UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

INVESTIGATION OF THE DRIVERS OF SKIN MICROBIAL DIVERSITY IN SIX CO-OCCURRING SALAMANDER SPECIES IN THE PRESENCE OF AMPHIBIAN PATHOGENS

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfilment of the requirements for the

Degree of

MASTER OF SCIENCE

By

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Norman, OK

2023

INVESTIGATION OF THE DRIVERS OF SKIN MICROBIAL DIVERSITY IN SIX CO-OCCURRING SALAMANDER SPECIES IN THE PRESENCE OF AMPHIBIAN PATHOGENS

A THESIS APPROVED FOR THE

DEPARTMENT OF BIOLOGY

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ACKNOWLEDGMENTS

I would like to thank my committee members (Dr. Hayley Lanier, Dr. Daniel Becker, and Dr. Katharine Marske) for their guidance throughout the process of finishing my thesis. Dr. Claire Curry provided help and advice about statistics during this process, and I'm very grateful to her for sharing her knowledge.

The past members of the Brunner Lab at Washington State University (Erin Keller, Johnna Eilers, Emily Burton, Christian Yarber, and Dr. Jesse Brunner) provided so much support throughout my undergraduate years and are the main reason that I believed I could go to graduate school. Jesse and Erin continued to provide invaluable advice and support during my time at OU.

I would like to thank the past and present members and employees of the Siler Lab (Alex Fulton, Katherine Stroh, Sierra Smith, Sam Eliades, Forrest Nielsen, Gracie Hedgpeth, Claudia Goss, Emma Clary, Emma Franklin, Jessa Watters, and Dr. Cameron Siler), as well as the kind people at the Tinker Air Force Base (Ray Moody, John Krupovage, and Donna Nolan). I learned a lot during my first year of graduate school, and it is thanks to them that I did!

I would also like to thank the members of the Lanier Lab (Miranda Theriot, Marcus da Cruz, and Dr. Hayley Lanier) for their warm welcome, guidance, instruction, and support throughout the second half of my graduate experience.

This work was financially supported by the Oklahoma Department of Wildlife Conservation, OK-WILD Grants #F14F01225 (T-80-1), #F20AF00023 (T-116-R-1), and Grant #F20AF10405 (T-118-R-1); the Sam Noble Oklahoma Museum of Natural History; and the OU Department of Biology.

Without my wonderful family, especially Beth and Scott Kirsch, Holly Krings, and Greg Gabrielsen, I would not be here today. I'm also thankful for the help and advice from Dr. Rosa Dávila and Gerardo Muñoz. They all provided a great deal of support during the most difficult parts of graduate school. The biggest thank-you goes to Carlos Muñoz, for his unending support, patience, guidance, and care throughout my time at OU.

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ABSTRACT

Communities of symbiotic bacteria associated with a host, also known as microbiomes, have gained increased recognition for the myriad of key roles that they play. For example, microbiomes of the skin and gut have been linked to important host functions, such as immunity and digestion. The skin microbiome may be especially important in amphibians due to the extremely permeable nature of their skin through which they drink and respire. As a result, an imbalance in the composition of the skin microbiome, a condition known as "dysbiosis," could have strong fitness consequences. Thus, identifying baseline skin microbial communities in amphibians may provide insight into their health and conservation needs, in addition to furthering our knowledge of microbial diversity. Several amphibian pathogens, including *Batrachochytrium dendrobatidis* (*Bd*) and viruses from the genus *Ranavirus*, currently decimate amphibian populations and are linked to significant changes in amphibian skin microbiomes. While an association between pathogen presence and amphibian skin microbial diversity has been demonstrated, it is not clear how widespread this pattern is or how it relates to other drivers of skin microbial diversity. For example, environmental, ecological, and genetic variables all impact the skin microbiome, making it important to quantify their contributions to microbiome structure in the presence of infection. To understand the joint influence of these factors, I used 16S sequencing to characterize the skin microbiomes of six salamander species found in Oklahoma and contrasted the effects of infection status, phylogeny, host ecology, and host environment on skin microbiomes. The results indicated that there was no phylogenetic influence on skin microbial diversity present; rather, unknown differences at the level of the salamander family were the main factors differentiating microbiome diversity, with host ecology and environment becoming more important at the level of differences among species. They also revealed a slight decrease in microbial diversity on animals that tested positive for *Bd*, whereas there were no microbiome differences associated with ranavirus presence. Together, these results indicate a nuanced relationship between the number and type of microbes present in the skin and the various factors influencing them. This work also provides a baseline for the skin microbiomes of six salamander species that had not been previously investigated.

