of burrowing ($H = 7.613$, $q = 0.012$) and ground-dwelling ($H = 6.463$, $q = 0.017$) hosts (Appendix C, Figure S3). Results from our beta diversity analyses further confirmed these observed differences (Appendix C, Table S2). Island size also contributed to Shannon Diversity differences observed among our focal samples ($\chi^2 = 40.381$, p-value = 0.03). Similar to results from the Observed OTUs CART analysis, microbiomes from hosts on smaller islands ($\leq 13{,}310$ km^2) were more diverse compared to those of hosts from the large island of Luzon (Figure 3.2). Host microhabitat ($\chi^2 = 40.135$, $p = 0.018$) explained the remaining variance in Shannon Diversity, with freshwater, saxicolous, and upper-level arboreal hosts having more diverse microbiomes than those of lower-level arboreal and marine reptiles (Figure 3.2). Results from our univariate Kruskal-Wallis analysis supported many of these comparisons (Appendix C, Figure S2).

For Faith's Phylogenetic Diversity (Faith's PD), host ecology explained all of the gut microbiome variance, with burrowing and ground-dwelling reptiles having more diverse microbiomes than aquatic and arboreal species ($\chi^2 = 69.353$, $p < 0.001$; Figure 3.2), which was consistent with the Shannon Diversity CART analysis and further confirmed by our univariate Kruskal-Wallis (Appendix C, Figure S3) and beta diversity analysis (Appendix C, Table S2). Results from our Kruskal-Wallis test also indicated that aquatic reptiles harbored significantly less-diverse microbiomes than burrowing ($H = 8.879$, $q = 0.006$) and ground-dwelling ($H = 4.464$, $q = 0.052$) species. Further, arboreal individuals had significantly lower microbial diversity when compared to burrowing ($H = 18.222$, $q = 0.0001$) and ground-dwelling ($H = 10.282$, $q = 0.004$) reptilian microbiomes (Appendix C, Figure S3).

The species-area relationship (SAR) describes the widely accepted and frequently observed pattern that the number of species increases with the area sampled (Rosenzweig, 1995; Matthews et al., 2019). However, results from our Observed OTUs and Shannon Diversity CART analyses indicated the inverse pattern, where microbial diversity was the lowest among hosts from the largest island sampled, Luzon (Figure 3.2). We further explored this by explicitly testing the SAR on the number of Observed OTUs and Shannon Diversity present within the gut

microbiomes of hosts spanning the four focal islands ranging in size from 166–109,965 km^2. We did not test the SAR using Faith's Phylogenetic Diversity, because the CART analysis did not identify island size as a key predictor for this diversity metric. Results from our log-log SAR analyses confirmed a slight, but significant, inverse relationship between island size and microbial alpha diversity (Table 3.1) which was further supported by our PGLMM island size regression results (Table 3.1).

	Observed OTUs	**Shannon Diversity**
Species-Area Relationship (*log-log power model*)	z = -0.04 F = 15.31 **p-value = 0.0001**	z = -0.02 F = 8.63 **p-value = 0.004**
PGLMM Island Size Regression	β = -6.25 l-95% CI = -11.63 u-95% CI = -1.72	β = -0.08 l-95% CI = -0.17 u-95% CI = 0.00

Table 3.1 | Evaluation of the Species-Area Relationship (SAR) using Observed OTUs and Shannon Diversity. Bold values indicate significant differences. The log-log power model found a significant, negative relationship between log-transformed island size and log-transformed microbial diversity which was also confirmed by the negative slopes of the PGLMM island size regressions.

Previous studies have found a positive relationship between host body mass and microbial diversity (Godon et al., 2016; Sherrill-Mix et al., 2018). Additionally, island size has been linked to insular reptilian body size variation, with more pronounced differences observed among reptiles inhabiting smaller islands (Boback, 2003; Benítez-López et al., 2021). Therefore, to rule out this potential confounding variable, we assessed the relationship between log-transformed host body mass and microbiome diversity using a PGLMM. Our results indicated that there was a negligible relationship between log-transformed host body mass and two of the diversity metrics: Observed OTUs: β = 0.01, 95% CI [-7.44, 8.13] and Shannon Diversity: β = -0.03, 95% CI [-0.23, 0.15]. However, we found a slight positive relationship when we compared log-transformed host body mass and Faith's PD (β = 0.11, 95% CI [-0.68, 0.85]).

We also used Kruskal-Wallis tests to assess if geographic location contributed to microbiome variation, given that our eight sites were clustered within four islands (Figure 3.1). Across all three alpha diversity metrics, we found no significant differences in the gut microbiomes of individuals from the same island. For example, microbiome diversity did not differ between samples collected from the two sites on Negros Island (Hinoba-an and Valencia) or the four Luzon Island sites (Albay, Labo, Quezon, and Tabaco; Appendix C, Table S3). Further, when comparing sites across all four islands, only the Observed OTUs analysis found any significant differences between the sites sampled (Appendix C, Table S3). Inter-island comparisons of Observed OTUs indicated that individuals from Calayan, Camiguin Norte, and Negros islands possessed significantly different gut microbiomes when compared to reptiles from Luzon Island (Appendix C, Table S3). Similarly, Shannon Diversity analysis found significant differences between the gut microbiomes of individuals from Luzon Island and both Calayan and Negros islands (Appendix C, Table S3). These differences were supported by our microbiome composition results which indicated that several microbial phyla were found among samples

from Calayan, Camiguin Norte, and Negros islands only, and were not identified within Luzon Island samples (Appendix C, Figure S4; Supplementary Data).

Our CART analyses indicated that host order, host suborder, host family, and host species did not contribute significantly to microbiome variation (Figure 3.2), which was also supported by our microbiome composition results (Supplementary Data). To explicitly test the host evolutionary history hypothesis, we used PGLMMs to quantify phylogenetic signal, and found no evidence of phylogenetic signal across all measures of alpha diversity (Observed OTUs: h^2 = 0.14, Estimated Error = 0.11; Shannon Diversity: h^2 = 0.16, Estimated Error = 0.10; Faith's Phylogenetic Diversity: h^2 = 0.14, Estimated Error = 0.1). Further, we did not find evidence of co-diversification between our focal reptilian hosts and their gut microbiomes (ParaFit: ParaFitGlobal = 211.58, p = 0.785, 999 permutations; PACo: Sum of Squares = 708.24, p = 1, 999 permutations; Appendix C, Figure S5). Based on these findings, we rejected the hypothesis that host evolutionary history contributed significantly to gut microbiome variation among our focal samples.

3.5 Discussion

Our study sampled the gut microbiomes of reptile communities spanning true islands in the Philippines to provide the first application of island biogeography theory to investigate patterns of host-associated microbiome diversity within a true island archipelago system. Results from our CART and regression analyses indicated an inverse relationship between reptile gut microbiome diversity and island size (Table 3.1; Figure 3.2). These findings do not align with the foundational ETIB expectation that diversity increases with increased island size (MacArthur and Wilson, 1963; Rosenzweig, 1995; Matthews et al., 2019). Although many previous studies have found a positive correlation between 'island' size and microbial diversity (Bell et al., 2005; Reche et al., 2005; Van Der Gast et al., 2005, 2006; Peay et al., 2007; Glassman et al., 2017), few have also observed an inverse relationship (Hessen et al., 2006; Logue et al., 2012). Indeed, when testing the SAR within a lake 'island' system, a negative relationship between lake area and zooplankton (Hessen et al., 2006) richness was observed. Logue et al. (2012) also found an inverse relationship between bacterioplankton richness and 'island' size. However, due to the general paucity of published studies which analyze patterns of microbial diversity spanning true island systems, it remains difficult to determine if microbial communities, particularly host-associated microbiomes, adhere to ETIB expectations established for insular plant and animal biodiversity. The continued application of these theories to help explain patterns of microbial diversity among diverse host organisms will help illuminate the ecological and geographic processes that shape these complex microbial communities.

We also found that host ecology was a primary contributor to gut microbiome diversity differences observed among the 47 focal reptilian species, and host microhabitat also explained some of the variance (Figure 3.2; Appendix C, Figure S2, S3). The CART, Kruskal-Wallis, and beta diversity analyses all confirmed that burrowing and ground-dwelling reptiles possessed significantly more diverse microbiomes when compared to aquatic and arboreal species (Figure 3.2; Appendix C, Figure S3; Table S2). Among many arboreal samples, Proteobacteria comprised over 80% of the gut microbiome composition (N = 50 out of 129 arboreal reptiles), and the microbiomes of some aquatic and arboreal individuals were over dominated by Campylobacterota (50–96%, N = 9). The overabundance of these phyla within aquatic and arboreal gut microbiome samples could explain why the microbiomes of those taxa were found to be less diverse than the microbiomes of burrowing and ground-dwelling species. Previous

findings have indicated that differences in reptile gut microbiome diversity correlate with distinct host ecologies (Smith et al., 2021; Vasconcelos et al., 2023), and processes such as differential selection may contribute to the observed relationship between host ecology and microbiome variation (Mazel et al., 2018; Kohl, 2020; Mallott and Amato, 2021). When applied to host-associated microbiomes, differential selection predicts that hosts occupying similar ecological niches will be exposed to similar resident pools of microorganisms, and the immune systems of the hosts then filter and select certain microbes to persist as symbionts (Kohl, 2020). The idea of hosts obtaining certain members of their microbiomes from the surrounding environment has been discussed extensively in the vertebrate skin microbiome literature (Belden and Harris, 2007; Becker et al., 2014; Loudon et al., 2014; Bletz et al., 2017; Jiménez and Sommer, 2017; Kueneman et al., 2019; Rebollar and Harris, 2019; Hernández-Gómez et al., 2020; Barnes et al., 2021; Smith et al., 2023), and our study further supports that host ecology impacts the composition and diversity of vertebrate gut microbiomes.

Other evolutionary mechanisms that are proposed to contribute to host-associated microbiome variation include dispersal (i.e., horizontal and vertical transmission), ecological drift, and host diversification, otherwise known as co-speciation (Kohl, 2020). The process of co-speciation refers to when hosts speciate over evolutionary time and their communities of symbiotic microbes also evolve in a way that matches the host phylogeny (Brooks et al., 2016; Lim and Bordenstein, 2020). This concept is also referred to as phylosymbiosis (Mazel et al., 2018; Lim and Bordenstein, 2020), and many organisms have been shown to exhibit phylosymbiosis with their associated microbiomes, including insects (Sanders et al., 2014; Brooks et al., 2016; Leigh et al., 2018; Tinker and Ottesen, 2020; Qin et al., 2023), mammals (Ochman et al., 2010; Kohl et al., 2018a, 2018b; Amato et al., 2019), sponges (Easson and Thacker, 2014; Thomas et al., 2016), corals (Pollock et al., 2018), and plants (Bell et al., 2014; Bouffaud et al., 2014; Yeoh et al., 2017; Fitzpatrick et al., 2018). To our knowledge, our study is the first to test this phenomenon on reptilian microbiomes; however, we did not find signatures of phylosymbiosis or co-diversification among our focal samples.

Applied historically to simple host-symbiont systems (i.e., one host clade and a single symbiont clade), evaluations of co-diversification between hosts and entire communities of associated microorganisms are far less common and pose many challenges (Moeller et al., 2023). For example, revealing signals of co-diversification often requires a well-resolved symbiont phylogeny, often at a fine taxonomic scale (i.e., strain-level), and most microorganisms within a host's microbiome lack fossil records, making it difficult to accurately date the timing of diversification events within a symbiont phylogeny. Our use of a high-level symbiont phylogeny (i.e., phylum-level) may have inhibited our ability to detect a significant association between our focal reptilian hosts and their gut microbiota; therefore, our findings do not rule out the possibility of historical codivergence between these lineages (Moeller et al., 2023). As such, future studies should continue sampling the microbiomes of diverse reptilian hosts and consider isolating individual microbial lineages to conduct more fine-scale evaluations of co-diversification and phylosymbiosis among reptilian hosts and their associated microbiota. We argue that the rich, and often endemic, reptilian diversity of the Philippine archipelago represents an ideal system to further explore these paradigms.

Although the Philippines has long been considered an exceptional island system for investigating a myriad of ecological and evolutionary concepts (Gillespie, 2007; Losos and Ricklefs, 2009; Vences et al., 2009; Heaney et al., 2011; Linkem et al., 2011, 2013; Setiadi et al., 2011; Siler and Brown, 2011; Siler et al., 2012; Blackburn et al., 2013; Brown et al., 2013a,

2014; Welton et al., 2014), few published studies to date have explored the gut microbiomes of the archipelago's vertebrate taxa (De Leon et al., 2018; Smith et al., 2021; Eliades et al., 2022), and our study is the first to integrate fundamental theories in island biogeography and evolutionary biology to help identify the factors that contribute to microbiome variation among host communities spanning numerous islands in this diverse archipelago. The Philippines represents an excellent island system to conduct biogeographic studies of host-associated microbial communities in a systematic way, allowing future research to analyze community-level microbiome differences across various geographic scales. For example, the Philippine islands can be grouped into various faunal regions which are groups of islands that are proposed to have been joined together at some point in their geological history (Brown et al., 2013a). There are seven defined faunal regions within the Philippines, each comprising numerous different islands varying in size from large islands such as Luzon and Mindanao to the small islands of the Sulu faunal region (Brown et al., 2013a). Sampling host communities from a single faunal region would help control for variation in the geological history of the islands and the biogeographical history of the insular hosts, allowing for more controlled investigations of island biogeography theory within a host-associated microbiome context. For example, although there has been mixed evidence of microbial communities adhering to the fundamental principle that diversity on islands decreases with increased distance from the mainland (Grilli et al., 2015; Teittinen and Soininen, 2015; Da Silva et al., 2017; Davison et al., 2018; Spiesman et al., 2018), no published studies to date have investigated this paradigm among vertebrate host-associated microbial communities, and the Philippines represents an optimal archipelago for future work to explore this phenomenon within and among faunal regions.

Continued field surveys across the Philippines would enable expanded comparisons of host-associated microbiomes, both taxonomically (across orders, families, etc.) and geographically (within and among islands, within and among faunal regions), allowing for future investigations to identify the environmental, evolutionary, and biogeographical mechanisms that shape the assemblage of vertebrate gut microbiomes. Results from this study suggest that multiple factors shape the reptile gut microbiome; however, host ecology and island size were the primary contributors to gut microbial community differences observed among our focal samples. Comparative studies of gut microbiomes spanning complex landscapes, such as oceanic islands, allow us to apply well-established and foundational theories in ecology, evolutionary biology, and biogeography to explain patterns of host-associated microbial diversity. The investigation of reptile gut microbiomes remains critical, especially in the Philippines, where the vertebrate group continues to face challenges imposed by habitat loss, climatic changes, and the international wildlife trade.

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Appendix A

Supplementary Material for Venomous snakes reveal ecological and phylogenetic factors influencing variation in gut and oral microbiomes

Supplementary Figures

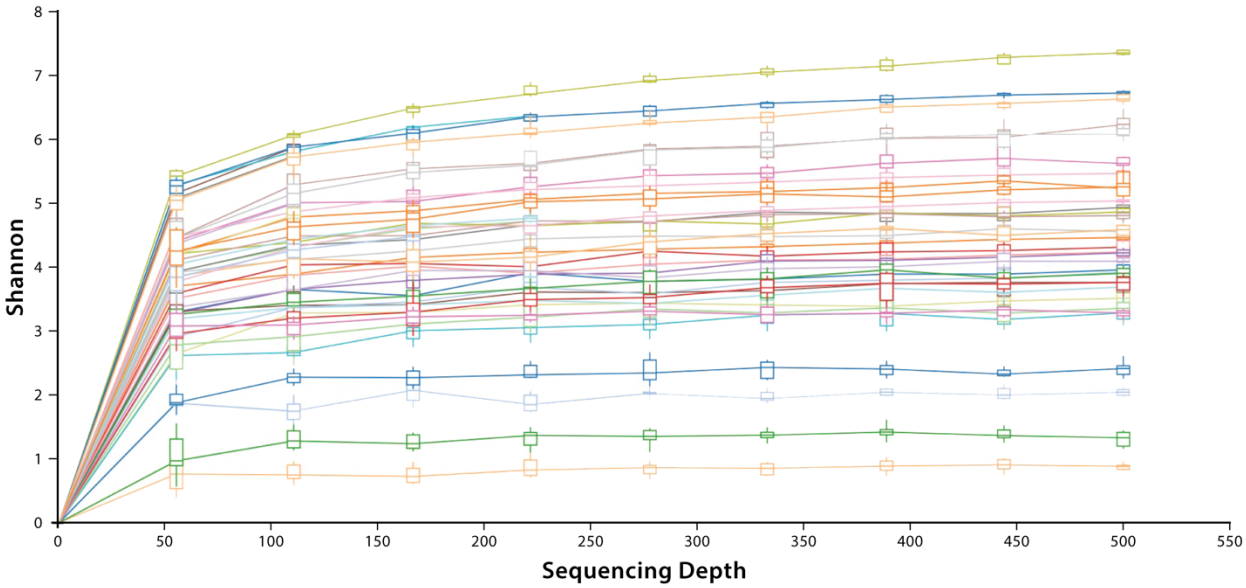

Figure S1 | Alpha diversity rarefaction curves based on Shannon diversity. Each curve is representative of a single swab. Based on these curves, we decided to rarefy all samples to a sequencing depth of 500 sequences.

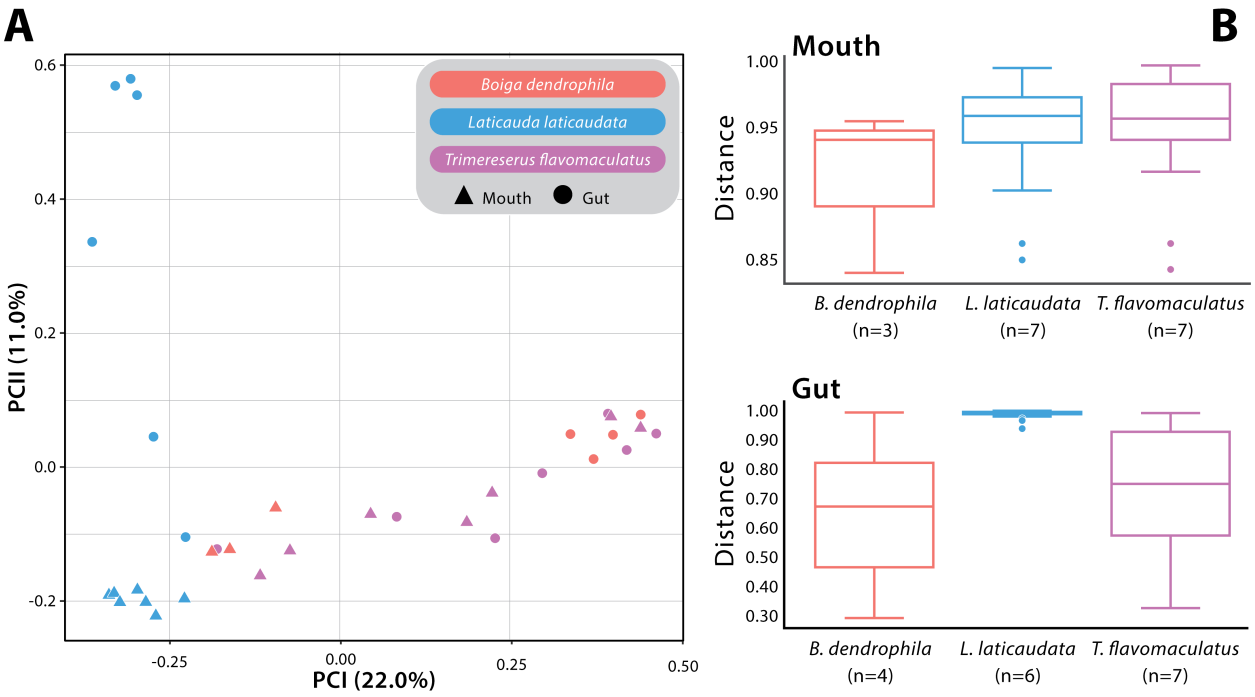

Figure S2 | Beta diversity analysis based on the Bray-Curtis dissimilarity matrix. **(A)** PCoA of all samples with the different body sites represented as triangles = mouth samples and circle = gut samples, and point color representing the different host species: red = *Boiga dendrophila*, blue = *Laticauda laticaudata*, and purple = *Trimeresurus flavomaculatus*. **(B)** Boxplots generated by the PERMANOVA comparing the mouth and gut samples from each host species.