INTRODUCTION

Microbiomes, or microbial communities that live symbiotically with host organisms, are crucial for host health, aiding in processes such as digestion and protection from disease (Moloney et al., 2014). There is increasing recognition that even small changes in microbial communities can result in the loss of innate functions (metabolic, physiological, and immune) — a condition referred to as dysbiosis—with large negative effects on a host's overall fitness (e.g., Chimenos-Küstner et al., 2017, Jiménez & Sommer, 2017). Dysbiosis can lead to behavioral and developmental changes as well as decreased immune function; for example, even a slight change in the abundance or community balance of microbiota is linked illness across a wide range of taxa (e.g., humans, amphibians, insects; Bletz et al., 2018; Brucker & Bordenstein, 2012; Chimenos-Küstner et al., 2017; Hooper & Gordon, 2001; Kueneman et al., 2016). A specific instance of this was observed in coral, where the likelihood that coral will experience bleaching can be predicted by evaluating the microbiome's diversity (Gardner et al., 2019). Thus, investigating microbial communities associated with an organism provides a greater understanding of its evolution and susceptibility to disease (Hird, 2017; Varela et al., 2018). Developing this baseline understanding and identifying the role of host-associated microbiomes in shaping susceptibility to infection is particularly critical for species of conservation concern (Jiménez & Sommer, 2017).

Amphibians are currently the most threatened vertebrates on the planet, with 41% of amphibian species threatened with extinction due to climate change, habitat loss, and infectious disease (IUCN 2020). At the end of the twentieth century, two infectious diseases, in particular, began to be linked to widespread declines in amphibian populations: chytridiomycosis (caused by *Batrachochytrium dendrobatidis* [*Bd*] or *B. salamandrivorans* [*Bsal*]) and ranavirus disease (caused by infection from one of the several viruses in the genus *Ranavirus*; Daszak et al., 1999; Fisher & Garner, 2020; Lips et al., 2006; Scheele et al., 2019; Skerratt et al., 2007). Amphibians' susceptibility to infection is influenced by their extremely permeable skin, which gases and water pass through (Campbell et al., 2012; Rollins-Smith, 2009; Smith et al., 2018). Vast microbial diversity is present on the skin of amphibian species; these can directly influence host immunity by producing antifungal or antimicrobial compounds (Campbell et al., 2012; Woodhams et al.,

2015). For example, the presence of anti-*Bd* microbes present on the skin of amphibians is linked to reduced fungal infection, indicating that beneficial bacteria may play a role in susceptibility and immunity (Mutnale et al., 2021; Nava-González et al., 2021). Amphibians vary in their susceptibility to pathogens (DiRenzo et al., 2020); because the varying susceptibility of amphibian species to emergent pathogens may be the result of both the host's immune system but also the varied microbial diversity found in the skin microbiome, it is important to characterize this diversity across a range of species.

Complicating our understanding of the role of amphibian microbiomes in inhibiting infection is the fact that infection with pathogens can change the skin microbiome's structure (Wilber et al., 2020). Amphibians experience a significant reduction in the skin microbiome's diversity after clearing infection with *Bd* (Bates et al., 2019; Jani and Briggs, 2014; Jani et al., 2021; Medina et al., 2017; Muletz-Wolz et al., 2019; Rebollar et al., 2016; Ruthsatz et al., 2020). Likewise, the skin microbiomes of amphibians that experienced ranavirus infection exhibit a shift in the species present in the skin microbiome compared to virus-free populations (Campbell et al., 2019; Harrison et al., 2019). However, some species' skin microbiomes do not appear to be strongly impacted by fungal infection, such as that of the red-backed salamander (*Plethodon cinereus*; Barnes et al., 2021; Bates et al., 2022; Becker & Harris, 2010). Given these discrepancies, additional studies documenting the diversity of microbes found in skin microbiomes and their relationship to host infection are needed.

Fully understanding the relationship between amphibian infectious disease and host microbiomes requires first determining the baseline composition of the microbiome and the factors driving its diversity. Three main potential contributors to skin microbiomes have been identified—environment, ecology, and phylogeny—each with a unique set of predictions. If the microbes in an amphibian's physical environment are the main determinant of microbial diversity in the skin, then amphibian species living in the same habitat should show the same or very similar microbial diversity and composition on the skin. A comparison of the skin microbiomes of 71 species of tree frogs across Brazil found that temperature, elevation, and precipitation, but not species identity, were significantly correlated with microbiome diversity, supporting this prediction (Ruthsatz et al., 2020). Similar patterns are evident in the skin microbiomes of the slimy salamander (*Plethodon glutinosus*) complex, where microbial

communities become increasingly distinct with geographic distance among individuals, indicating that changes in geographic location could be impacting the microbiome's diversity (Walker et al., 2020). Thus, there is evidence to suggest that the host's environment is an important driver of diversity in amphibian skin microbiomes.

However, if the ecology of the host species (including its habitat or life stage) is a greater predictor of skin microbial diversity than simply the animal's surrounding environment, then species' skin microbiomes would differ based on a species' ecological niche and/or microhabitat preferences. Groups of amphibian species that utilize similar microhabitats (e.g., arboreal or aquatic) would exhibit similar skin microbiomes, regardless of geographical location. This could be due to lateral transfer among hosts or exposure to the same stressors (Bletz et al., 2017). Thus, biotic or abiotic differences in their specific microhabitat use, even within the same geographic region, could be significant enough to result in statistically distinct skin microbiomes when compared to species in different niches. In Madagascar, the most important predictor of microbiome structure and diversity across 96 frog species was the host's life history, with evidence of significant differences between the skin microbiomes of arboreal, terrestrial, and aquatic frogs (Bletz et al., 2017). Host habitat type was also the greatest predictor of skin microbial diversity in a study of multiple co-distributed amphibian species (from genera *Batrachoseps* and *Ensatina*; Bird et al., 2018). Differences in host microbiome could also be a result of life history behaviors: e.g., during the breeding season, when adult frogs migrate to bodies of water, their skin microbiomes have been shown to contain more water-sourced microbes when compared to their terrestrial life stages (Xu et al., 2020).

In contrast to findings that implicate environment or host ecology as driving skin microbial diversity, multiple studies have found that host species identity is the strongest predictor of diversity in amphibian skin microbiomes (McKenzie et al., 2012; Prado-Irwin et al., 2017). It may be that certain microbes are conserved as part of the skin microbiome due to evolutionary differences between amphibian species, and this could dictate which microbes become part of the microbiome. In a study on plethodontid salamander species belonging to genera *Batrachoseps* and *Ensatina,* the genus of the host was the main predictor of the species richness of the amphibian skin microbiome (Buttimer et al., 2022; a contrast to the results from Bird et al. (2018)). This phenomenon could be an example of phylosymbiosis, in which the

difference in skin microbiome makeup of two species may correspond to the evolutionary history and phylogenetic distance between the hosts (Lim & Bordenstein, 2020). Changes in the skin microbiome of a species can happen as quickly as in several generations, as demonstrated in *Drosophila* (Rudman et al., 2019), or through long-term coevolution (Pollock et al., 2018). Therefore, a long evolutionary history could provide an abundance of time for coevolution to induce changes in the amphibian skin microbiome, especially due to its potential importance in maintaining a healthy host.

Ultimately, multiple drivers of microbial diversity are likely to impact the skin microbiome's composition simultaneously. When considering multiple, interacting factors in four frog and one newt species, species identity correlated most strongly with microbial community composition differences, but habitat location was a secondary result of significant variation within each species (Kueneman et al., 2014). Similarly, a study of 49 frog (genus *Plectrohyla*) and 23 salamander (genera *Bolitoglossa* and *Pseudoeurycea*) species, found that phylogeny was the most important indicator of skin microbial diversity between different orders and families, but between genera and species, microbial diversity was most impacted by the habitat of the host (Ellison et al., 2019). Despite numerous studies, a consensus on the main driver of amphibian skin microbial diversity has not been reached. Thus, examining multiple interacting drivers simultaneously is needed to understand how the assembly of microbiomes and how they intersect with amphibian disease.