Supplementary Tables

Host Species	Habitat Type	Venom Toxin	Mouth sample no. (pre-rarefaction)	Mouth sample no. (post-rarefaction)	Gut sample no. (pre-rarefaction)	Gut sample no. (post-rarefaction)
Boiga dendrophila	Semi-Arboreal	Denmotoxin	8	3	8	4
Laticauda laticaudata	Marine	Neurotoxin	7	7	7	6
Trimeresurus flavomaculatus	Arboreal	Hemotoxin	7	7	7	7
Total			**22**	**17**	**22**	**17**

Table S1 | Sample sizes for the total number of mouth and gut samples before and after rarefaction to exclude samples with low sequence number (i.e. <500 reads per sample).

Host Species	Acidobacteria	Actinobacteria	Bacteroidetes	Firmicutes	Proteobacteria
Boiga dendrophila	6.33%	20.65%	3.98%	9.68%	50.26%
Laticauda laticaudata	1.61%	9.76%	19.90%	11.54%	38.94%
Trimeresurus flavomaculatus	1.52%	3.45%	8.13%	2.93%	82.28%

Table S2 | Relative abundances of the five dominant (relative abundance > 1%) microbial phyla present in all mouth samples from the three host species.

Beta diversity analysis	Host ecology (gut samples)	Species (mouth samples)	Body site (marine samples)	Body site (arboreal samples)	Body site (semi-arboreal samples)
Unweighted-Unifrac	Semi-Arboreal vs. Marine: pseudo-F = 3.68; **p-value = 0.004**	*B. dendrophila* vs. *L. laticaudata*: pseudo-F = 1.33; p-value = 0.131	Mouth vs. Gut: pseudo-F = 3.39; p-value = **0.002**	Mouth vs. Gut: pseudo-F = 1.39; p-value = 0.111	Mouth vs. Gut: pseudo-F = 2.89; p-value = **0.032**
	Semi-Arboreal vs. Arboreal: pseudo-F = 2.09; p-value = **0.004**	*B. dendrophila* vs. *T. flavomaculatus*: pseudo-F = 1.76; p-value = 0.062			
	Marine vs. Arboreal: pseudo-F = 3.08; p-value = **0.001**	*L. laticaudata* vs. *T. flavomaculatus*: pseudo-F = 2.13; p-value = **0.011**			
Weighted-Unifrac	Semi-Arboreal vs. Marine: pseudo-F = 3.83; p-value = **0.039**	*B. dendrophila* vs. *L. laticaudata*: pseudo-F = 2.47; p-value = **0.048**	Mouth vs. Gut: pseudo-F = 4.41; p-value = 0.01	Mouth vs. Gut: pseudo-F = 0.694; p-value = 0.765	Mouth vs. Gut: pseudo-F = 3.27; p-value = 0.122
	Semi-Arboreal vs. Arboreal: pseudo-F = 1.11; p-value = 0.352	*B. dendrophila* vs. *T. flavomaculatus*: pseudo-F = 3.92; p-value = **0.028**			
	Marine vs. Arboreal: pseudo-F =	*L. laticaudata* vs. *T. flavomaculatus*:			

	6.14; p-value = **0.003**	pseudo-F = 6.60; p-value = **0.002**			
Bray-Curtis	Semi-Arboreal vs. Marine: pseudo-F = 5.40; p-value = **0.003**	*B. dendrophila* vs. *L. laticaudata*: pseudo-F = 2.18; p-value = **0.02**	Mouth vs. Gut: pseudo-F = 3.67; p-value = **0.001**	Mouth vs. Gut: pseudo-F = 0.857; p-value = 1.0	Mouth vs. Gut: pseudo-F = 2.35; p-value = 0.169
	Semi-Arboreal vs. Arboreal: pseudo-F = 1.16; p-value = 0.295	*B. dendrophila* vs. *T. flavomaculatus*: pseudo-F = 1.78; p-value = **0.034**			
	Marine vs. Arboreal: pseudo-F = 4.88; p-value = **0.001**	*L. laticaudata* vs. *T. flavomaculatus*: pseudo-F = 3.71; p-value = **0.003**			

Table S3 | Beta diversity results for each analysis (Unweighted-Unifrac, Weighted-Unifrac, and Bray-Curtis). Bold values indicate significant differences.

Appendix B

Supplementary Material for Host ecology drives frog skin microbiome diversity across ecotone in South-Central North America

Supplementary Data: Detailed Unweighted- and Weighted-Unifrac beta diversity results

Unweighted- and Weighted-Unifrac distance matrices were used to analyze beta diversity among skin microbiome samples. The Unweighted-Unifrac analysis indicated that the skin microbiomes of *A. blanchardi* were significantly different from both *Rana* species (*R. catesbeiana*: pseudo-F = 2.81; q-value = 0.003; *R. sphenocephala*: pseudo-F = 2.27; q-value = 0.006; Appendix B, Figure S7) but were not significantly different from either *Hyla* species (*H. chrysoscelis*/*versicolor*: pseudo-F = 1.34; q-value = 0.094; *H. cinerea*: pseudo-F = 1.39; q-value = 0.105). Additionally, the skin microbial communities of *H. chrysoscelis*/*versicolor* were significantly different from *H. cinerea* (pseudo-F = 2.00; q-value = 0.014), *R. catesbeiana* (pseudo-F = 3.81; q-value = 0.003), and *R. sphenocephala* (pseudo-F = 2.40; q-value = 0.006). Also, results from this analysis indicated that *H. cinerea* had significantly different skin microbial diversity when compared to both *Rana* species (*R. catesbeiana*: pseudo-F = 5.10; q-value = 0.003; *R. sphenocephala*: pseudo-F = 4.30; q-value = 0.003) and both *Rana* species differed from one another (*R. catesbeiana* vs. *R. sphenocephala*: pseudo-F = 2.00; q-value = 0.006). Based on the species-to-species comparisons, it is not surprising that skin microbiomes differed by host family (Hylidae vs. Ranidae: pseudo-F = 5.94; p-value = 0.001). When we grouped the data into ecoregions, we found that samples from each ecoregion were significantly different from one another (Arkansas Valley vs. Central Great Plains: pseudo-F = 2.28; q-value = 0.002, Crosstimbers: pseudo-F = 4.69; q-value = 0.002, and South Central Plains: pseudo-F = 3.67; q-value = 0.002; Central Great Plains vs. Crosstimbers: pseudo-F = 4.49; q-value = 0.002 and South Central Plains: pseudo-F = 2.08; q-value = 0.004; Crosstimbers vs. South Central Plains: pseudo-F = 8.45; q-value = 0.002). More specifically, within each host species, except *H. chrysoscelis*/*versicolor* (Arkansas Valley vs. Crosstimbers: pseudo-F = 1.56; p-value = 0.057), significant differences were found among skin microbiome samples collected from the same species located in different ecoregions (*A. blanchardi*: Arkansas Valley vs. Crosstimbers: pseudo-F = 3.87; p-value = 0.001; *H. cinerea*: Arkansas Valley vs. Crosstimbers: pseudo-F = 3.09; p-value = 0.003; *R. catesbeiana*: Central Great Plains vs. South Central Plains: pseudo-F = 2.30; p-value = 0.002; *R. sphenocephala*: Central Great Plains vs. South Central Plains: pseudo-F = 1.34; p-value = 0.048; Appendix B, Figure S7). Lastly, when we grouped samples based on the ecology of the hosts, we found significant differences between each habitat type: aquatic vs. arboreal (pseudo-F = 5.34; q-value = 0.002), aquatic vs. semi-aquatic (pseudo-F = 2.45; q-value = 0.002), and arboreal vs. semi-aquatic (pseudo-F = 2.33; q-value = 0.003; Appendix B, Figure S7).

Weighted-Unifrac analysis of the skin microbiome samples found significant differences among all host species we compared with the exception of *A. blanchardi* vs. *H. cinerea* (pseudo-F = 1.75; q- value = 0.111). Additionally, significant differences were found among samples from each host family (Hylidae vs. Ranidae: pseudo-F = 8.96; p-value = 0.001). Skin microbiomes differed among all species found at distinct ecoregions (Arkansas Valley vs.

Central Great Plains: pseudo-F = 9.83; q-value = 0.002, Crosstimbers: pseudo-F = 9.74; q-value = 0.002, and South Central Plains: pseudo-F = 4.52; q-value = 0.002; Central Great Plains vs. Crosstimbers: pseudo-F = 7.59; q-value = 0.002 and South Central Plains: pseudo-F = 2.89; q-value = 0.009; Crosstimbers vs. South Central Plains: pseudo-F = 8.12; q-value = 0.002). With the exception of *R. sphenocephala* (Central Great Plains vs. South Central Plains: pseudo-F = 1.98; p-value = 0.094), the skin microbial communities of each host species differed between ecoregions (*A. blanchardi*: Arkansas Valley vs. Crosstimbers: pseudo-F = 3.14; p-value = 0.011; *H. chrysoscelis*/*versicolor*: Arkansas Valley vs. Crosstimbers: pseudo-F = 5.79; p-value = 0.001; *H. cinerea*: Arkansas Valley vs. Crosstimbers: pseudo-F = 4.92; p-value = 0.001; R. *catesbeiana*: Central Great Plains vs. South Central Plains: pseudo-F = 2.29; p-value = 0.038; Figure 2.3). Additionally, skin microbiomes differed based on host ecology (aquatic vs. arboreal: pseudo-F = 8.49; q-value = 0.003; aquatic vs. semi-aquatic: pseudo-F = 2.64; q-value = 0.019; arboreal vs. semi-aquatic: pseudo-F = 4.79; q-value = 0.003; Figure 2.3).

Supplementary Figures

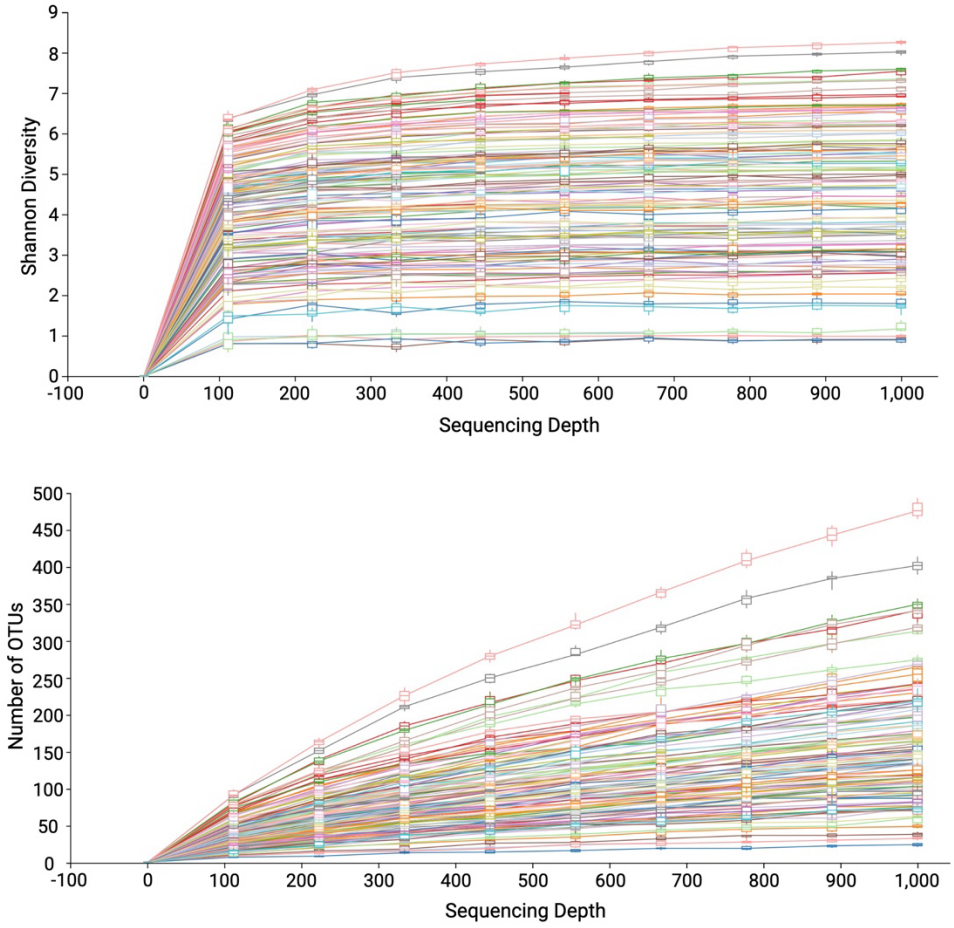

Figure S1 | Alpha diversity rarefaction curves based on Shannon Diversity and Number of OTUs. Each curve is representative of a single swab. Based on these curves, we decided to rarefy all samples to a sequencing depth of 1,000 sequences.

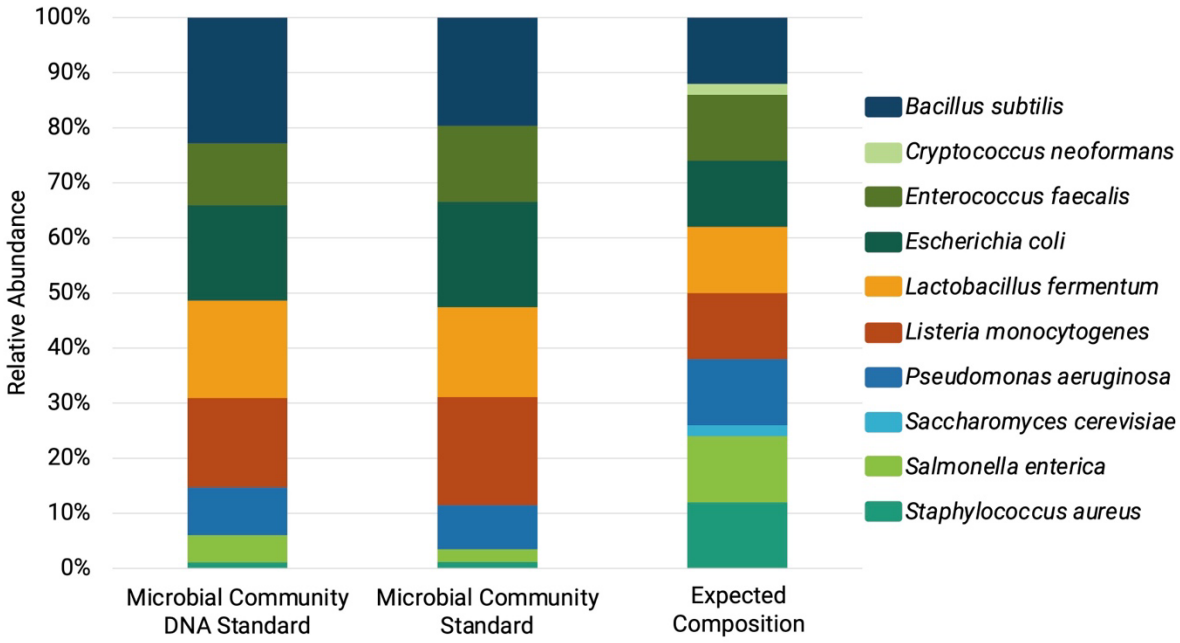

Figure S2 | Compositional comparison of two positive control samples (Zymo Research Products, Irvine, CA, USA): the ZymoBIOMICS microbial community DNA standard (Cat. No. D6305, Zymo Research Products) and the ZymoBIOMICS microbial community standard (Cat. No. D6300, Zymo Research Products) alongside the expected composition of both standards. The microbial community standard was extracted alongside the focal skin microbiome samples whereas the microbial DNA standard contained pre-extracted DNA that was amplified and sequenced with the other focal samples. The compositions of both standards were compared to assess any bias or error in our extraction and amplification methods. We found that there were little compositional differences between the two standards; however, two microbial species that comprised a small portion of the expected composition, *Cryptococcus neoformans* and *Saccharomyces cerevisiae* were missing from the two positive control samples. This could indicate that our methodologies may have been less sensitive to certain microbial taxa that comprised a small percentage of the overall microbiome composition within our samples.

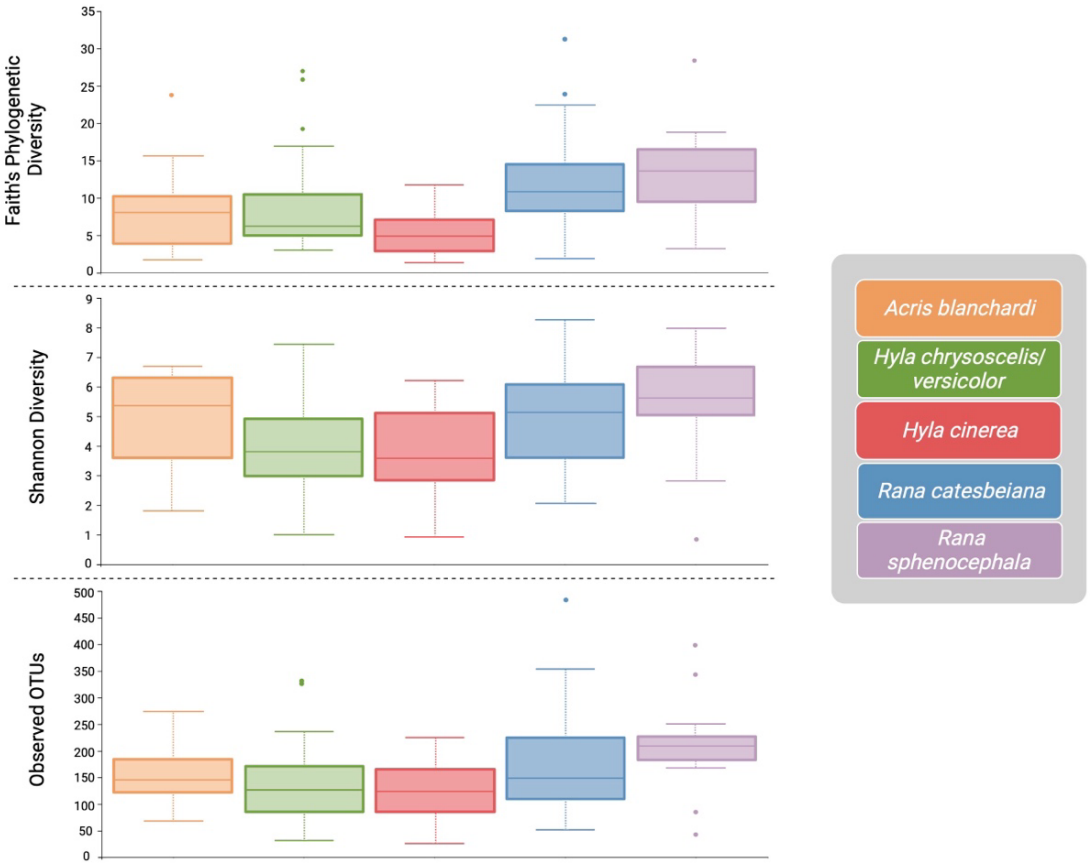

Figure S3 | Alpha diversity (Faith's Phylogenetic Diversity, Top; Shannon Diversity, Middle; Observed OTUs, Bottom) comparisons of microbial OTUs by host species. The host species are represented by distinct colors as indicated in the figure legend. Figure was created with BioRender.com.