In this study, skin microbiome data were collected from six focal, co-distributed species of salamanders representing two salamander families and three genera across four ecoregions in Oklahoma (Fig. 1, Table 1). My goals were to (1) characterize the skin microbiomes of these six salamander species; (2) evaluate the role of the host's environment, host ecomorphology, and phylogeny on skin microbiome composition; and (3) determine the relationship between pathogen presence and microbial abundance and diversity. This study will provide baseline knowledge about skin microbial composition in salamander species across regions of the United States for which little is known. Additionally, the results add to a growing body of literature focused on the effect of infectious disease in the skin microbiomes of amphibian hosts.

METHODS

Sample Populations

This project relied on museum-vouchered specimens with associated epithelial swab samples and pathogen data from 2015–2021, available through the Herpetology Department of the Sam Noble Museum (Davis et al., 2019; Marhanka et al., 2017; Watters et al., 2018). We identified 287 samples (Supplementary Table 1) representing six species of salamanders with sufficient sampling for robust characterization of variation in species-specific skin microbiomes: *Eurycea longicauda melanopleura* (hereafter *Eurycea longicauda*), *E. lucifuga, E. tynerensis, Plethodon albagula*, and *P. angusticlavius* (family Plethodontidae), and *Notophthalmus viridescens* (family Salamandridae). Samples were selected from four unique Level III ecoregions across eastern Oklahoma: Arkansas Valley, Boston Mountains, Ouachita Mountains, and Ozark Highlands (Fig. 1; Hoagland & Stoodley, 1998). Where available, pathogen status (i.e., presence/absence of *Bd* and ranavirus) was ascertained from prior amphibian surveys containing published pathogen data (Davis et al., 2019; Marhanka et al., 2017; Watters et al., 2018).

Sample Collection

During each collection effort (comprising 24–72 hours of effort), salamanders were captured in ponds, wetlands, and streams by hand, dipnet, or seine. Animals were kept individually in sterile plastic bags before being swabbed and either released or euthanized and vouchered in the Herpetology Collection in the Sam Noble Oklahoma Museum of Natural History. Microbiome samples were taken by rubbing a cotton swab tip (Puritan Medical Products) along the ventral and dorsal portions of the trunk, limbs, and toes five times each to collect microbial DNA, following the methods of Lannoo et al. (2011). Swabs were placed into empty vials and flash-frozen using liquid nitrogen prior to long-term storage at –20ºC.

Life History Stage and Host Ecology

The life history stage was assessed during sampling or determined later using vouchered specimens. Individuals were considered to be adults if the snout-vent length (SVL) was greater than or equal to 31 mm for *Eurycea longicauda*, 26 mm for *E. tynerensis*, 55 mm for *Plethodon albagula*, and 30 mm for *P. angusticlavius* (Trauth et al., 2004). *Eurycea lucifuga* were labeled as adults if the total length (TL) was 100 mm or more (Petranka, 1998). Individuals from either

Plethodon species were labeled as juveniles if below the length requirement. Members of *Eurycea* were considered larval if they were below the SVL or TL length requirement and had gills; they were classified as juveniles if they had no external gills but were below the length requirement. Adults belonging to *Eurycea* were determined to be paedomorphic if external gills could be seen. All vouchered newts in the SNOMNH Herpetology Collection were adults; they were distinct from larval or eft forms, based upon comparison to images from *Amphibians and Reptiles of Arkansas* (Trauth et al., 2004).

Samples were labeled as coming from terrestrial or aquatic individuals based on the age and physical characteristics of the animal. If gills were present in the plethodontids, samples were listed as coming from aquatic individuals. Since all newts were determined to be adults, they were listed as aquatic (Trauth et al., 2004). Any other animals were assigned a terrestrial label.

Environmental Data

Latitude and longitude were recorded for each sample at the time of sample collection. Using these coordinates, elevation was calculated using an online map tool (U. S. Geological Survey, 2017). Environmental data—average annual, seasonal, and monthly precipitation and temperature data–associated with sample coordinates and month/year of sampling were secondarily collected using ClimateNA (Wang et al., 2016). Before use in analyses of alpha and beta diversity, environmental data were centered and scaled, with a mean of zero, in R software (R Core Team, 2021) to ensure that a one-unit change in one variable would have the same importance as a one-unit change in another.