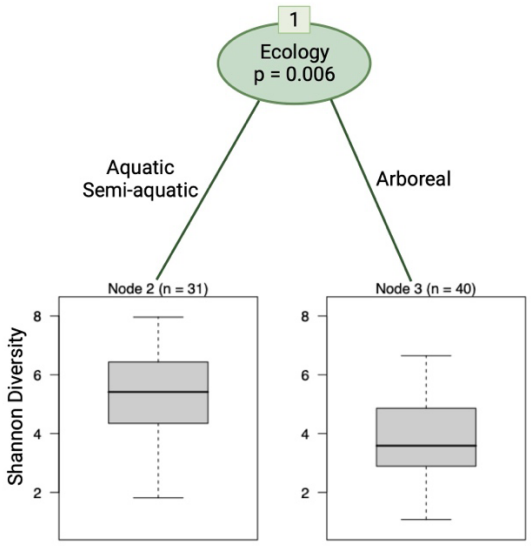

Figure S4 | Results of the Classification and Regression Tree analysis indicated that host ecology explained all of the variance in Shannon Diversity among our focal samples. Both aquatic and semi-aquatic frogs had significantly higher diversity than the arboreal species.

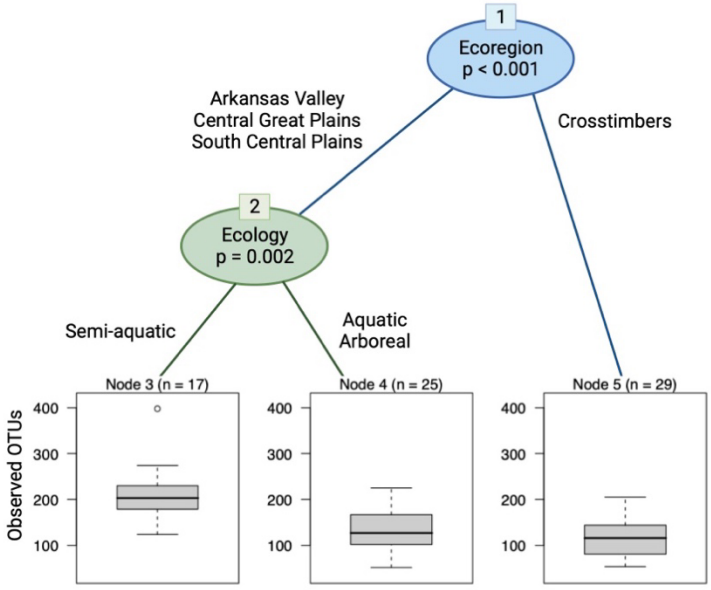

Figure S5 | Classification and Regression Tree analysis revealed that ecoregion explained most of the variance found in the number of Operational Taxonomic Units (i.e., Observed OTUs) within our focal samples. Samples from the Crosstimbers ecoregion had significantly less OTUs when compared to samples from the other three ecoregions. Additionally, among frogs from the Arkansas Valley, Central Great Plains, and South Central Plains ecoregions, ecology explained the remaining variance in the number of OTUs, with semi-aquatic frogs having significantly more OTUs when compared to aquatic and arboreal frogs.

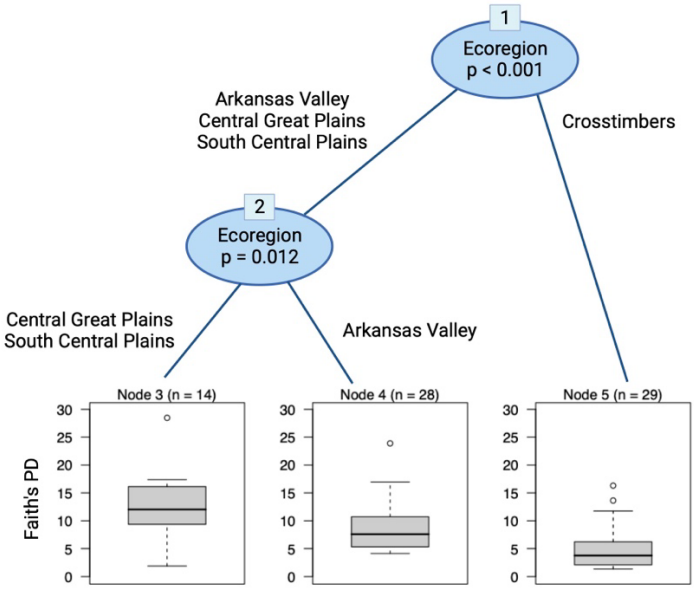

Figure S6 | Results of the Classification and Regression Tree analysis show that ecoregion was the only variable that explained the variance in Faith's Phylogenetic Diversity (PD) among our skin microbiome samples. Samples collected from the Crosstimbers ecoregion were less diverse when compared to the other three ecoregions. Similarly, Arkansas Valley samples had less diversity than those from Central Great Plains and South Central Plains ecoregions.

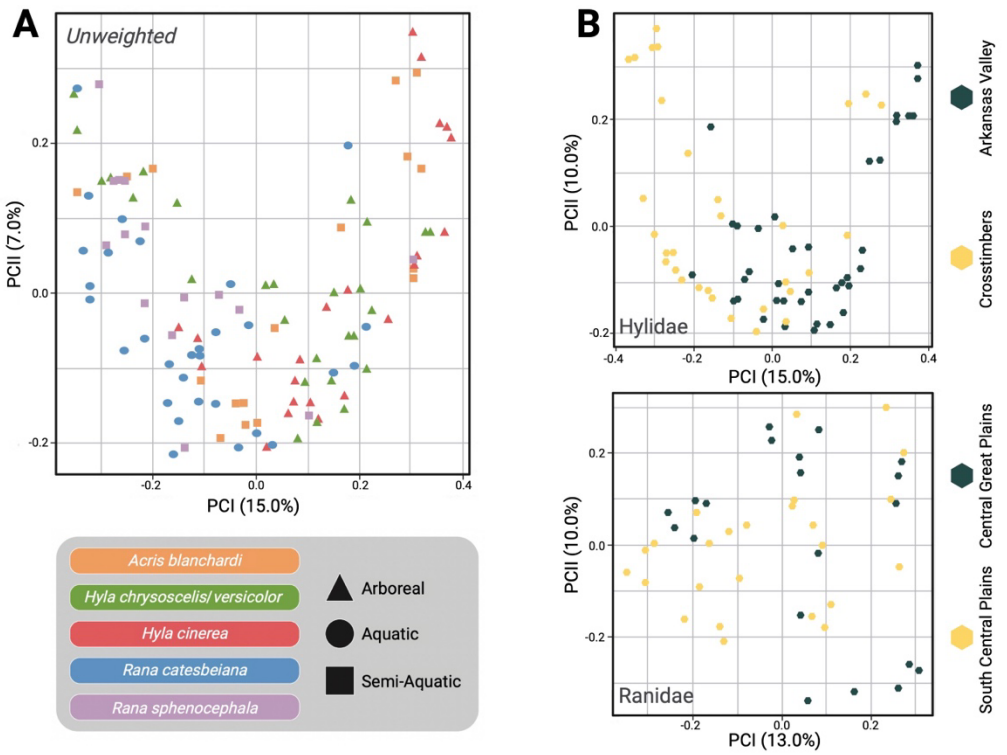

Figure S7 | Beta diversity comparisons based on Unweighted-Unifrac distances. **(A)** Principal Coordinates Analysis (PCoA) of all samples with point color indicating the host species: orange = *Acris blanchardi*, green = *Hyla chrysoscelis/versicolor*, red = *Hyla cinerea*, blue = *Rana catesbeiana*, purple = *Rana sphenocephala*, and shapes representing the different host ecological preferences: triangle = arboreal, circle = aquatic, and square = semi-aquatic. **(B)** PCoAs of each host family separately with Hylidae samples on top and Ranidae samples on the bottom. Color indicates the ecoregion where sampling occurred: dark gray = Arkansas Valley and Central Great Plains, yellow = Crosstimbers and South Central Plains.

Supplementary Tables

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	**Family**
Acris	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	**Genus**
blanchardi	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	**County**
34.78279	34.68206	34.68206	34.68206	34.68206	34.68206	34.76459	34.76459	35.33709	**Latitude**
-94.69408	-94.74407	-94.74407	-94.74407	-94.74407	-94.74407	-94.69794	-94.69794	-94.77384	**Longitude**
Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	**Ecoregion**
Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	**Ecology**
17	28	28	28	28	28	27	27	18	**Day**
April	May	May	May	May	May	May	May	April	**Month**
2021	2015	2015	2015	2015	2015	2015	2015	2015	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
4256	1161	1160	1159	1158	1133	1111	1107	464	**Field ID #**
No	No	No	No	No	No	No	No	No	**Removed from downstream analyses?**
N/A	Positive	Positive	Negative	Negative	Negative	Positive	Positive	Positive	**Bd Status**
N/A	621129.9479	294154.5573	0	0	0	263.8721848	318864.1992	78620.67057	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	**Family**
Acris	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	**Genus**
blanchardi	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	**County**
34.78162	34.78162	34.78162	34.78162	34.78434	34.76917	34.76464	34.76464	34.76464	**Latitude**
-94.71484	-94.71484	-94.71484	-94.71484	-94.69495	-94.71061	-94.69873	-94.69873	-94.69873	**Longitude**
Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	**Ecoregion**
Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	**Ecology**
25	25	25	25	17	17	16	16	16	**Day**
June	June	June	June	April	April	April	April	April	**Month**
2021	2021	2021	2021	2021	2021	2021	2021	2021	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
4368	4344	4343	4342	4253	4250	4236	4223	4217	**Field ID #**
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	**Removed from downstream analyses?**
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**Bd Status**
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	**Family**
Acris	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	**Genus**
blanchardi	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	**County**
33.87991	33.99694	33.99664	33.99808	33.99757	33.9966	33.99684	33.99664	33.8868	**Latitude**
-96.80509	-96.82703	-96.82803	-96.82843	-96.8277	-96.82818	-96.82803	-96.8207	-96.7955	**Longitude**
Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	**Ecoregion**
Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	**Ecology**
8	12	12	12	12	12	12	12	1	**Day**
July	July	July	July	July	July	July	July	August	**Month**
2018	2019	2019	2019	2019	2019	2019	2019	2017	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
3681	4117	4113	4112	4111	4110	4109	4106	3354	**Field ID #**
Yes	No	No	No	No	No	No	No	No	**Removed from downstream analyses?**
Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	**Bd Status**
0	0	0	0	0	0	0	0	0	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	**Family**
Acris	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	*Acris*	**Genus**
blanchardi	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	*blanchardi*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	**County**
33.99781	33.9966	33.99694	33.8854	33.88374	33.87986	33.87976	33.87986	33.87983	**Latitude**
-96.82808	-96.82818	-96.82748	-96.86639	-96.86744	-96.80494	-96.80611	-96.80494	-96.80577	**Longitude**
Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	**Ecoregion**
Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	**Ecology**
12	12	12	10	10	10	10	10	8	**Day**
July	July	July	July	July	July	July	July	July	**Month**
2019	2019	2019	2018	2018	2018	2018	2018	2018	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
4114	4108	4107	3693	3691	3688	3686	3685	3682	**Field ID #**
Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	**Removed from downstream analyses?**
Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	**Bd Status**
0	0.685749829	0	0	0	0	0	0	0	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	**Family**
Hyla	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Acris*	*Acris*	**Genus**
chrysoscelis/ versicolor	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*blanchardi*	*blanchardi*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Marshall	Marshall	**County**
35.33709	35.33709	35.33709	35.33709	35.33709	35.33709	35.33709	33.99768	33.99664	**Latitude**
-94.77384	-94.77384	-94.77384	-94.77384	-94.77384	-94.77384	-94.77384	-96.82803	-96.82803	**Longitude**
Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Crosstimbers	Crosstimbers	**Ecoregion**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Semi-Aquatic	Semi-Aquatic	**Ecology**
18	18	18	18	18	18	18	12	12	**Day**
April	April	April	April	April	April	April	July	July	**Month**
2015	2015	2015	2015	2015	2015	2015	2019	2019	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
471	469	461	439	436	432	431	4116	4115	**Field ID #**
No	No	No	No	No	No	No	Yes	Yes	**Removed from downstream analyses?**
N/A	N/A	Positive	Positive	Positive	Negative	Negative	Negative	Negative	**Bd Status**
N/A	N/A	1248084.479	9014.440104	101607.5	0	0	0	0	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	**Family**
Hyla	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	**Genus**
chrysoscelis/ versicolor	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	**County**
34.68206	34.68206	34.68206	34.68206	34.76459	34.76459	35.33709	35.33709	35.33709	**Latitude**
-94.74407	-94.74407	-94.74407	-94.74407	-94.69794	-94.69794	-94.77384	-94.77384	-94.77384	**Longitude**
Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	**Ecoregion**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
28	28	28	28	27	27	18	18	18	**Day**
May	May	May	May	May	May	April	April	April	**Month**
2015	2015	2015	2015	2015	2015	2015	2015	2015	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
1152	1151	1150	1149	1105	1102	474	473	472	**Field ID #**
No	No	No	No	No	No	No	No	No	**Removed from downstream analyses?**
Positive	Positive	Negative	Negative	Negative	Negative	N/A	N/A	N/A	**Bd Status**
519360.1042	6676.481527	0	0	0	0	N/A	N/A	N/A	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	**Family**
Hyla	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	**Genus**
chrysoscelis/ versicolor	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Marshall	Marshall	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	**County**
33.88006	33.88234	34.68206	34.68206	34.76459	35.33709	35.33709	35.33709	34.68206	**Latitude**
-96.80241	-96.79803	-94.74407	-94.74407	-94.69794	-94.77384	-94.77384	-94.77384	-94.74407	**Longitude**
Crosstimbers	Crosstimbers	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	**Ecoregion**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
1	30	28	28	27	18	18	18	28	**Day**
August	July	May	May	May	April	April	April	May	**Month**
2017	2017	2015	2015	2015	2015	2015	2015	2015	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
3339	3331	1156	1153	1103	470	462	438	1155	**Field ID #**
No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	**Removed from downstream analyses?**
Negative	Negative	N/A	Negative	Positive	N/A	N/A	Negative	N/A	**Bd Status**
0	0	N/A	0	3483.372803	N/A	N/A	0	N/A	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	**Family**
Hyla	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	**Genus**
chrysoscelis/ versicolor	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	**County**
33.89543	33.88181	33.88181	33.87989	33.88228	33.88228	33.88228	33.89543	33.89543	**Latitude**
-96.81023	-96.80077	-96.8007	-96.80614	-96.798	-96.798	-96.798	-96.81023	-96.81023	**Longitude**
Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	**Ecoregion**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
11	7	7	13	2	2	2	11	11	**Day**
May	July	July	July	August	August	August	May	May	**Month**
2015	2019	2019	2018	2016	2016	2016	2015	2015	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
871	4039	4038	3704	2638	2636	2635	870	861	**Field ID #**
Yes	No	No	No	No	No	No	No	No	**Removed from downstream analyses?**
N/A	Negative	Negative	Negative	Negative	Negative	Negative	N/A	N/A	**Bd Status**
N/A	0	0	0	0	0	0	N/A	N/A	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	**Family**
Hyla	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	**Genus**
cinerea	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	*chrysoscelis/ versicolor*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Marshall	Marshall	Marshall	**County**
34.68206	34.76459	34.76459	34.76459	34.76459	34.76459	33.882796	33.87983	33.88228	**Latitude**
-94.74407	-94.69794	-94.69794	-94.69794	-94.69794	-94.69794	-96.801248	-96.80226	-96.798	**Longitude**
Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Crosstimbers	Crosstimbers	Crosstimbers	**Ecoregion**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
28	27	27	27	27	27	13	12	2	**Day**
May	May	May	May	May	May	July	July	August	**Month**
2015	2015	2015	2015	2015	2015	2018	2018	2016	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
1144	1080	1079	1078	1077	1074	3703	3701	2637	**Field ID #**
No	No	No	No	No	No	Yes	Yes	Yes	**Removed from downstream analyses?**
Negative	Negative	Negative	N/A	Negative	N/A	Negative	Negative	Negative	**Bd Status**
0	0	0	N/A	0	N/A	0	0	0	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	**Family**
Hyla	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	**Genus**
cinerea	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	Le Flore	**County**
34.76464	34.76464	34.76464	34.76464	34.76623	34.68206	34.68206	34.68206	34.68206	**Latitude**
-94.69873	-94.69873	-94.69873	-94.69873	-94.69916	-94.74407	-94.74407	-94.74407	-94.74407	**Longitude**
Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	Arkansas Valley	**Ecoregion**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
25	25	25	16	29	28	28	28	28	**Day**
June	June	June	April	April	May	May	May	May	**Month**
2021	2021	2021	2021	2017	2015	2015	2015	2015	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
4322	4319	4299	4212	2991	1148	1147	1146	1145	**Field ID #**
Yes	Yes	Yes	Yes	No	No	No	No	No	**Removed from downstream analyses?**
N/A	N/A	N/A	N/A	N/A	Negative	Negative	Negative	Negative	**Bd Status**
N/A	N/A	N/A	N/A	N/A	0	0	0	0	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	**Family**
Hyla	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	**Genus**
cinerea	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Le Flore	Le Flore	**County**
33.88412	33.88105	33.87979	33.882845	33.88206	33.88234	33.88234	34.76464	34.76464	**Latitude**
-96.86461	-96.80141	-96.8018	-96.800221	-96.79809	-96.79803	-96.79803	-94.69873	-94.69873	**Longitude**
Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Arkansas Valley	Arkansas Valley	**Ecoregion**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
10	10	7	8	1	30	30	25	25	**Day**
July	July	July	July	August	July	July	June	June	**Month**
2019	2019	2019	2018	2017	2017	2017	2021	2021	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
4086	4074	4033	3673	3350	3334	3333	4325	4324	**Field ID #**
No	No	No	No	No	No	No	Yes	Yes	**Removed from downstream analyses?**
Negative	Negative	Negative	Negative	Negative	Negative	Negative	N/A	N/A	**Bd Status**
0	0	0	0	0	0	0	N/A	N/A	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	Hylidae	**Family**
Hyla	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	**Genus**
cinerea	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	Marshall	**County**
33.87976	33.88446	33.88452	33.88429	33.88404	33.88393	33.88404	33.88424	33.88412	**Latitude**
-96.80547	-96.79694	-96.86549	-96.80508	-96.8651	-96.8651	-96.8651	-96.86461	-96.86461	**Longitude**
Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	**Ecoregion**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
8	1	28	10	10	10	10	10	10	**Day**
July	August	July	July	July	July	July	July	July	**Month**
2018	2017	2016	2019	2019	2019	2019	2019	2019	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
3678	3353	2648	4096	4093	4092	4091	4088	4087	**Field ID #**
Yes	Yes	Yes	No	No	No	No	No	No	**Removed from downstream analyses?**
N/A	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	**Bd Status**
N/A	0	0	0	0	0	0	0	0	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Hylidae	Hylidae	Hylidae	Hylidae	**Family**
Rana	*Rana*	*Rana*	*Rana*	*Rana*	*Hyla*	*Hyla*	*Hyla*	*Hyla*	**Genus**
catesbeiana	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*cinerea*	*cinerea*	*cinerea*	*cinerea*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Cleveland	Cleveland	Cleveland	Cleveland	Cleveland	Marshall	Marshall	Marshall	Marshall	**County**
35.21137	35.21107	35.21107	35.2115	35.21153	33.88424	33.88437	33.88456	33.87986	**Latitude**
-97.39058	-97.39036	-97.39036	-97.39036	-97.39037	-96.86461	-96.8662	-96.86604	-96.80549	**Longitude**
Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	Crosstimbers	Crosstimbers	Crosstimbers	Crosstimbers	**Ecoregion**
Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
24	23	23	23	23	10	10	10	8	**Day**
March	March	March	March	March	July	July	July	July	**Month**
2015	2015	2015	2015	2015	2019	2018	2018	2018	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
60	54	53	52	50	4090	3695	3694	3679	**Field ID #**
No	No	No	No	No	Yes	Yes	Yes	Yes	**Removed from downstream analyses?**
Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	**Bd Status**
0	0	0	0	3399.82605	0	0	0	0	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	**Family**
Rana	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	**Genus**
catesbeiana	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Cleveland	Cleveland	Cleveland	Cleveland	Cleveland	Cleveland	Cleveland	Cleveland	Cleveland	**County**
35.21137	35.21153	35.2548	35.2548	35.2548	35.2548	35.2548	35.2548	35.11789	**Latitude**
-97.39058	-97.39037	-97.454	-97.454	-97.454	-97.454	-97.454	-97.454	-97.25175	**Longitude**
Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	**Ecoregion**
Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	**Ecology**
24	23	25	25	25	25	25	25	21	**Day**
March	March	June	June	June	June	June	June	March	**Month**
2015	2015	2018	2018	2018	2018	2018	2018	2015	**Year**
ODWC	ODWC	SJE	SJE	SJE	SJE	SJE	SJE	ODWC	**Field ID Series**
57	51	29	28	27	26	25	24	61	**Field ID #**
Yes	Yes	No	No	No	No	No	No	No	**Removed from downstream analyses?**
Negative	Positive	N/A	N/A	N/A	N/A	N/A	N/A	Negative	**Bd Status**
0	34.7027421	N/A	N/A	N/A	N/A	N/A	N/A	0	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	**Family**
Rana	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	**Genus**
catesbeiana	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	Cleveland	Cleveland	Cleveland	Cleveland	**County**
33.72146	33.72146	33.72146	33.72146	33.75126	35.2548	35.2548	35.11775	35.21137	**Latitude**
-94.63931	-94.63931	-94.63931	-94.63931	-94.649	-97.454	-97.454	-97.25195	-97.39058	**Longitude**
South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	**Ecoregion**
Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	**Ecology**
5	5	5	5	5	25	25	22	24	**Day**
April	April	April	April	April	June	June	March	March	**Month**
2019	2019	2019	2019	2019	2018	2018	2015	2015	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	SJE	SJE	ODWC	ODWC	**Field ID Series**
3812	3809	3808	3807	3788	30	21	62	58	**Field ID #**
No	No	No	No	No	Yes	Yes	Yes	Yes	**Removed from downstream analyses?**
N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative	Negative	**Bd Status**
N/A	N/A	N/A	N/A	N/A	N/A	N/A	0	0	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	**Family**
Rana	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	**Genus**
catesbeiana	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	**County**
33.72277	33.72277	33.72329	33.72329	33.72404	33.72404	33.72491	33.72155	33.72146	**Latitude**
-94.64215	-94.64215	-94.64215	-94.64215	-94.64212	-94.64212	-94.64214	-94.63744	-94.63931	**Longitude**
South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	**Ecoregion**
Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	**Ecology**
5	5	5	5	5	5	5	5	5	**Day**
April	April	April	April	April	April	April	April	April	**Month**
2019	2019	2019	2019	2019	2019	2019	2019	2019	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
3838	3837	3834	3833	3832	3831	3828	3814	3813	**Field ID #**
No	No	No	No	No	No	No	No	No	**Removed from downstream analyses?**
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**Bd Status**
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	**Family**
Rana	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	**Genus**
catesbeiana	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	*catesbeiana*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	**County**
33.7231	33.72491	33.72146	33.72166	33.73329	33.72115	33.72277	33.72277	33.72277	**Latitude**
-94.64213	-94.64214	-94.63931	-94.64043	-94.64207	-94.64227	-94.64215	-94.64215	-94.64215	**Longitude**
South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	**Ecoregion**
Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	**Ecology**
5	5	5	5	2	6	5	5	5	**Day**
April	April	April	April	April	April	April	April	April	**Month**
2019	2019	2019	2019	2017	2019	2019	2019	2019	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
3836	3829	3811	3805	2857	3883	3841	3840	3839	**Field ID #**
Yes	Yes	Yes	Yes	Yes	No	No	No	No	**Removed from downstream analyses?**
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**Bd Status**
N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	**Family**
Rana	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	**Genus**
sphenocephala	*sphenocephala*	*sphenocephala*	*sphenocephala*	*sphenocephala*	*sphenocephala*	*sphenocephala*	*sphenocephala*	*catesbeiana*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
Cleveland	Cleveland	Cleveland	Cleveland	Cleveland	Cleveland	Cleveland	Cleveland	McCurtain	**County**
35.24443	35.24413	35.24266	35.156	35.156	35.1431	35.1431	35.11926	33.73328	**Latitude**
-97.4297	-97.42655	-97.42689	-97.254	-97.254	-97.2535	-97.2535	-97.25085	-94.6423	**Longitude**
Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	South Central Plains	**Ecoregion**
Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Aquatic	**Ecology**
12	23	23	23	23	23	23	22	6	**Day**
April	June	June	June	June	June	June	March	April	**Month**
2019	2015	2015	2015	2015	2015	2015	2015	2019	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
3937	1444	1431	1248	1247	1245	1244	63	3855	**Field ID #**
No	No	No	No	No	No	No	No	Yes	**Removed from downstream analyses?**
N/A	Positive	Negative	Negative	Negative	Negative	Negative	N/A	N/A	**Bd Status**
N/A	6809.448649	0	0	0	0	0	N/A	N/A	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	**Family**
Rana	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	**Genus**
sphenocephala	*sphenocephala*	*sphenocephala*	*sphenocephala*	*sphenocephala*	*sphenocephala*	*sphenocephala*	*sphenocephala*	*sphenocephala*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
McCurtain	McCurtain	McCurtain	Cleveland	Cleveland	Cleveland	Cleveland	Cleveland	Cleveland	**County**
33.74849	33.78324	33.78324	35.156	35.24413	35.156	35.0486	35.06915	35.11933	**Latitude**
-94.64227	-94.76353	-94.76353	-97.254	-97.42655	-97.254	-97.21264	-97.24023	-97.25086	**Longitude**
South Central Plains	South Central Plains	South Central Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	Central Great Plains	**Ecoregion**
Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	**Ecology**
31	29	28	23	23	23	22	10	22	**Day**
March	March	March	June	June	June	June	May	March	**Month**
2017	2015	2015	2015	2015	2015	2015	2015	2015	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
2741	212	186	1445	1443	1246	1222	806	64	**Field ID #**
No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	**Removed from downstream analyses?**
N/A	Positive	Positive	Negative	Negative	Positive	Negative	Positive	N/A	**Bd Status**
N/A	40976.11003	561717.8646	0	0	1332.671916	0	14014.12598	N/A	**Infection Load**

Anura	Anura	Anura	Anura	Anura	Anura	Anura	**Order**
Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	Ranidae	**Family**
Rana	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	*Rana*	**Genus**
sphenocephala	*sphenocephala*	*sphenocephala*	*sphenocephala*	*sphenocephala*	*sphenocephala*	*sphenocephala*	**Species**
Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	Oklahoma	**State**
McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	McCurtain	**County**
33.73391	33.73391	33.74849	33.78381	33.75126	33.74839	33.74851	33.74855	**Latitude**
-94.64393	-94.64393	-94.64225	-94.76373	-94.649	-94.64198	-94.64524	-94.64172	**Longitude**
South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	South Central Plains	**Ecoregion**
Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	Semi-Aquatic	**Ecology**
5	5	1	28	5	5	31	31	**Day**
April	April	April	March	April	April	March	March	**Month**
2019	2019	2017	2015	2019	2019	2017	2017	**Year**
ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	ODWC	**Field ID Series**
3844	3843	2843	189	3789	3784	2743	2742	**Field ID #**
Yes	Yes	Yes	Yes	No	No	No	No	**Removed from downstream analyses?**
N/A	N/A	N/A	Positive	N/A	N/A	N/A	N/A	**Bd Status**
N/A	N/A	N/A	85259.78516	N/A	N/A	N/A	N/A	**Infection Load**

Table S1 | Metadata for 179 skin microbiome samples that were collected, extracted, and sequenced. Each horizontal row represents an individual sample.

Host Species	Ecology	Ecoregions	Host Family	Skin Sample No. (pre-rarefaction)	Skin Sample No. (post-rarefaction)
Acris blanchardi	Semi-Aquatic	Arkansas Valley; Crosstimbers	Hylidae	38	17
Hyla chrysoscelis/versicolor	Arboreal	Arkansas Valley; Crosstimbers	Hylidae	37	27
Hyla cinerea	Arboreal	Arkansas Valley; Crosstimbers	Hylidae	37	24
Rana catesbeiana	Aquatic	Central Great Plains; South Central Plains	Ranidae	42	30
Rana sphenocephala	Semi-Aquatic	Central Great Plains; South Central Plains	Ranidae	25	15
Total				**179**	**113**

Table S2 | Sample sizes for the number of skin microbiome samples before and after rarefaction to exclude samples with low sequence number.

Appendix C

Supplementary Material for Bending the rules of island biogeography: Microbiome diversity decreases with increased island size among insular reptilian hosts in the Philippines

SUPPLEMENTARY DATA

Detailed microbiome composition results

Among most of our focal gut microbiome samples (N = 212), Proteobacteria emerged as the most abundant phyla (56.83%). Three other dominant (relative abundance ≥ 1%) phyla were found among the majority of samples: Actinobacteriota (4.68%; N = 136), Bacteroidota (16.54%; N = 184), and Firmicutes (21.41%; N = 179). Viperidae gut microbiome samples were dominated by Proteobacteria (84%), but also contained Bacteroidota (11%), Firmicutes (4%), and Actinobacteriota (2%) in lower abundances. Similarly, Colubridae samples had high relative abundances of Proteobacteria (72%), and Firmicutes (10%), Bacteroidota (9%), Actinobacteriota (4%), and Verrucomicrobiota (1%) made up smaller portions of the overall composition. Most samples from the family Homalopsidae contained only Proteobacteria (61%), Bacteroidota (19%), and Firmicutes (17%), whereas six microbial phyla, Actinobacteriota (3%), Bacteroidota (17%), Campilobacterota (29%), Firmicutes (19%), Fusobacteriota (3%), and Proteobacteria (44%) dominated the gut microbiomes of most lizards in the family Agamidae. Samples from the Dibamidae family had the lowest percentage of Proteobacteria (4%), and were instead dominated by Firmicutes (54%), Bacteroidota (36%), Desulfobacterota (8%), and also contained Verrucomicrobiota (2%) and Cyanobacteria (1%). Campilobacterota were the most abundant phylum among Elapidae samples (63%) which also contained Proteobacteria (23%), Bacteroidota (21%), Verrucomicrobiota (13%), Firmicutes (8%), Fusobacteriota (2%), and Actinobacteriota (1%). Two samples from the turtle family Geoemydidae and the lizard family Gekkonidae had high abundances of Campilobacterota (Geoemydidae: 33% and 73%; Gekkonidae: 44% and 58%), but most Geoemydidae samples were dominated by Proteobacteria (45%), Firmicutes (23%), Bacteroidota (13%), Actinobacteriota (3%), and Bdellovibrionota (2%) while Gekkonidae samples comprised Proteobacteria (49%), Firmicutes (24%), Bacteroidota (16%), Actinobacteriota (4%), Cyanobacteria (1%), and Desulfobacterota (1%). Similar to compositions of Geoemydidae and Gekkonidae samples, Typhlopidae snakes possessed low relative abundances of Bdellovibrionota (2%) and Desulfobacterota (1%), respectively, but they also contained Proteobacteria (55%), Bacteroidota (30%), Firmicutes (10%), and Actinobacteriota (3%). Although the average relative abundance was low (1%), Acidobacteriota was found among Scincidae samples only which also contained six other phyla: Proteobacteria (48%), Firmicutes (26%), Bacteroidota (17%), Actinobacteriota (3%), Verrucomicrobiota (2%), and Desulfobacterota (1%).

Composition results for the four focal islands

Desulfobacterota was detected among Calayan and Camiguin Norte samples, and Verrucomicrobiota was present within the gut microbiomes of individuals from Calayan and Negros islands. Desulfobacterota and Verrucomicrobiota have been identified as dominant members of some snake species (Tang et al., 2019; Smith et al., 2021; Hoffbeck et al., 2023); therefore, it is not surprising that these phyla were detected within our focal samples. Further, Bdellovibrionota, Cyanobacteria, and Patescibacteria were found within samples from Negros Island only (Appendix C, Figure S3). Patescibacteria has been identified as a dominant member of the *Laticauda laticaudata* oral microbiome, but no other studies to date have reported Patescibacteria within reptilian gut microbiome samples, but members of this large microbial radiation are found in various aquatic environments such as groundwater (Brown et al., 2015) and seawater (Takebe et al., 2020), and activated sludge collected from wastewater tanks (Fujii et al., 2022). Similarly, Bdellovibrionota has not yet been reported as a dominant member of the reptilian gut microbiome, but members of the phylum are found commonly within the environment (i.e., soil, freshwater, ocean). In contrast, other reptilian gut microbiome studies have detected Cyanobacteria in low relative abundances (Hong et al., 2011; Tang et al., 2019; Zhang et al., 2019). Only one phylum was unique to samples from Luzon Island (Spirochaetota; Appendix C, Figure S3), and members of this phylum have been found within the gut microbiomes of other reptile species (Hong et al., 2011; Keenan et al., 2013; Arizza et al., 2019; Zhang et al., 2019).

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Supplementary Figures

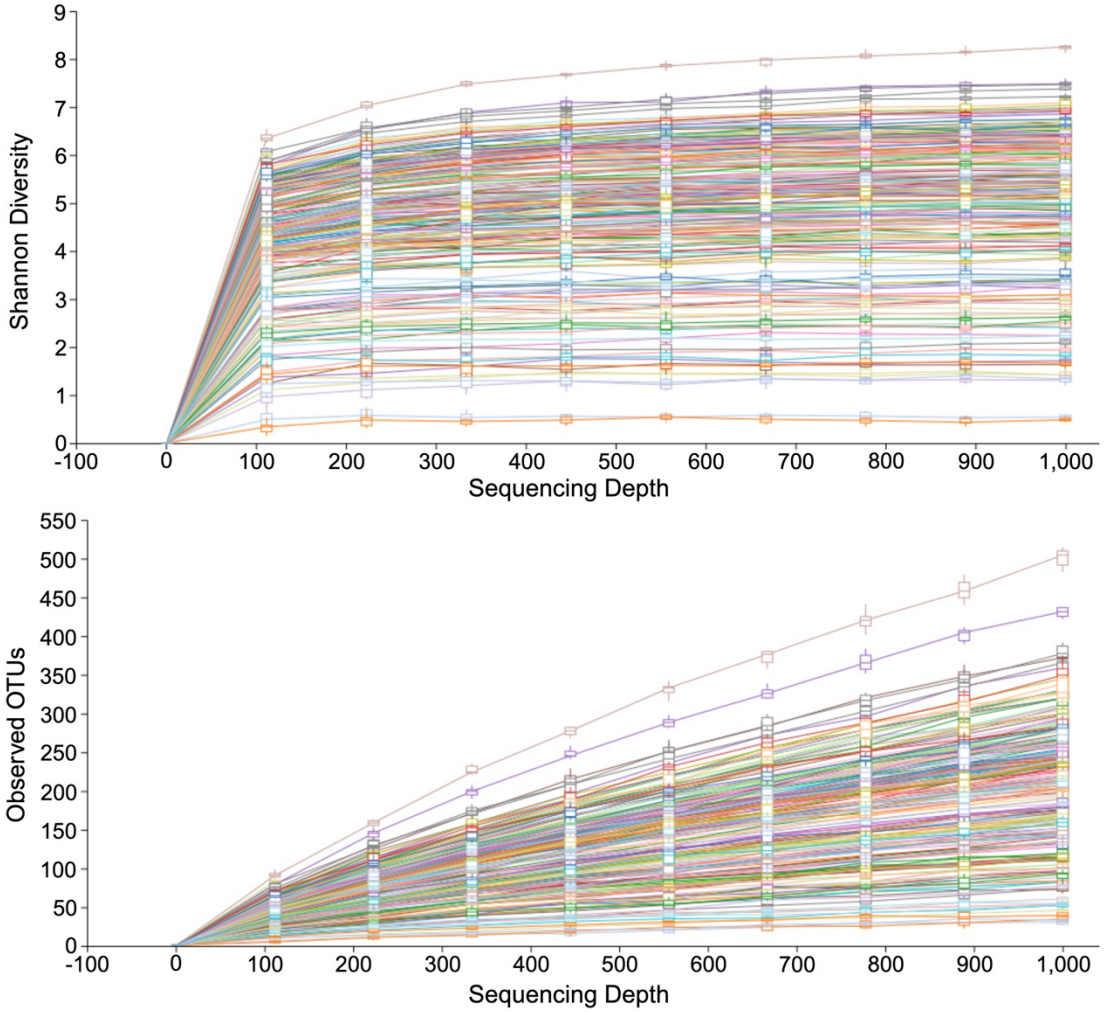

Figure S1 | Rarefaction curves based on Shannon Diversity and Observed OTUs alpha diversity metrics. Each curve is representative of a single swab. Based on these curves, we rarefied all samples to a sequencing depth of 1,000 sequences.