DNA Extraction and Sequencing

Genomic DNA from epithelial samples was extracted using ZymoBiomics DNA Miniprep Kits (Zymo Research Products, Irvine, CA, United States). Extraction negatives were run with each extraction, and PCR negative and positive controls were run on each PCR plate. Positive controls consisted of the ZymoBIOMICS Microbial Community Standard and the ZymoBIOMICS Microbial Community DNA Standard (Zymo Research Products, Irvine, CA, United States). Extracted DNA was amplified using one-step Polymerase Chain Reaction barcoded primers targeting the ribosomal 16S subregion V4 (Kozich et al., 2013). Two microliters of several

samples per plate were then visualized via gel electrophoresis to ensure amplification, after which all samples were cleaned using KAPA Pure Beads (Roche Sequencing Solutions, Pleasanton, CA, United States). The DNA concentration for each sample was determined using a Quantus Fluorometer (Promega, Madison, WI, United States), and samples were normalized to 10 nM of DNA before pooling all samples in a 1.5 mL sterile microcentrifuge tube. The pooled library was then sequenced through a single run on an Illumina MiSeq platform using 2 × 250 bp paired-end sequencing at the University of Oklahoma Consolidated Core Lab. Pathogen presence was sourced from previous surveys (Davis et al., 2019; Marhanka et al., 2017; Watters et al., 2018). Recent samples that had not yet been evaluated for pathogens were processed following the methods of Watters et al. (2018). In brief, this involved DNA extraction using the PrepMan Ultra reagent (*Bd* samples; Life Technologies; Cheng et al., 2011) and a method of high salt DNA extraction (for ranavirus liver or toe samples; Esselstyn et al., 2008). Samples were then amplified following a previously published qualitative real-time polymerase chain reaction procedure (Kerby et al., 2013). Primers targeted the internal transcribed spacer (ITS-1) ribosomal RNA gene (forward primer: ITS1-3 Chytr; reverse primer: 5.8S) for *Bd,* and the major capsid protein (MCP) gene for ranavirus (Boyle et al., 2004; Forson & Storfer, 2006).

Analysis

Paired-end sequencing reads were trimmed using AdapterRemoval v2 (Schubert et al., 2016), and sequences were prepared for analysis using USEARCH version 2.21.1 (Edgar, 2010). Amplicon sequence variants (ASVs) were classified against the EzTaxon database (Yoon et al., 2017) and the antifungal isolates database compiled by Woodhams et al. (2015). If an ASV was a 99% or greater match to the EzTaxon database, it was identified to species; 94.5–99% matches were identified to genus; and matches that were 88–94.5% identical were identified to family. We included only matches of 99% or above to the antifungal database, following the methods of Barnes et al. (2021). Sequences were aligned with MAFFT (Katoh et al., 2002). The resulting ASV table, taxa table, metadata, and phylogenetic tree of microbes were imported into R and combined into a phyloseq object using the package *phyloseq* (McMurdie & Holmes, 2013). Samples were rarefied to 200, 500, 1000, and 2000 reads per sample; results listed in the main text refer to rarefaction at 1000 reads, a threshold chosen to remove samples with low sequence counts (Weiss et al., 2017), and the other results are present in the supplementary data.

We calculated three alpha diversity metrics (observed ASVs, Shannon-Wiener Index, and Inverse Simpson's Index) using the R package *phyloseq*. These metrics were chosen to provide a range of complimentary insights into the microbiome data. For example, observed ASVs provide the number of unique sequence variant clusters in a sample, which yields a direct measure of taxonomic diversity. In contrast, the Shannon-Wiener Index, another commonly used alpha diversity metric, is a species richness metric that also accounts for evenness, reducing the impact of rare taxa on the overall metric. Finally, we calculated the Inverse Simpson's Index, which accounts for species richness and evenness but weights microbe species by abundance, which prevents rare microbes with low abundances from strongly influencing the results. The Shannon-Wiener Index and Inverse Simpson's Index are slightly more sensitive to species richness and species evenness, respectively (Johnson & Burnet, 2016). Diversity metric values were analyzed using Kruskal-Wallis tests for explanatory variables with only two qualitative responses, and a pairwise Wilcoxon rank-sum test with the Holm *p*-value correction to compare the remaining qualitative variables.