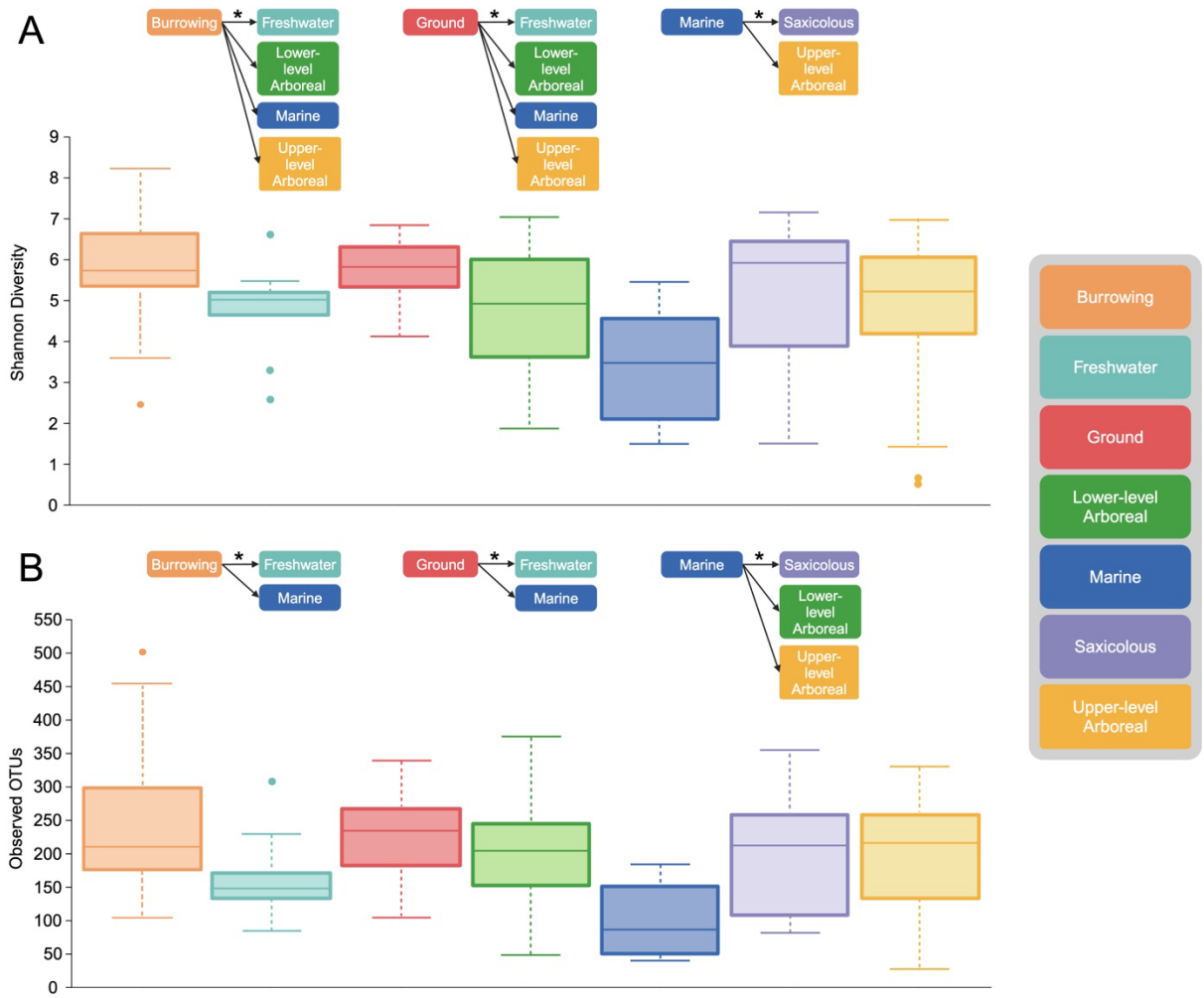

Figure S2 | **(A)** Shannon Diversity and **(B)** Observed OTUs comparisons between different host microhabitat groups. The different groups are distinguished based on color with burrowing = orange, freshwater = teal, ground-dwelling = red, lower-level arboreal = dark green, marine = dark blue, saxicolous (rock-dwelling) = purple, and upper-level arboreal = yellow. **(A)** Shannon diversity was significantly higher among burrowing reptiles when compared to all other groups with the exception of ground-dwelling and saxicolous species. Similarly, ground-dwelling reptiles possessed significantly more diverse microbiomes than individuals from all other microhabitat groups except burrowing and saxicolous species. Microbiomes of marine species were also significantly less diverse than saxicolous and upper-level arboreal individuals. **(B)** Burrowing and ground-dwelling reptiles possessed significantly more OTUs when compared to freshwater and marine species. The microbiomes of marine species were also significantly less diverse than individuals from both arboreal groups (lower and upper) and the saxicolous group.

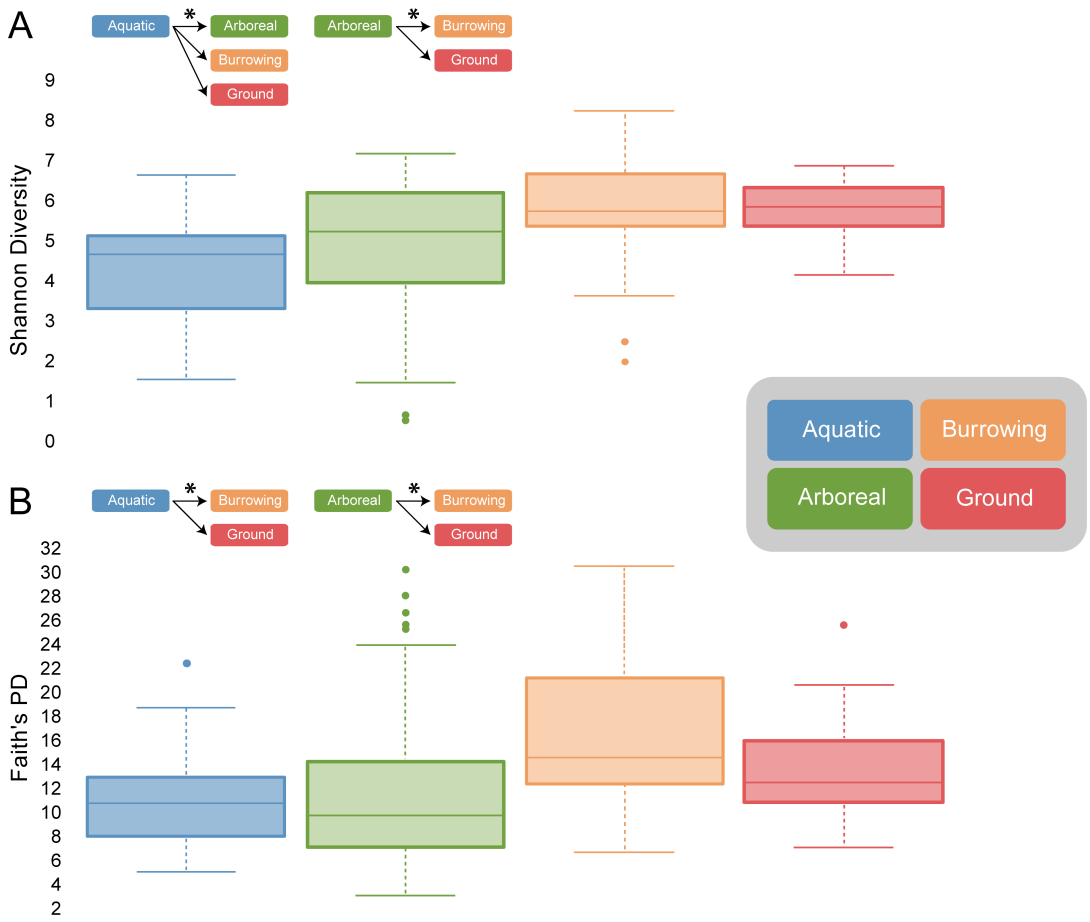

Figure S3 | Alpha diversity comparisons between the four host ecology groups using **(A)** Shannon Diversity and **(B)** Faith's Phylogenetic Diversity (PD). Different host ecologies are distinguished by color with aquatic = blue, arboreal = green, burrowing = orange, and ground-dwelling = red. Shannon diversity was significantly lower when comparing the microbiomes of aquatic reptiles to all other ecological groups. Arboreal species also possessed significantly lower gut microbiome diversity when compared to burrowing and ground-dwelling reptiles, but ground-dwelling and burrowing species did not differ from one another. Similar results were found using Faith's PD, but there was not a significant difference when comparing the microbiomes of aquatic and arboreal reptiles.

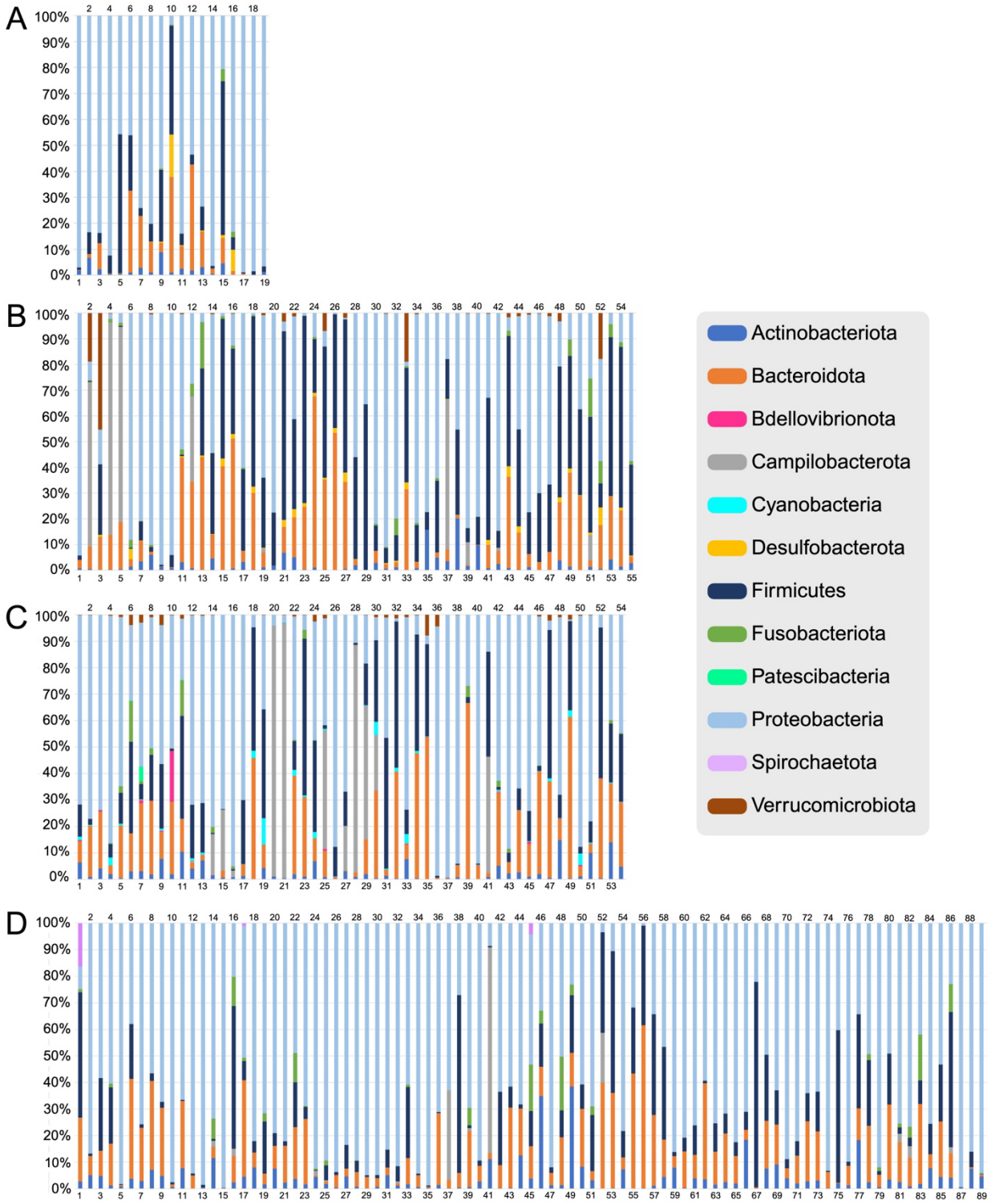

Figure S4 | Relative abundances of the dominant microbial phyla present within our focal gut microbiome samples. Each vertical bar represents a single reptile individual, and samples are grouped based on the geographic locations of the hosts: **(A)** Camiguin Norte, **(B)** Calayan, **(C)** Negros, and **(D)** Luzon.

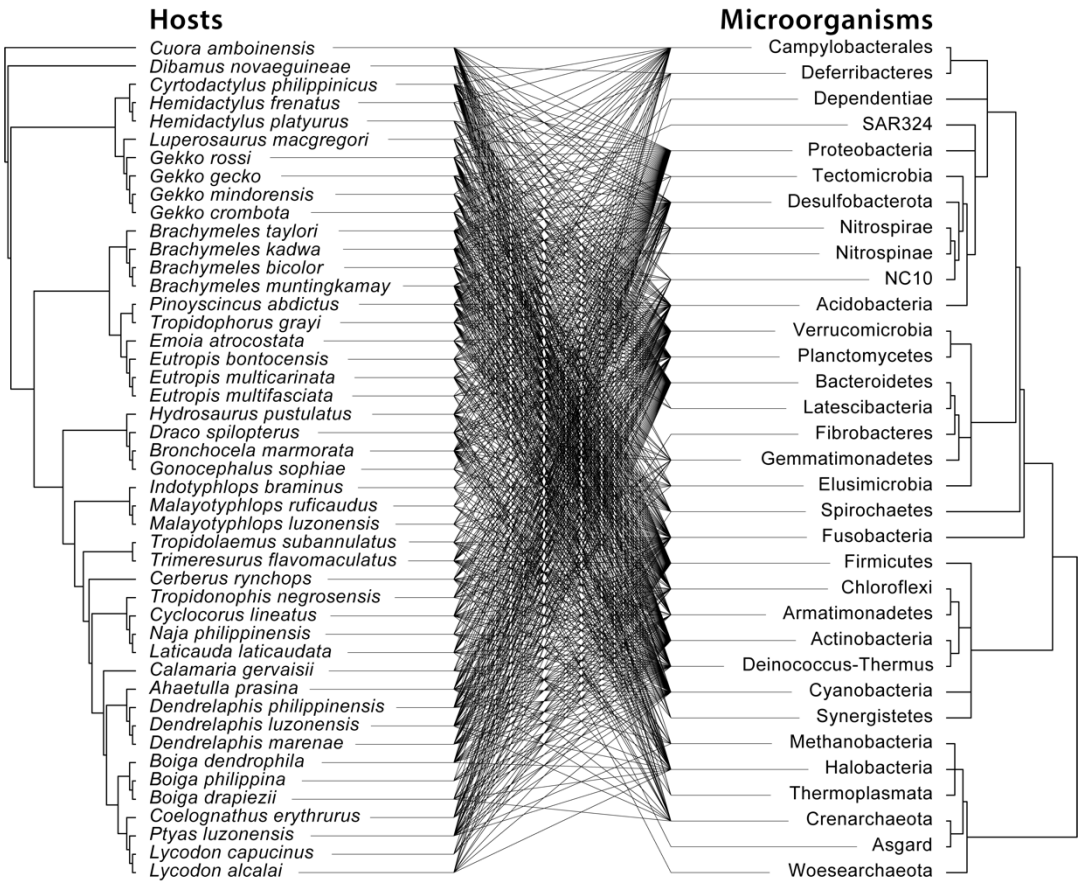

Figure S5 | Tanglegram showing the correspondence between the phylogeny of our focal microbial phyla and the reptile host phylogeny. We did not find evidence of cophylogeny between our focal hosts and their associated microbiota, as indicated by the numerous crossing lines linking both trees.

Supplementary Tables

Ahaetulla prasina	*Ahaetulla prasina*	*Ahaetulla prasina*	*Ahaetulla prasina*	*Ahaetulla prasina*	*Ahaetulla prasina*	*Ahaetulla prasina*	*Ahaetulla prasina*	*Ahaetulla prasina*	**SpeciesID**
Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	**HostFamily**
Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**Island**
Labo	Labo	Labo	Labo	Labo	Labo	Gattaran	Gattaran	Gattaran	**County**
Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**FaunalRegion**
Bicol	Bicol	Bicol	Bicol	Bicol	Bicol	Nasipping	Nasipping	Nasipping	**SubFaunalRegions**
14.07577	14.07577	14.07577	14.07577	14.07577	17.97014	17.97014	17.97014	17.97014	**Latitude**
122.7691	122.7691	122.7691	122.7691	122.7691	121.6544	121.6544	121.6544	121.6544	**Longitude**
109965	109965	109965	109965	109965	109965	109965	109965	109965	**Island Size (km^2)**
2016	2016	2016	2016	2016	2016	2017	2017	2017	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Unknown	Adult	**AgeClass**
Male	Unknown	Male	Female	Male	Female	Male	Unknown	Male	**Sex**
D54	D48	D47	D30	D29	D21	Not Included	Not Included	Not Included	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
						Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	**Notes**

Boiga dendrophila	*Boiga dendrophila*	*Boiga cf. philippina*	*Boiga cf. philippina*	*Boiga cf. drapiezii*	*Ahaetulla prasina*	*Ahaetulla prasina*	*Ahaetulla prasina*	*Ahaetulla prasina*	**SpeciesID**
Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	**HostFamily**
Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Luzon	Negros	Negros	Luzon	Camiguin Norte	Camiguin Norte	Camiguin Norte	Luzon	**Island**
Labo	Labo	Valencia	Valencia	Labo	Camiguin Norte	Camiguin Norte	Camiguin Norte	Labo	**County**
Luzon	Luzon	NegrosPanay	NegrosPanay	Luzon	Babuyan	Babuyan	Babuyan	Luzon	**FaunalRegion**
Bicol	Bicol	Valencia	Valencia	Bicol	Babuyan	Babuyan	Babuyan	Bicol	**SubFaunalRegions**
14.07577	14.07577	9.28753	9.28753	14.07577	18.91781	18.91781	18.91781	14.07577	**Latitude**
122.7691	122.7691	123.209	123.209	122.7691	121.8925	121.8925	121.8925	122.7691	**Longitude**
109965	109965	13310	13310	109965	166	166	166	109965	**Island Size (km^2)**
2016	2016	2016	2016	2016	2018	2018	2018	2016	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Lower Level Arboreal	Lower Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Male	Male	Female	Unknown	Unknown	Female	Unknown	Unknown	Female	**Sex**
D44	D24	C26	C3	D31	A19	A18	A17	D57	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
		Assigned Boiga philippina for phylo analyses	Assigned Boiga philippina for phylo analyses	Assigned Boiga drapiezii for phylo analyses					**Notes**

Boiga drapiezii	*Boiga dendrophila*	*Boiga dendrophila*	*Boiga dendrophila*	*Boiga dendrophila*	*Boiga dendrophila*	*Boiga dendrophila*	*Boiga dendrophila*	*Boiga dendrophila*	**SpeciesID**
Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	**HostFamily**
Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**Island**
Labo	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**County**
Luzon	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**FaunalRegion**
Bicol	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**SubFaunalRegions**
14.07577	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	**Latitude**
122.7691	121.447	121.447	121.447	121.447	121.447	121.447	121.447	121.447	**Longitude**
109965	196	196	196	196	196	196	196	196	**Island Size (km^2)**
2016	2018	2018	2018	2018	2018	2018	2018	2018	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Upper Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Female	Male	Male	Female	Male	Female	Male	Female	Female	**Sex**
D50	Not Included	Not Included	Not Included	Not Included	B10	B9	B8	B7	**Code for Supp. Fig. X**
No	Yes	Yes	Yes	Yes	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
									Notes

Brachymeles kadwa	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles bicolor*	*Brachymeles bicolor*	**SpeciesID**
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Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Calayan	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**Island**
Calayan	Albay	Albay	Albay	Albay	Gattaran	Gattaran	Quezon	Quezon	**County**
Babuyan	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**FaunalRegion**
Babuyan	Bicol	Bicol	Bicol	Bicol	Nasipping	Nasipping	Palali	Palali	**SubFaunalRegions**
19.27482	13.31476	13.31476	13.31476	13.31476	17.97014	17.97014	16.46231	16.46231	**Latitude**
121.447	123.6955	123.6955	123.6955	123.6955	121.6544	121.6544	121.2198	121.2198	**Longitude**
196	109965	109965	109965	109965	109965	109965	109965	109965	**Island Size (km^2)**
2018	2016	2016	2016	2016	2017	2017	2017	2017	**Year**
Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	**Ecology**
Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Unknown	Juvenile	**AgeClass**
Female	Male	Female	Male	Male	Male	Female	Unknown	Unknown	**Sex**
Not Included	Not Included	D65	D62	D59	Not Included	Not Included	D1	Not Included	**Code for Supp. Fig. X**
Yes	Yes	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
					Removed from analyses poor sequencing data	Removed from analyses poor sequencing data		Removed from analyses poor sequencing data	**Notes**

Brachymeles kadwa	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles kadwa*	**SpeciesID**
Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**Island**
Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**County**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**FaunalRegion**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**SubFaunalRegions**
19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	**Latitude**
121.447	121.447	121.447	121.447	121.447	121.447	121.447	121.447	121.447	**Longitude**
196	196	196	196	196	196	196	196	196	**Island Size (km^2)**
2018	2018	2018	2018	2018	2018	2018	2018	2018	**Year**
Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	**Ecology**
Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Female	Unknown	Male	Unknown	Female	Unknown	Female	Unknown	Female	**Sex**
Not Included	Not Included	B26	B25	B24	B23	Not Included	B22	B21	**Code for Supp. Fig. X**
Yes	Yes	No	No	No	No	Yes	No	No	**Removed due to low sequence counts (<1,000)?**
									Notes

Brachymeles muntingkamay	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles kadwa*	*Brachymeles kadwa*	**SpeciesID**
Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Luzon	Luzon	Luzon	Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Calayan	**Island**
Quezon	Quezon	Quezon	Quezon	Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Calayan	**County**
Luzon	Luzon	Luzon	Luzon	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**FaunalRegion**
Palali	Palali	Palali	Palali	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**SubFaunalRegions**
16.46231	16.46231	16.46231	16.46231	18.91781	18.91781	18.91781	18.91781	19.27482	**Latitude**
121.2198	121.2198	121.2198	121.2198	121.8925	121.8925	121.8925	121.8925	121.447	**Longitude**
109965	109965	109965	109965	166	166	166	166	196	**Island Size (km^2)**
2017	2017	2017	2017	2018	2018	2018	2018	2018	**Year**
Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	**Ecology**
Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	**Microhabitat**
Unknown	Unknown	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Female	Unknown	Female	Female	Unknown	Unknown	Unknown	Female	Female	**Sex**
Not Included	Not Included	Not Included	Not Included	A6	Not Included	A5	Not Included	B27	**Code for Supp. Fig. X**
No	No	No	No	No	Yes	No	Yes	No	**Removed due to low sequence counts (<1,000)?**
Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	Removed from analyses poor sequencing data						**Notes**

Brachymeles muntingkamay	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	**SpeciesID**
Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**Island**
Quezon	Quezon	Quezon	Quezon	Quezon	Quezon	Quezon	Quezon	Quezon	**County**
Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**FaunalRegion**
Palali	Palali	Palali	Palali	Palali	Palali	Palali	Palali	Palali	**SubFaunalRegions**
16.46231	16.46231	16.46231	16.46231	16.46231	16.46231	16.46231	16.46231	16.46231	**Latitude**
121.2198	121.2198	121.2198	121.2198	121.2198	121.2198	121.2198	121.2198	121.2198	**Longitude**
109965	109965	109965	109965	109965	109965	109965	109965	109965	**Island Size (km^2)**
2017	2017	2017	2017	2017	2017	2017	2017	2017	**Year**
Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	**Ecology**
Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	**Microhabitat**
Unknown	Adult	Unknown	Adult	Adult	Unknown	Adult	Unknown	Unknown	**AgeClass**
Unknown	Female	Unknown	Female	Female	Unknown	Female	Unknown	Unknown	**Sex**
Not Included	Not Included	Not Included	Not Included	Not Included	Not Included	Not Included	Not Included	Not Included	**Code for Supp. Fig. X**
Yes	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
	Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	**Notes**

Brachymeles muntingkamay	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	*Brachymeles muntingkamay*	**SpeciesID**
Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**Island**
Quezon	Quezon	Quezon	Quezon	Quezon	Quezon	Quezon	Quezon	Quezon	**County**
Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**FaunalRegion**
Palali	Palali	Palali	Palali	Palali	Palali	Palali	Palali	Palali	**SubFaunalRegions**
16.46231	16.46231	16.46231	16.46231	16.46231	16.46231	16.46231	16.46231	16.46231	**Latitude**
121.2198	121.2198	121.2198	121.2198	121.2198	121.2198	121.2198	121.2198	121.2198	**Longitude**
109965	109965	109965	109965	109965	109965	109965	109965	109965	**Island Size (km^2)**
2017	2017	2017	2017	2017	2017	2017	2017	2017	**Year**
Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	**Ecology**
Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	**Microhabitat**
Unknown	Adult	Unknown	Unknown	Unknown	Adult	Unknown	Adult	Adult	**AgeClass**
Unknown	Female	Unknown	Unknown	Unknown	Female	Unknown	Female	Female	**Sex**
D19	D11	D7	D6	D5	D4	D3	D2	Not Included	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	No	Yes	**Removed due to low sequence counts (<1,000)?**
									Notes

Bronchocela marmorata	*Bronchocela marmorata*	*Bronchocela marmorata*	*Bronchocela marmorata*	*Brachymeles taylori*	*Brachymeles taylori*	*Brachymeles taylori*	*Brachymeles taylori*	*Brachymeles taylori*	**SpeciesID**
Agamidae	Agamidae	Agamidae	Agamidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Negros	Negros	Luzon	Negros	Negros	Negros	Negros	Negros	**Island**
Albay	Valencia	Valencia	Gattaran	Valencia	Valencia	Valencia	Hinoba-an	Hinoba-an	**County**
Luzon	NegrosPanay	NegrosPanay	Luzon	NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	**FaunalRegion**
Bicol	Valencia	Valencia	Nasipping	Valencia	Valencia	Valencia	Valencia	Valencia	**SubFaunalRegions**
13.31476	9.28753	9.28753	17.97014	9.28753	9.28753	9.28753	9.61275	9.6119	**Latitude**
123.6955	123.209	123.209	121.6544	123.209	123.209	123.209	122.4648	122.4642	**Longitude**
109965	13310	13310	109965	13310	13310	13310	13310	13310	**Island Size (km^2)**
2017	2016	2016	2017	2016	2016	2016	2016	2016	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	**Ecology**
Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Burrowing	Burrowing	Burrowing	Burrowing	Burrowing	**Microhabitat**
Juvenile	Adult	Adult	Adult	Adult	Adult	Juvenile	Adult	Adult	**AgeClass**
Unknown	Unknown	Unknown	Female	Male	Male	Unknown	Unknown	Unknown	**Sex**
D79	C8	C7	Not Included	C51	C50	C48	C13	C12	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
			Removed from analyses poor sequencing data						**Notes**

Bronchocela marmorata	*Bronchocela marmorata*	*Bronchocela marmorata*	*Bronchocela marmorata*	*Bronchocela marmorata*	*Bronchocela marmorata*	*Bronchocela marmorata*	*Bronchocela marmorata*	*Bronchocela marmorata*	**SpeciesID**
Agamidae	Agamidae	Agamidae	Agamidae	Agamidae	Agamidae	Agamidae	Agamidae	Agamidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Negros	Negros	Negros	Negros	Negros	Negros	Negros	Luzon	Luzon	**Island**
Valencia	Valencia	Valencia	Valencia	Valencia	Valencia	Valencia	Albay	Albay	**County**
NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	Luzon	Luzon	**FaunalRegion**
Valencia	Valencia	Valencia	Valencia	Valencia	Valencia	Valencia	Bicol	Bicol	**SubFaunalRegions**
9.28753	9.28753	9.28753	9.28753	9.28753	9.28753	9.28753	13.31476	13.31476	**Latitude**
123.209	123.209	123.209	123.209	123.209	123.209	123.209	123.6955	123.6955	**Longitude**
13310	13310	13310	13310	13310	13310	13310	109965	109965	**Island Size (km^2)**
2016	2016	2016	2016	2016	2016	2016	2017	2017	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Male	Unknown	Female	Male	Unknown	Male	Unknown	Male	Female	**Sex**
C30	C29	C28	C27	C25	C21	C20	D84	D80	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
									Notes

Cuora amboinensis	*Coelognathus erythrurus*	*Coelognathus erythrurus*	*Coelognathus erythrurus*	*Chrysopelea paradisi*	*Cerberus rynchops*	*Cerberus rynchops*	*Cerberus rynchops*	*Calamaria gervaisii*	**SpeciesID**
Geoemydidae	Colubridae	Colubridae	Colubridae	Colubridae	Homalopsida[illegible]	Homalopsida[illegible]	Homalopsida[illegible]	Colubridae	**HostFamily**
Cryptodira	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	**HostSubOrder**
Testudines	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Negros	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**Island**
Labo	Valencia	Albay	Labo	Gattaran	Labo	Labo	Labo	Quezon	**County**
Luzon	NegrosPanay	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**FaunalRegion**
Bicol	Valencia	Bicol	Bicol	Nasipping	Bicol	Bicol	Bicol	Palali	**SubFaunalRegions**
14.07577	9.28753	13.31476	14.07577	17.97014	14.07577	14.07577	14.07577	16.46231	**Latitude**
122.7691	123.209	123.6955	122.7691	121.6544	122.7691	122.7691	122.7691	121.2198	**Longitude**
109965	13310	109965	109965	109965	109965	109965	109965	109965	**Island Size (km^2)**
2016	2016	2017	2016	2017	2016	2016	2016	2017	**Year**
Aquatic	Ground	Ground	Ground	Arboreal	Aquatic	Aquatic	Aquatic	Burrowing	**Ecology**
Freshwater	Ground	Ground	Ground	Upper Level Arboreal	Marine	Marine	Marine	Burrowing	**Microhabitat**
Unknown	Adult	Adult	Adult	Adult	Unknown	Unknown	Unknown	Unknown	**AgeClass**
Unknown	Female	Male	Female	Female	Unknown	Unknown	Unknown	Unknown	**Sex**
Not Included	C44	D83	D25	Not Included	D43	D42	Not Included	D20	**Code for Supp. Fig. X**
Yes	No	No	No	No	No	No	Yes	No	**Removed due to low sequence counts (<1,000)?**
				Removed from analyses poor sequencing data					**Notes**

Cyclocorus lineatus	*Cuora amboinensis*	*Cuora amboinensis*	*Cuora amboinensis*	*Cuora amboinensis*	*Cuora amboinensis*	*Cuora amboinensis*	*Cuora amboinensis*	*Cuora amboinensis*	**SpeciesID**
Colubridae	Geoemydidae	Geoemydidae	Geoemydidae	Geoemydidae	Geoemydidae	Geoemydidae	Geoemydidae	Geoemydidae	**HostFamily**
Serpentes	Cryptodira	Cryptodira	Cryptodira	Cryptodira	Cryptodira	Cryptodira	Cryptodira	Cryptodira	**HostSubOrder**
Squamata	Testudines	Testudines	Testudines	Testudines	Testudines	Testudines	Testudines	Testudines	**HostOrder**
Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**Island**
Gattaran	Labo	Labo	Labo	Labo	Labo	Labo	Labo	Labo	**County**
Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**FaunalRegion**
Nasipping	Bicol	Bicol	Bicol	Bicol	Bicol	Bicol	Bicol	Bicol	**SubFaunalRegions**
17.97014	14.07577	14.07577	14.07577	14.07577	14.07577	14.07577	14.07577	14.07577	**Latitude**
121.6544	122.7691	122.7691	122.7691	122.7691	122.7691	122.7691	122.7691	122.7691	**Longitude**
109965	109965	109965	109965	109965	109965	109965	109965	109965	**Island Size (km^2)**
2017	2016	2016	2016	2016	2016	2016	2016	2016	**Year**
Ground	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	**Ecology**
Ground	Freshwater	Freshwater	Freshwater	Freshwater	Freshwater	Freshwater	Freshwater	Freshwater	**Microhabitat**
Adult	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	**AgeClass**
Female	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	**Sex**
Not Included	D64	D53	D51	D45	D41	D38	D37	D36	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
Removed from analyses poor sequencing data									**Notes**

Cyrtodactylus philippinicus	*Cyrtodactylus philippinicus*	*Cyrtodactylus philippinicus*	*Cyclocorus lineatus*	*Cyclocorus lineatus*	*Cyclocorus lineatus*	*Cyclocorus lineatus*	*Cyclocorus lineatus*	*Cyclocorus lineatus*	**SpeciesID**
Gekkonidae	Gekkonidae	Gekkonidae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Luzon	Luzon	Luzon	Negros	Negros	Luzon	Luzon	Luzon	**Island**
Quezon	Quezon	Quezon	Albay	Valencia	Valencia	Labo	Gattaran	Gattaran	**County**
Luzon	Luzon	Luzon	Luzon	NegrosPanay	NegrosPanay	Luzon	Luzon	Luzon	**FaunalRegion**
Palali	Palali	Palali	Bicol	Valencia	Valencia	Bicol	Nasipping	Nasipping	**SubFaunalRegions**
16.46231	16.46231	16.46231	13.31476	9.28753	9.28753	14.07577	17.97014	17.97014	**Latitude**
121.2198	121.2198	121.2198	123.6955	123.209	123.209	122.7691	121.6544	121.6544	**Longitude**
109965	109965	109965	109965	13310	13310	109965	109965	109965	**Island Size (km^2)**
2017	2017	2017	2017	2016	2016	2016	2017	2017	**Year**
Arboreal	Arboreal	Arboreal	Ground	Ground	Ground	Ground	Ground	Ground	**Ecology**
Saxicolous	Saxicolous	Saxicolous	Ground	Ground	Ground	Ground	Ground	Ground	**Microhabitat**
Adult	Unknown	Juvenile	Adult	Adult	Adult	Adult	Unknown	Unknown	**AgeClass**
Female	Male	Unknown	Female	Female	Male	Female	Female	Male	**Sex**
Not Included	Not Included	Not Included	D82	C2	C1	D40	Not Included	Not Included	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	Renamed Oxyrhabdium leporinum for phylo analyses	Renamed Oxyrhabdium leporinum for phylo analyses	Renamed Oxyrhabdium leporinum for phylo analyses	Renamed Oxyrhabdium leporinum for phylo analyses	Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	**Notes**

Cyrtodactylus philippinicus	*Cyrtodactylus philippinicus*	*Cyrtodactylus philippinicus*	*Cyrtodactylus philippinicus*	*Cyrtodactylus philippinicus*	*Cyrtodactylus philippinicus*	*Cyrtodactylus philippinicus*	*Cyrtodactylus philippinicus*	*Cyrtodactylus philippinicus*	**SpeciesID**
Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**Island**
Albay	Albay	Albay	Labo	Labo	Labo	Quezon	Quezon	Quezon	**County**
Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	**FaunalRegion**
Bicol	Bicol	Bicol	Bicol	Bicol	Bicol	Palali	Palali	Palali	**SubFaunalRegions**
13.31476	13.31476	13.31476	14.07577	14.07577	14.07577	16.46231	16.46231	16.46231	**Latitude**
123.6955	123.6955	123.6955	122.7691	122.7691	122.7691	121.2198	121.2198	121.2198	**Longitude**
109965	109965	109965	109965	109965	109965	109965	109965	109965	**Island Size (km^2)**
2016	2016	2016	2016	2016	2016	2017	2017	2017	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Unknown	Unknown	Unknown	**AgeClass**
Male	Female	Female	Male	Male	Male	Unknown	Male	Male	**Sex**
D78	D77	D72	D56	D55	D34	D14	D13	D12	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
									Notes

Dendrelaphis cf. philippinensis	*Dendrelaphis cf. philippinensis*	*Dendrelaphis cf. philippinensis*	*Dendrelaphis cf. philippinensis*	*Dendrelaphis cf. philippinensis*	*Dendrelaphis cf. marenae*	*Cyrtodactylus philippinicus*	*Cyrtodactylus philippinicus*	*Cyrtodactylus philippinicus*	**SpeciesID**
Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Gekkonidae	Gekkonidae	Gekkonidae	**HostFamily**
Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Negros	Negros	Negros	Negros	Negros	Negros	Negros	Negros	Luzon	**Island**
Valencia	Valencia	Valencia	Valencia	Valencia	Valencia	Valencia	Valencia	Albay	**County**
NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	Luzon	**FaunalRegion**
Valencia	Valencia	Valencia	Valencia	Valencia	Valencia	Valencia	Valencia	Bicol	**SubFaunalRegions**
9.28753	9.28753	9.28753	9.28753	9.28753	9.28753	9.28753	9.28753	13.31476	**Latitude**
123.209	123.209	123.209	123.209	123.209	123.209	123.209	123.209	123.6955	**Longitude**
13310	13310	13310	13310	13310	13310	13310	13310	109965	**Island Size (km^2)**
2016	2016	2016	2016	2016	2016	2016	2016	2017	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Saxicolous	Saxicolous	Saxicolous	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Female	Male	Female	Female	Male	Female	Male	Female	Male	**Sex**
C16	C15	C14	C9	C5	C4	C33	C19	D81	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
Assigned Dendrelaphis philippinensis for phylo	Assigned Dendrelaphis philippinensis for phylo	Assigned Dendrelaphis philippinensis for phylo	Assigned Dendrelaphis philippinensis for phylo	Assigned Dendrelaphis philippinensis for phylo	Assigned Dendrelaphis marenae for phylo analyses				**Notes**

Dendrelaphis philippinensis	*Dendrelaphis philippinensis*	*Dendrelaphis marenae*	*Dendrelaphis marenae*	*Dendrelaphis luzonensis*	*Dendrelaphis luzonensis*	*Dendrelaphis cf. philippinensis*	*Dendrelaphis cf. philippinensis*	*Dendrelaphis cf. philippinensis*	**SpeciesID**
Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	**HostFamily**
Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	Negros	Negros	Negros	**Island**
Tabaco	Labo	Tabaco	Tabaco	Labo	Labo	Valencia	Valencia	Valencia	**County**
Luzon	Luzon	Luzon	Luzon	Luzon	Luzon	NegrosPanay	NegrosPanay	NegrosPanay	**FaunalRegion**
Bicol	Bicol	Bicol	Bicol	Bicol	Bicol	Valencia	Valencia	Valencia	**SubFaunalRegions**
13.30558	14.07577	13.30558	13.30558	14.07577	14.07577	9.28753	9.28753	9.28753	**Latitude**
123.689	122.7691	123.689	123.689	122.7691	122.7691	123.209	123.209	123.209	**Longitude**
109965	109965	109965	109965	109965	109965	13310	13310	13310	**Island Size (km^2)**
2016	2016	2016	2016	2016	2016	2016	2016	2016	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Female	Female	Male	Male	Unknown	Male	Male	Female	Female	**Sex**
D70	D26	D75	D69	D61	D60	C42	Not Included	C37	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	Yes	No	**Removed due to low sequence counts (<1,000)?**
						Assigned Dendrelaphis philippinensis for phylo		Assigned Dendrelaphis philippinensis for phylo	**Notes**

Draco spilopterus	*Draco spilopterus*	*Draco spilopterus*	*Draco spilopterus*	*Draco spilopterus*	*Dibamus sp.*	*Dibamus sp.*	*Dendrelaphis sp.*	*Dendrelaphis philippinensis*	**SpeciesID**
Agamidae	Agamidae	Agamidae	Agamidae	Agamidae	Dibamidae	Dibamidae	Colubridae	Colubridae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Serpentes	Serpentes	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Luzon	Luzon	Luzon	Luzon	Negros	Negros	Luzon	Luzon	**Island**
Labo	Labo	Labo	Labo	Gattaran	Valencia	Valencia	Labo	Tabaco	**County**
Luzon	Luzon	Luzon	Luzon	Luzon	NegrosPanay	NegrosPanay	Luzon	Luzon	**FaunalRegion**
Bicol	Bicol	Bicol	Bicol	Nasipping	Valencia	Valencia	Bicol	Bicol	**SubFaunalRegions**
14.07577	14.07577	14.07577	14.07577	17.97014	9.28753	9.28753	14.07577	13.30558	**Latitude**
122.7691	122.7691	122.7691	122.7691	121.6544	123.209	123.209	122.7691	123.689	**Longitude**
109965	109965	109965	109965	109965	13310	13310	109965	109965	**Island Size (km^2)**
2016	2016	2016	2016	2017	2016	2016	2016	2016	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Burrowing	Burrowing	Arboreal	Arboreal	**Ecology**
Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Burrowing	Burrowing	Lower Level Arboreal	Lower Level Arboreal	**Microhabitat**
Adult	Adult	Adult	Adult	Unknown	Adult	Adult	Adult	Adult	**AgeClass**
Female	Female	Male	Female	Female	Unknown	Unknown	Male	Male	**Sex**
D52	D35	D33	D32	Not Included	C52	C47	D49	D76	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
				Removed from analyses poor sequencing data	Assigned Dibamus novaeguineae for phylo analyses	Assigned Dibamus novaeguineae for phylo analyses	Assigned Dendrelaphis luzonensis for phylo analyses		**Notes**