We took two approaches to understand the combined effects of the phylogenetic, environmental, and ecological explanatory variables. Firstly, we created a Classification and Regression Tree (CART; R package *partykit* (Hothorn & Zeileis, 2015), which treats the phylogenetic variables of family, genus, and species as categorical variables. The CART analysis was performed using the conditional inference tree command (ctree) with the number of input variables per tree randomly sampled and no restrictions applied to the number of splits in the tree. The CART and PGLMM analyses were run on each alpha diversity index separately, to determine the most important drivers of alpha diversity. Secondly, we used a phylogenetic generalized linear mixed model (PGLMM; R packages *brms*, *geiger*, and *PhyloOrchard*; Bürkner, 2017; O'Meara et al., 2013; Pennell et al., 2014) to look at patterns in alpha diversity while accounting for phylogenetic non-independence, using a phylogeny from Pyron & Wiens (2011). To prepare data for the PGLMM, explanatory variables (phylogenetic variables of family, genus, and species; ecological variables of habitat and life stage; and environmental variables of mean temperature, mean precipitation, and elevation) were tested for collinearity and removed if they had a variance inflation factor (VIF) greater than four, which represents a conservative metric for minimizing covarying explanatory data (Johnston et al., 2018). The PGLMM was run with two random effects (phylogeny and species), ten chains, 20,000

iterations, thinning set to ten, and using Gaussian response distribution. The effects of phylogenetic signal were parsed from the PGLMM by comparing the genetic variation between species to the alpha diversity values. This analysis was run solely with the random effects of phylogeny and species, delta set to 0.95, two chains, two cores, and iterations set to 4,000.

Microbial community composition was evaluated in the program QIIME2 (Bolyen et al., 2019). To identify specific microbes that were more abundant between different families or in the presence/absence of infection, differential abundances were calculated using the package DEseq2 in *R* (Love et al., 2014). To understand differences among microbial communities and the factors driving them beta diversity metrics were calculated using unweighted- and weighted-UniFrac distances in the *phyloseq* package (Lozupone & Knight, 2005). The unweighted test evaluates the presence and absence of ASVs, regardless of microbial phylogenetic affinity; the weighted test accounts for the abundance and phylogenetic identity of ASVs. Beta diversity was analyzed using Permutational Analysis of Variance tests (PERMANOVA) with Holm *p*-value correction and permutations set to 10,000. To investigate how beta diversity changes with environmental variables while controlling for geographic distance, we conducted partial Mantel tests using the Bray-Curtis matrix of beta diversity values and a geodesic distance matrix, and controlled for the geographic distance using the Euclidean distance between sample locations with the R packages *vegan* and *ecodist* (Goslee & Urban, 2007; Oksanen et al., 2022).

RESULTS

The initial dataset consisted of 287 epithelial swab samples (Supplementary Table 1). After the sequencing and quality filtering, there were 280 usable samples. In total, 5,256,288 sequences were obtained from these 280 samples, with a total of 13,038 ASVs. The total number of ASVs present in each sample varied widely, from 1 to 429,627, with an average of 11,883 ASVs ± 3,252. To adjust for differences in ASV sample sizes, samples were rarefied to 1000 reads per sample. After rarefaction, 179 samples remained, with the number of unique ASVs ranging from 22–610 (average 103; Supplementary Table 1). Of these, 147 animals were tested for *Bd* presence (59 *Bd*-positive and 88 *Bd*-negative) and 151 were tested for ranavirus (21 positive and 130 negative).

Microbial Composition of Salamander Skin Microbiomes

Animals in the family Salamandridae (*n* = 71) had a skin microbial community composition of 82.13% Proteobacteria, 8.23% Firmicutes, 4.65% Bacteroidetes, and 2.10% Actinobacteria; none of the other microbial phyla comprised more than 1% of the community. Salamanders from family Plethodontidae (*n* = 108) had a skin microbial composition of 59.20% Proteobacteria, 12.14% Firmicutes, 13.90% Bacteroidetes, 5.92% Actinobacteria, 2.71% Cyanobacteria, and 2.44% Verrucomicrobia (Fig. 2). There were 79 differentially abundant microbes between the Plethodontidae and Salamandridae (Supplementary Table 1). Two hundred fifty-nine of the 13,038 ASVs were identified to sequences in the antifungal isolates database. Of these, ten were differentially abundant between animals with and without *Bd* (Supplementary Table 2).