Emoia atrocostata	*Emoia atrocostata*	*Emoia atrocostata*	*Emoia atrocostata*	*Emoia atrocostata*	*Draco spilopterus*	*Draco spilopterus*	*Draco spilopterus*	*Draco spilopterus*	**SpeciesID**
Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Agamidae	Agamidae	Agamidae	Agamidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Calayan	Calayan	Calayan	Calayan	Calayan	Negros	Luzon	Luzon	Luzon	**Island**
Calayan	Calayan	Calayan	Calayan	Calayan	Valencia	Labo	Labo	Labo	**County**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	NegrosPanay	Luzon	Luzon	Luzon	**FaunalRegion**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Valencia	Bicol	Bicol	Bicol	**SubFaunalRegions**
19.27482	19.27482	19.27482	19.27482	19.27482	9.28753	14.07577	14.07577	14.07577	**Latitude**
121.447	121.447	121.447	121.447	121.447	123.209	122.7691	122.7691	122.7691	**Longitude**
196	196	196	196	196	13310	109965	109965	109965	**Island Size (km^2)**
2018	2018	2018	2018	2018	2016	2016	2016	2016	**Year**
Ground	Ground	Ground	Ground	Ground	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Ground	Ground	Ground	Ground	Ground	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Female	Female	Male	Male	Male	Male	Male	Male	Male	**Sex**
B36	B35	B34	Not Included	Not Included	C45	D67	Not Included	Not Included	**Code for Supp. Fig. X**
No	No	No	Yes	Yes	No	No	Yes	Yes	**Removed due to low sequence counts (<1,000)?**
									Notes

Eutropis bontocensis	*Eutropis bontocensis*	*Eutropis bontocensis*	*Eutropis bontocensis*	*Eutropis bontocensis*	*Eutropis bontocensis*	*Eutropis bontocensis*	*Eutropis bontocensis*	*Emoia atrocostata*	**SpeciesID**
Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**Island**
Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**County**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**FaunalRegion**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**SubFaunalRegions**
19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	**Latitude**
121.447	121.447	121.447	121.447	121.447	121.447	121.447	121.447	121.447	**Longitude**
196	196	196	196	196	196	196	196	196	**Island Size (km^2)**
2018	2018	2018	2018	2018	2018	2018	2018	2018	**Year**
Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	**Ecology**
Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Male	Male	Male	Female	Female	Unknown	Unknown	Unknown	Female	**Sex**
Not Included	B50	B49	Not Included	B33	Not Included	Not Included	Not Included	Not Included	**Code for Supp. Fig. X**
Yes	No	No	Yes	No	Yes	Yes	Yes	Yes	**Removed due to low sequence counts (<1,000)?**
									Notes

Eutropis multifasciata	*Eutropis multifasciata*	*Eutropis multicarinata*	*Eutropis multicarinata*	*Eutropis multicarinata*	*Eutropis multicarinata*	*Eutropis multicarinata*	*Eutropis multicarinata*	*Eutropis bontocensis*	**SpeciesID**
Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Negros	Luzon	Calayan	Calayan	Camiguin Norte	Camiguin Norte	Luzon	Luzon	Calayan	**Island**
Valencia	Gattaran	Calayan	Calayan	Camiguin Norte	Camiguin Norte	Labo	Gattaran	Calayan	**County**
NegrosPanay	Luzon	Babuyan	Babuyan	Babuyan	Babuyan	Luzon	Luzon	Babuyan	**FaunalRegion**
Valencia	Nasipping	Babuyan	Babuyan	Babuyan	Babuyan	Bicol	Nasipping	Babuyan	**SubFaunalRegions**
9.28753	17.97014	19.27482	19.27482	18.91781	18.91781	14.07577	17.97014	19.27482	**Latitude**
123.209	121.6544	121.447	121.447	121.8925	121.8925	122.7691	121.6544	121.447	**Longitude**
13310	109965	196	196	166	166	109965	109965	196	**Island Size (km^2)**
2016	2017	2018	2018	2018	2018	2016	2017	2018	**Year**
Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	**Ecology**
Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	**Microhabitat**
Juvenile	Adult	Adult	Adult	Adult	Adult	Adult	Unknown	Adult	**AgeClass**
Unknown	Female	Male	Female	Unknown	Unknown	Female	Unknown	Male	**Sex**
C35	Not Included	B53	B43	A8	A7	Not Included	Not Included	B54	**Code for Supp. Fig. X**
No	No	No	No	No	No	Yes	No	No	**Removed due to low sequence counts (<1,000)?**
	Removed from analyses poor sequencing data						Removed from analyses poor sequencing data		**Notes**

Gekko crombota	*Gekko crombota*	*Gekko crombota*	*Gekko crombota*	*Gekko crombota*	*Gekko crombota*	*Gekko crombota*	*Gekko crombota*	*Gekko cf. kikuchii*	**SpeciesID**
Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Luzon	**Island**
Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Gattaran	**County**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Luzon	**FaunalRegion**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Nasipping	**SubFaunalRegions**
18.91781	18.91781	18.91781	18.91781	18.91781	18.91781	18.91781	18.91781	17.97014	**Latitude**
121.8925	121.8925	121.8925	121.8925	121.8925	121.8925	121.8925	121.8925	121.6544	**Longitude**
166	166	166	166	166	166	166	166	109965	**Island Size (km^2)**
2018	2018	2018	2018	2018	2018	2018	2018	2017	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Saxicolous	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Male	Male	Female	Male	Female	Female	Female	Unknown	Female	**Sex**
A15	Not Included	A14	A13	A12	A11	A10	A9	Not Included	**Code for Supp. Fig. X**
No	Yes	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
								Removed from analyses poor sequencing data	**Notes**

Gekko mindorensis	*Gekko mindorensis*	*Gekko mindorensis*	*Gekko mindorensis*	*Gekko kikuchii*	*Gekko gecko*	*Gekko gecko*	*Gekko gecko*	*Gekko gecko*	**SpeciesID**
Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Luzon	Luzon	Luzon	Luzon	Negros	Negros	Negros	Negros	**Island**
Quezon	Quezon	Quezon	Quezon	Gattaran	Valencia	Valencia	Valencia	Valencia	**County**
Luzon	Luzon	Luzon	Luzon	Luzon	NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	**FaunalRegion**
Palali	Palali	Palali	Palali	Nasipping	Valencia	Valencia	Valencia	Valencia	**SubFaunalRegions**
16.46231	16.46231	16.46231	16.46231	17.97014	9.28753	9.28753	9.28753	9.28753	**Latitude**
121.2198	121.2198	121.2198	121.2198	121.6544	123.209	123.209	123.209	123.209	**Longitude**
109965	109965	109965	109965	109965	13310	13310	13310	13310	**Island Size (km^2)**
2017	2017	2017	2017	2017	2016	2016	2016	2016	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	**Microhabitat**
Unknown	Adult	Unknown	Unknown	Juvenile	Adult	Adult	Adult	Adult	**AgeClass**
Male	Female	Female	Female	Unknown	Unknown	Female	Unknown	Male	**Sex**
D18	D17	D16	D15	Not Included	C32	C31	C18	C17	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
				Removed from analyses poor sequencing data					**Notes**

Gekko rossi	*Gekko rossi*	*Gekko rossi*	*Gekko rossi*	*Gekko rossi*	*Gekko rossi*	*Gekko rossi*	*Gekko rossi*	*Gekko rossi*	**SpeciesID**
Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**Island**
Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**County**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**FaunalRegion**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**SubFaunalRegions**
19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	**Latitude**
121.447	121.447	121.447	121.447	121.447	121.447	121.447	121.447	121.447	**Longitude**
196	196	196	196	196	196	196	196	196	**Island Size (km^2)**
2018	2018	2018	2018	2018	2018	2018	2018	2018	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Male	Male	Male	Male	Male	Female	Female	Male	Male	**Sex**
B20	B19	B18	B17	Not Included	B16	B15	B14	B13	**Code for Supp. Fig. X**
No	No	No	No	Yes	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
									Notes

Hemidactylus frenatus	*Hemidactylus frenatus*	*Hemidactylus frenatus*	*Gonocephalus sp.*	*Gonocephalus sp.*	*Gonocephalus sp.*	*Gekko rossi*	*Gekko rossi*	*Gekko rossi*	**SpeciesID**
Gekkonidae	Gekkonidae	Gekkonidae	Agamidae	Agamidae	Agamidae	Gekkonidae	Gekkonidae	Gekkonidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Negros	Luzon	Luzon	Luzon	Luzon	Luzon	Calayan	Calayan	Calayan	**Island**
Valencia	Quezon	Gattaran	Albay	Albay	Labo	Calayan	Calayan	Calayan	**County**
NegrosPanay	Luzon	Luzon	Luzon	Luzon	Luzon	Babuyan	Babuyan	Babuyan	**FaunalRegion**
Valencia	Palali	Nasipping	Bicol	Bicol	Bicol	Babuyan	Babuyan	Babuyan	**SubFaunalRegions**
9.28753	16.46231	17.97014	13.31476	13.31476	14.07577	19.27482	19.27482	19.27482	**Latitude**
123.209	121.2198	121.6544	123.6955	123.6955	122.7691	121.447	121.447	121.447	**Longitude**
13310	109965	109965	109965	109965	109965	196	196	196	**Island Size (km^2)**
2016	2017	2017	2017	2017	2016	2018	2018	2018	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Saxicolous	Saxicolous	Saxicolous	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	**Microhabitat**
Adult	Unknown	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Male	Unknown	Male	Male	Male	Unknown	Male	Male	Female	**Sex**
C41	Not Included	Not Included	D89	D86	Not Included	B55	Not Included	Not Included	**Code for Supp. Fig. X**
No	Yes	No	No	No	Yes	No	Yes	Yes	**Removed due to low sequence counts (<1,000)?**
		Removed from analyses poor sequencing data	Assigned Gonocephalus sophiae for phylo analyses	Assigned Gonocephalus sophiae for phylo analyses					**Notes**

Hydrosaurus pustulatus	*Hydrosaurus pustulatus*	*Hydrosaurus pustulatus*	*Hydrosaurus pustulatus*	*Hydrosaurus pustulatus*	*Hydrosaurus pustulatus*	*Hemidactylus platyurus*	*Hemidactylus platyurus*	*Hemidactylus platyurus*	**SpeciesID**
Agamidae	Agamidae	Agamidae	Agamidae	Agamidae	Agamidae	Gekkonidae	Gekkonidae	Gekkonidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Negros	Negros	Luzon	Luzon	Luzon	Luzon	Negros	Luzon	Luzon	**Island**
Valencia	Valencia	Labo	Labo	Labo	Labo	Valencia	Gattaran	Gattaran	**County**
NegrosPanay	NegrosPanay	Luzon	Luzon	Luzon	Luzon	NegrosPanay	Luzon	Luzon	**FaunalRegion**
Valencia	Valencia	Bicol	Bicol	Bicol	Bicol	Valencia	Nasipping	Nasipping	**SubFaunalRegions**
9.28753	9.28753	14.07577	14.07577	14.07577	14.07577	9.28753	17.97014	17.97014	**Latitude**
123.209	123.209	122.7691	122.7691	122.7691	122.7691	123.209	121.6544	121.6544	**Longitude**
13310	13310	109965	109965	109965	109965	13310	109965	109965	**Island Size (km^2)**
2016	2016	2016	2016	2016	2016	2016	2017	2017	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	Saxicolous	**Microhabitat**
Adult	Juvenile	Juvenile	Adult	Adult	Adult	Adult	Unknown	Unknown	**AgeClass**
Female	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	**Sex**
C23	C22	Not Included	D39	D23	D22	C6	Not Included	Not Included	**Code for Supp. Fig. X**
No	No	Yes	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
							Removed from analyses poor sequencing data	Removed from analyses poor sequencing data	**Notes**

Laticauda laticaudata	*Laticauda laticaudata*	*Laticauda laticaudata*	*Laticauda laticaudata*	*Laticauda laticaudata*	*Hydrosaurus pustulatus*	*Hydrosaurus pustulatus*	*Hydrosaurus pustulatus*	*Hydrosaurus pustulatus*	**SpeciesID**
Elapidae	Elapidae	Elapidae	Elapidae	Elapidae	Agamidae	Agamidae	Agamidae	Agamidae	**HostFamily**
Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Calayan	Calayan	Calayan	Calayan	Calayan	Negros	Negros	Negros	Negros	**Island**
Calayan	Calayan	Calayan	Calayan	Calayan	Valencia	Valencia	Valencia	Valencia	**County**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	**FaunalRegion**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Valencia	Valencia	Valencia	Valencia	**SubFaunalRegions**
19.27482	19.27482	19.27482	19.27482	19.27482	9.28753	9.28753	9.28753	9.28753	**Latitude**
121.447	121.447	121.447	121.447	121.447	123.209	123.209	123.209	123.209	**Longitude**
196	196	196	196	196	13310	13310	13310	13310	**Island Size (km^2)**
2018	2018	2018	2018	2018	2016	2016	2016	2016	**Year**
Aquatic	Aquatic	Aquatic	Aquatic	Aquatic	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Marine	Marine	Marine	Marine	Marine	Saxicolous	Saxicolous	Saxicolous	Saxicolous	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Juvenile	Juvenile	Juvenile	Adult	**AgeClass**
Unknown	Unknown	Male	Unknown	Male	Unknown	Unknown	Unknown	Female	**Sex**
Not Included	B5	B4	B3	B2	C53	C46	C34	C24	**Code for Supp. Fig. X**
Yes	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
									Notes

Lycodon alcalai	*Lycodon alcalai*	*Luperosaurus macgregori*	*Luperosaurus macgregori*	*Luperosaurus macgregori*	*Luperosaurus macgregori*	*Luperosaurus macgregori*	*Laticauda laticaudata*	*Laticauda laticaudata*	**SpeciesID**
Colubridae	Colubridae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Gekkonidae	Elapidae	Elapidae	**HostFamily**
Serpentes	Serpentes	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Serpentes	Serpentes	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**Island**
Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**County**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**FaunalRegion**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**SubFaunalRegions**
19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	**Latitude**
121.447	121.447	121.447	121.447	121.447	121.447	121.447	121.447	121.447	**Longitude**
196	196	196	196	196	196	196	196	196	**Island Size (km^2)**
2018	2018	2018	2018	2018	2018	2018	2018	2018	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Aquatic	Aquatic	**Ecology**
Lower Level Arboreal	Lower Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Marine	Marine	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Male	Unknown	Unknown	Female	Female	Unknown	Female	Male	Male	**Sex**
B30	B29	B40	B39	B38	B37	Not Included	B12	B11	**Code for Supp. Fig. X**
No	No	No	No	No	No	Yes	No	No	**Removed due to low sequence counts (<1,000)?**
									Notes

Lycodon capucinus	*Lycodon alcalai*	*Lycodon alcalai*	*Lycodon alcalai*	*Lycodon alcalai*	*Lycodon alcalai*	*Lycodon alcalai*	*Lycodon alcalai*	*Lycodon alcalai*	**SpeciesID**
Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	**HostFamily**
Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Camiguin Norte	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**Island**
Quezon	Camiguin Norte	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**County**
Luzon	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**FaunalRegion**
Palali	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**SubFaunalRegions**
16.46231	18.91781	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	**Latitude**
121.2198	121.8925	121.447	121.447	121.447	121.447	121.447	121.447	121.447	**Longitude**
109965	166	196	196	196	196	196	196	196	**Island Size (km^2)**
2017	2018	2018	2018	2018	2018	2018	2018	2018	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	Lower Level Arboreal	**Microhabitat**
Juvenile	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Unknown	Male	Male	Female	Female	Male	Female	Female	Unknown	**Sex**
Not Included	A16	Not Included	B52	B51	Not Included	B42	B32	B31	**Code for Supp. Fig. X**
No	No	Yes	No	No	Yes	No	No	No	**Removed due to low sequence counts (<1,000)?**
Removed from analyses poor sequencing data									**Notes**

Pinoyscincus abdictus	*Pinoyscincus abdictus*	*Pinoyscincus abdictus*	*Pinoyscincus abdictus*	*Naja philippinensis*	*Malayotyphlops sp.* 2	*Malayotyphlops sp.* 2	*Malayotyphlops sp.* 1	*Lycodon capucinus*	**SpeciesID**
Scincidae	Scincidae	Scincidae	Scincidae	Elapidae	Typhlopidae	Typhlopidae	Typhlopidae	Colubridae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Lacertilia	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Calayan	Calayan	Calayan	Calayan	Luzon	Luzon	Luzon	Luzon	Negros	**Island**
Calayan	Calayan	Calayan	Calayan	Albay	Quezon	Quezon	Quezon	Valencia	**County**
Babuyan	Babuyan	Babuyan	Babuyan	Luzon	Luzon	Luzon	Luzon	NegrosPanay	**FaunalRegion**
Babuyan	Babuyan	Babuyan	Babuyan	Bicol	Palali	Palali	Palali	Valencia	**SubFaunalRegions**
19.27482	19.27482	19.27482	19.27482	13.31476	16.46231	16.46231	16.46231	9.28753	**Latitude**
121.447	121.447	121.447	121.447	123.6955	121.2198	121.2198	121.2198	123.209	**Longitude**
196	196	196	196	109965	109965	109965	109965	13310	**Island Size (km^2)**
2018	2018	2018	2018	2017	2017	2017	2017	2016	**Year**
Ground	Ground	Ground	Ground	Ground	Burrowing	Burrowing	Burrowing	Arboreal	**Ecology**
Ground	Ground	Ground	Ground	Ground	Burrowing	Burrowing	Burrowing	Lower Level Arboreal	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Unknown	Unknown	Unknown	Adult	**AgeClass**
Unknown	Unknown	Unknown	Unknown	Male	Unknown	Unknown	Unknown	Female	**Sex**
B28	Not Included	Not Included	Not Included	D85	D10	D9	D8	C36	**Code for Supp. Fig. X**
No	Yes	Yes	Yes	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
					Assigned Malayotyphlops ruficaudus for phylo analyses	Assigned Malayotyphlops ruficaudus for phylo analyses	Assigned Malayotyphlops luzonensis for phylo analyses		**Notes**

Ptyas luzonensis	*Psammodynastes pulverulentus*	*Psammodynastes pulverulentus*	*Pinoyscincus abdictus*	*Pinoyscincus abdictus*	*Pinoyscincus abdictus*	*Pinoyscincus abdictus*	*Pinoyscincus abdictus*	*Pinoyscincus abdictus*	**SpeciesID**
Colubridae	Lamprophiida	Lamprophiida	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	Scincidae	**HostFamily**
Serpentes	Serpentes	Serpentes	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	Lacertilia	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Luzon	Luzon	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**Island**
Labo	Quezon	Quezon	Calayan	Calayan	Calayan	Calayan	Calayan	Calayan	**County**
Luzon	Luzon	Luzon	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**FaunalRegion**
Bicol	Palali	Bicol	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	**SubFaunalRegions**
14.07577	16.46231	14.07577	19.27482	19.27482	19.27482	19.27482	19.27482	19.27482	**Latitude**
122.7691	121.2198	122.7691	121.447	121.447	121.447	121.447	121.447	121.447	**Longitude**
109965	109965	109965	196	196	196	196	196	196	**Island Size (km^2)**
2016	2017	2017	2018	2018	2018	2018	2018	2018	**Year**
Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	**Ecology**
Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	**Microhabitat**
Adult	Unknown	Unknown	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Female	Female	Female	Female	Female	Male	Female	Female	Male	**Sex**
D46	Not Included	Not Included	B48	B47	B46	B45	B44	B41	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
	Removed from analyses poor sequencing data	Removed from analyses poor sequencing data							**Notes**

Ptyas luzonensis	*Ptyas luzonensis*	*Ptyas luzonensis*	*Ptyas luzonensis*	*Ptyas luzonensis*	*Ptyas luzonensis*	*Ptyas luzonensis*	*Ptyas luzonensis*	*Ptyas luzonensis*	**SpeciesID**
Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	Colubridae	**HostFamily**
Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Negros	Negros	Negros	Negros	Luzon	Luzon	Luzon	Luzon	Luzon	**Island**
Valencia	Valencia	Valencia	Valencia	Albay	Labo	Labo	Labo	Labo	**County**
NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	Luzon	Luzon	Luzon	Luzon	Luzon	**FaunalRegion**
Valencia	Valencia	Valencia	Valencia	Bicol	Bicol	Bicol	Bicol	Bicol	**SubFaunalRegions**
9.28753	9.28753	9.28753	9.28753	13.31476	14.07577	14.07577	14.07577	14.07577	**Latitude**
123.209	123.209	123.209	123.209	123.6955	122.7691	122.7691	122.7691	122.7691	**Longitude**
13310	13310	13310	13310	109965	109965	109965	109965	109965	**Island Size (km^2)**
2016	2016	2016	2016	2017	2016	2016	2016	2016	**Year**
Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	**Ecology**
Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	Ground	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Unknown	Unknown	Female	Female	Female	Male	Female	Male	Female	**Sex**
C54	Not Included	C11	C10	D88	Not Included	D68	Not Included	D63	**Code for Supp. Fig. X**
No	Yes	No	No	No	Yes	No	Yes	No	**Removed due to low sequence counts (<1,000)?**
									Notes

Trimeresurus flavomaculatus	*Trimeresurus flavomaculatus*	*Trimeresurus flavomaculatus*	*Trimeresurus flavomaculatus*	*Trimeresurus flavomaculatus*	*Trimeresurus flavomaculatus*	*Trimeresurus flavomaculatus*	*Trimeresurus flavomaculatus*	*Trimeresurus flavomaculatus*	**SpeciesID**
Viperidae	Viperidae	Viperidae	Viperidae	Viperidae	Viperidae	Viperidae	Viperidae	Viperidae	**HostFamily**
Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Calayan	Luzon	Luzon	Luzon	Luzon	**Island**
Camiguin Norte	Camiguin Norte	Camiguin Norte	Camiguin Norte	Calayan	Albay	Tabaco	Tabaco	Tabaco	**County**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Luzon	Luzon	Luzon	Luzon	**FaunalRegion**
Babuyan	Babuyan	Babuyan	Babuyan	Babuyan	Bicol	Bicol	Bicol	Bicol	**SubFaunalRegions**
18.91781	18.91781	18.91781	18.91781	19.27482	13.31476	13.30558	13.30558	13.30558	**Latitude**
121.8925	121.8925	121.8925	121.8925	121.447	123.6955	123.689	123.689	123.689	**Longitude**
166	166	166	166	196	109965	109965	109965	109965	**Island Size (km^2)**
2018	2018	2018	2018	2018	2017	2016	2016	2016	**Year**
Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	**Microhabitat**
Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Unknown	Unknown	Female	Female	Female	Female	Female	Female	Male	**Sex**
A3	Not Included	A2	A1	B1	D87	D74	D73	D71	**Code for Supp. Fig. X**
No	Yes	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
									Notes

Tropidophorus grayi	*Tropidophorus grayi*	*Tropidophorus grayi*	*Tropidonophis negrosensis*	*Tropidolaemus subannulatus*	*Tropidolaemus subannulatus*	*Tropidolaemus subannulatus*	*Trimeresurus flavomaculatus*	*Trimeresurus flavomaculatus*	**SpeciesID**
Scincidae	Scincidae	Scincidae	Colubridae	Viperidae	Viperidae	Viperidae	Viperidae	Viperidae	**HostFamily**
Lacertilia	Lacertilia	Lacertilia	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	Serpentes	**HostSubOrder**
Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	Squamata	**HostOrder**
Luzon	Luzon	Luzon	Negros	Negros	Negros	Negros	Calayan	Camiguin Norte	**Island**
Labo	Labo	Labo	Valencia	Valencia	Valencia	Valencia	Calayan	Camiguin Norte	**County**
Luzon	Luzon	Luzon	NegrosPanay	NegrosPanay	NegrosPanay	NegrosPanay	Babuyan	Babuyan	**FaunalRegion**
Bicol	Bicol	Bicol	Valencia	Valencia	Valencia	Valencia	Babuyan	Babuyan	**SubFaunalRegions**
14.07577	14.07577	14.07577	9.28753	9.28753	9.28753	9.28753	19.27482	18.91781	**Latitude**
122.7691	122.7691	122.7691	123.209	123.209	123.209	123.209	121.447	121.8925	**Longitude**
109965	109965	109965	13310	13310	13310	13310	196	166	**Island Size (km^2)**
2016	2016	2016	2016	2016	2016	2016	2018	2018	**Year**
Ground	Ground	Ground	Aquatic	Arboreal	Arboreal	Arboreal	Arboreal	Arboreal	**Ecology**
Ground	Ground	Ground	Freshwater	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	Upper Level Arboreal	**Microhabitat**
Adult	Unknown	Adult	Adult	Adult	Adult	Adult	Adult	Adult	**AgeClass**
Unknown	Unknown	Unknown	Female	Male	Male	Unknown	Unknown	Female	**Sex**
D58	D28	D27	C43	C40	C39	C38	B6	A4	**Code for Supp. Fig. X**
No	No	No	No	No	No	No	No	No	**Removed due to low sequence counts (<1,000)?**
									Notes

SpeciesID	*Tropidophorus grayi*	*Typhlops sp.*
HostFamily	Scincidae	Typhlopidae
HostSubOrder	Lacertilia	Serpentes
HostOrder	Squamata	Squamata
Island	Luzon	Negros
County	Labo	Valencia
FaunalRegion	Luzon	NegrosPanay
SubFaunalRegions	Bicol	Valencia
Latitude	14.07577	9.28753
Longitude	122.7691	123.209
Island Size (km^2)	109965	13310
Year	2016	2016
Ecology	Ground	Burrowing
Microhabitat	Ground	Burrowing
AgeClass	Adult	Adult
Sex	Female	Female
Code for Supp. Fig. X	D66	C49
Removed due to low sequence counts (<1,000)?	No	No
Notes		Assigned Indotyphlops braminus for phylo analyses

Table S1 | Metadata for 299 gut microbiome samples that were extracted, amplified, and sequenced.

	Unweighted-Unifrac	Weighted-Unifrac
Host Microhabitat	Burrowing vs. Freshwater: pseudo-F = 1.031, q-value = 0.326	Burrowing vs. Freshwater: pseudo-F = 1.159, q-value = 0.350
	Burrowing vs. Ground: pseudo-F = 1.609, q-value = 0.039	Burrowing vs. Ground: pseudo-F = 0.881, q-value = 0.466
	Burrowing vs. Lower Level Arboreal: pseudo-F = 3.022, q-value = 0.002	**Burrowing vs. Lower Level Arboreal: pseudo-F = 7.319, q-value = 0.004**
	Burrowing vs. Marine: pseudo-F = 3.263, q-value = 0.002	**Burrowing vs. Marine: pseudo-F = 4.749, q-value = 0.008**
	Burrowing vs. Saxicolous: pseudo-F = 1.586, q-value = 0.059	Burrowing vs. Saxicolous: pseudo-F = 1.046, q-value = 0.385
	Burrowing vs. Upper Level Arboreal: pseudo-F = 3.267, q-value = 0.002	**Burrowing vs. Upper Level Arboreal: pseudo-F = 4.133, q-value = 0.012**
	Freshwater vs. Ground: pseudo-F = 1.274, q-value = 0.105	Freshwater vs. Ground: pseudo-F = 1.193, q-value = 0.348
	Freshwater vs. Lower Level Arboreal: pseudo-F = 1.326, q-value = 0.124	Freshwater vs. Lower Level Arboreal: pseudo-F = 1.791, q-value = 0.141
	Freshwater vs. Marine: pseudo-F = 2.210, q-value = 0.008	Freshwater vs. Marine: pseudo-F = 2.123, q-value = 0.110
	Freshwater vs. Saxicolous: pseudo-F = 1.125, q-value = 0.250	Freshwater vs. Saxicolous: pseudo-F = 1.238, q-value = 0.330
	Freshwater vs. Upper Level Arboreal: pseudo-F = 1.183, q-value = 0.187	Freshwater vs. Upper Level Arboreal: pseudo-F = 0.633, q-value = 0.721
	Ground vs. Lower Level Arboreal: pseudo-F = 2.561, q-value = 0.002	**Ground vs. Lower Level Arboreal: pseudo-F = 6.468, q-value = 0.004**
	Ground vs. Marine: pseudo-F = 3.588, q-value = 0.002	**Ground vs. Marine: pseudo-F = 6.125, q-value = 0.006**
	Ground vs. Saxicolous: pseudo-F = 1.320, q-value = 0.121	Ground vs. Saxicolous: pseudo-F = 0.882, q-value = 0.466
	Ground vs. Upper Level Arboreal: pseudo-F = 2.640, q-value = 0.002	**Ground vs. Upper Level Arboreal: pseudo-F = 3.072, q-value = 0.050**
	Lower Level Arboreal vs. Marine: pseudo-F = 2.371, q-value = 0.002	**Lower Level Arboreal vs. Marine: pseudo-F = 7.945, q-value = 0.004**
	Lower Level Arboreal vs. Saxicolous: pseudo-F = 1.738, q-value = 0.038	**Lower Level Arboreal vs. Saxicolous: pseudo-F = 5.952, q-value = 0.004**
	Lower Level Arboreal vs. Upper Level Arboreal: pseudo-F = 1.384, q-value = 0.076	Lower Level Arboreal vs. Upper Level Arboreal: pseudo-F = 2.029, q-value = 0.110
	Marine vs. Saxicolous: pseudo-F = 2.701, q-value = 0.002	**Marine vs. Saxicolous: pseudo-F = 4.170, q-value = 0.004**
	Marine vs. Upper Level Arboreal: pseudo-F = 2.919, q-value = 0.002	**Marine vs. Upper Level Arboreal: pseudo-F = 5.488, q-value = 0.004**
	Saxicolous vs. Upper Level Arboreal: pseudo-F = 1.554, q-value = 0.059	**Saxicolous vs. Upper Level Arboreal: pseudo-F = 2.850, q-value = 0.038**
Host Ecology	**Aquatic vs. Arboreal: pseudo-F = 1.835, q-value = 0.018**	**Aquatic vs. Arboreal: pseudo-F = 3.765, q-value = 0.008**
	Aquatic vs. Burrowing: pseudo-F = 2.084, q-value = 0.009	**Aquatic vs. Burrowing: pseudo-F = 3.188, q-value = 0.014**
	Aquatic vs. Ground: pseudo-F = 2.464, q-value = 0.002	**Aquatic vs. Ground: pseudo-F = 3.923, q-value = 0.006**
	Arboreal vs. Burrowing: pseudo-F = 3.205, q-value = 0.002	**Arboreal vs. Burrowing: pseudo-F = 4.402, q-value = 0.006**
	Arboreal vs. Ground: pseudo-F = 2.507, q-value = 0.002	**Arboreal vs. Ground: pseudo-F = 3.039, q-value = 0.028**
	Burrowing vs. Ground: pseudo-F = 1.609, q-value = 0.019	Burrowing vs. Ground: pseudo-F = 0.881, q-value = 0.388

Table S2 | Unweighted- and Weighted-Unifrac beta diversity comparisons between all host microhabitat and host ecology groups. Bold values represent significant differences.

		Observed OTUs	Shannon Diversity	Faith's Phylogenetic Diversity
Site	*Intra-Island*	Albay vs. Labo: H = 5.194, q-value = 0.0830	Albay vs. Labo: H = 4.093, q-value = 0.172	Albay vs. Labo: H = 4.811, q-value = 0.144
		Albay vs. Quezon: H = 4.221, q-value = 0.0830	Albay vs. Quezon: H = 2.700, q-value = 0.255	Albay vs. Quezon: H = 4.033, q-value = 0.166
		Albay vs. Tabaco: H = 3.202, q-value = 0.129	Albay vs. Tabaco: H = 3.857, q-value = 0.174	Albay vs. Tabaco: H = 3.857, q-value = 0.166
		Hinoba-an vs. Valencia: H = 2.873, q-value = 0.140	Hinoba-an vs. Valencia: H = 0.210, q-value = 0.755	Hinoba-an vs. Valencia: H = 0.839, q-value = 0.559
		Labo vs. Quezon: H = 0.315, q-value = 0.670	Labo vs. Quezon: H = 0.073, q-value = 0.816	Labo vs. Quezon: H = 0.0002, q-value = 0.990
		Labo vs. Tabaco: H = 0.188, q-value = 0.744	Labo vs. Tabaco: H = 0.143, q-value = 0.780	Labo vs. Tabaco: H = 0.052, q-value = 0.957
		Quezon vs. Tabaco: H = 0.011, q-value = 0.987	Quezon vs. Tabaco: H = 0.220, q-value = 0.755	Quezon vs. Tabaco: H = 0.0007, q-value = 0.990
	Inter-Island	Albay vs. Calayan: H = 2.552, q-value = 0.162	Albay vs. Calayan: H = 3.020, q-value = 0.230	Albay vs. Calayan: H = 7.596, q-value = 0.144
		Albay vs. Camiguin Norte: H = 1.108, q-value = 0.371	Albay vs. Camiguin Norte: H = 3.133, q-value = 0.230	Albay vs. Camiguin Norte: H = 6.428, q-value = 0.144
		Albay vs. Hinoba-an: H = 0, q-value = 1	Albay vs. Hinoba-an: H = 0, q-value = 1	Albay vs. Hinoba-an: H = 3, q-value = 0.194
		Albay vs. Valencia: H = 1.781, q-value = 0.255	Albay vs. Valencia: H = 1.496, q-value = 0.387	Albay vs. Valencia: H = 5.626, q-value = 0.144
		Calayan vs. Camiguin Norte: H = 1.562, q-value = 0.282	Calayan vs. Camiguin Norte: H = 0.124, q-value = 0.780	Calayan vs. Camiguin Norte: H = 0.227, q-value = 0.824
		Calayan vs. Hinoba-an: H = 4.156, q-value = 0.083	Calayan vs. Hinoba-an: H = 1.083, q-value = 0.426	Calayan vs. Hinoba-an: H = 3.478, q-value = 0.166
		Calayan vs. Labo: H = 18.934, q-value = 0.0002	Calayan vs. Labo: H = 9.230, q-value = 0.057	Calayan vs. Labo: H = 4.658, q-value = 0.144
		Calayan vs. Quezon: H = 4.887, q-value = 0.083	Calayan vs. Quezon: H = 1.769, q-value = 0.343	Calayan vs. Quezon: H = 1.833, q-value = 0.328
		Calayan vs. Tabaco: H = 5.643, q-value = 0.082	Calayan vs. Tabaco: H = 6.324, q-value = 0.109	Calayan vs. Tabaco: H = 1.390, q-value = 0.393
		Calayan vs. Valencia: H = 0.002, q-value = 1	Calayan vs. Valencia: H = 0.412, q-value = 0.663	Calayan vs. Valencia: H = 4.814, q-value = 0.144
		Camiguin Norte vs. Hinoba-an: H = 3.675, q-value = 0.103	Camiguin Norte vs. Hinoba-an: H = 1.163, q-value = 0.426	Camiguin Norte vs. Hinoba-an: H = 2.426, q-value = 0.257
		Camiguin Norte vs. Labo: H = 18.414, q-value = 0.0002	Camiguin Norte vs. Labo: H = 5.843, q-value = 0.109	Camiguin Norte vs. Labo: H = 3.455, q-value = 0.166
		Camiguin Norte vs. Quezon: H = 8.215, q-value = 0.028	Camiguin Norte vs. Quezon: H = 1.140, q-value = 0.426	Camiguin Norte vs. Quezon: H = 2.302, q-value = 0.258
		Camiguin Norte vs. Tabaco: H = 7.859, q-value = 0.028	Camiguin Norte vs. Tabaco: H = 4.872, q-value = 0.127	Camiguin Norte vs. Tabaco: H = 1.586, q-value = 0.364
		Camiguin Norte vs. Valencia: H = 1.053, q-value = 0.371	Camiguin Norte vs. Valencia: H = 0.529, q-value = 0.623	Camiguin Norte vs. Valencia: H = 3.401, q-value = 0.166
		Hinoba-an vs. Labo: H = 4.446, q-value = 0.083	Hinoba-an vs. Labo: H = 1.878, q-value = 0.341	Hinoba-an vs. Labo: H = 0.625, q-value = 0.623
		Hinoba-an vs. Quezon: H = 4.226, q-value = 0.083	Hinoba-an vs. Quezon: H = 1.057, q-value = 0.426	Hinoba-an vs. Quezon: H = 0.209, q-value = 0.824
		Hinoba-an vs. Tabaco: H = 3.048, q-value = 0.133	Hinoba-an vs. Tabaco: H = 2.012, q-value = 0.336	Hinoba-an vs. Tabaco: H = 0.583, q-value = 0.623
		Labo vs. Valencia: H = 13.189, q-value = 0.003	Labo vs. Valencia: H = 8.247, q-value = 0.057	Labo vs. Valencia: H = 0.001, q-value = 0.990
		Quezon vs. Valencia: H = 4.226, q-value = 0.083	Quezon vs. Valencia: H = 2.391, q-value = 0.285	Quezon vs. Valencia: H = 0.057, q-value = 0.957
		Tabaco vs. Valencia: H = 4.627, q-value = 0.083	Tabaco vs. Valencia: H = 5.082, q-value = 0.127	Tabaco vs. Valencia: H = 0.0001, q-value = 0.990
Island		Calayan vs. Camiguin Norte: H = 1.562, q-value = 0.317	Calayan vs. Camiguin Norte: H = 0.124, q-value = 0.724	Calayan vs. Camiguin Norte: H = 0.227, q-value = 0.761

		Calayan vs. Luzon: H = 15.958, q-value = 0.0003	**Calayan vs. Luzon: H = 8.679, q-value = 0.010**	**Calayan vs. Luzon: H = 6.178, q-value = 0.05**
		Calayan vs. Negros: H = 0.060, q-value = 0.806	Calayan vs. Negros: H = 0.592, q-value = 0.530	**Calayan vs. Negros: H = 5.731, q-value = 0.05**
		Camiguin Norte vs. Luzon: H = 15.411, q-value = 0.0003	Camiguin Norte vs. Luzon: H = 4.589, q-value = 0.064	Camiguin Norte vs. Luzon: H = 4.184, q-value = 0.077
		Camiguin Norte vs. Negros: H = 0.627, q-value = 0.514	Camiguin Norte vs. Negros: H = 0.668, q-value = 0.530	Camiguin Norte vs. Negros: H = 3.797, q-value = 0.077
		Luzon vs. Negros: H = 13.429, q-value = 0.0005	**Luzon vs. Negros: H = 8.989, q-value = 0.010**	Luzon vs. Negros: H = 0.076, q-value = 0.783

Table S3 | Alpha diversity comparisons between the sites and islands that host communities were sampled. Bold values indicate significant differences.