Alpha Diversity

Role of host phylogeny

When contrasting alpha diversity by taxonomic grouping, without considering other explanatory variables, the family Plethodontidae had consistently higher microbial diversity than Salamandridae across all three metrics: observed ASVs, Shannon-Wiener Index, and Inverse Simpson's Index (significance assessed with Kruskal-Wallis tests; Table 2). This same pattern was observed between genera and species, with plethodontid species generally exhibiting greater alpha diversity than newts in pairwise Wilcoxon rank-sum tests across all metrics; however, plethodontids were not significantly different from one another at the level of genera or species (Tables 2–4).

When host phylogeny was evaluated in the context of other explanatory variables, a similar split was evident in CART analyses of both observed ASVs and the Shannon-Wiener Index—family-level divergence was the predominant split in the dataset. For the Inverse Simpson's Index, the primary factor that categorized microbiome diversity was related to species, with *E. lucifuga* and *P. angusticlavius* differing from all other taxa, followed by differences between plethodontids and newts. (Fig. 4, Supplementary figs. 1 & 2). However, when testing for a phylogenetic signal using the PGLMM, the overall signal was very low (ASVs: 0.01 [0.0–0.05 credible interval]; Shannon: 0.01 [0.0–0.03 credible interval]; and Inverse Simpson: 0.01 [0.0–0.06 credible interval]). This indicates no relationship between skin microbial diversity and phylogeny, with family-level differences being the main driver of diversity.

Role of the host's environment

For univariate analyses of the effect of ecoregion on microbiome diversity, we accounted for family and only contrasted species present in more than one ecoregion. Newts were present in all four ecoregions, but ecoregion was not a significant predictor of skin microbiome diversity for all comparisons in all alpha diversity metrics (Table 3). We evaluated the ecoregion in plethodontids by removing species that weren't present in multiple ecoregions, leaving us with three species that were present in two ecoregions (Ozark Highlands and the Boston Mountains; Fig. 1; Table 1). Across these three species (combined), microbial diversity associated with individuals from the Ozark Highlands had significantly more ASVs than the Boston Mountains in Kruskal-Wallis tests (Table 4).

When contrasting multiple predictor variables, environmental variables pertaining to temperature and precipitation either were not important or were secondary predictors of skin microbial diversity in the CART analyses (Fig. 4, Supplementary figs. 1 & 2). However, for the PGLMM results, several environmental variables had weak but significant positive relationships with alpha diversity: elevation, mean temperature of the month of collection, mean precipitation of season of collection, and mean precipitation of year.

Role of host ecology

Univariate comparisons of host ecology were limited to contrasts of salamander species that had samples of both aquatic and terrestrial individuals (*E. longicauda* and *E. tynerensis*). For these species, habitat was not a significant factor ($\chi^2 < 0.76$, $p > 0.38$ for both species across all alpha diversity metrics; Kruskal-Wallis tests). When considered relative to other factors that partition variance in alpha diversity, CART analyses did not identify life stage or habitat as significant drivers of microbial diversity (Fig. 4, Supplementary figs. 1 & 2). However, when considered jointly with phylogeny and other explanatory factors in the PGLMM, a terrestrial ecology was negatively associated with alpha diversity for the observed ASVs and Inverse Simpson metrics (Fig. 3), and skin microbiomes of both paedomorphic adult and larval plethodontids were associated with lower alpha diversity, while those of juvenile plethodontids had slightly increased alpha diversity.

Beta Diversity

Role of host phylogeny

Differences in microbial community composition, as measured by beta diversity, varied significantly in pairwise comparisons between salamander families, genera, and some species in both the weighted- and unweighted-UniFrac analyses (Tables 5–7). *Notophthalmus viridescens* exhibited distinctly different community composition when compared to each of the other salamander species in both analyses ($p < 0.01$). Similarly, microbial communities on *E. tynerensis* differed significantly from all other species (unweighted: $p \leq 0.03$, $r^2 \leq 0.05$; weighted: $p \leq 0.045$, $r^2 \leq 0.05$), except when compared to *P. angusticlavius* ($p > 0.32$, $r^2 \leq 0.03$ in both tests). Besides *E. tynerensis*, none of the other plethodontids differed significantly at the level of species ($p > 0.1$, $r^2 \leq 0.05$ in either metric).

Role of the host's environment

To understand the role of the environment in shaping the composition of salamander microbiomes, we compared samples within species present in multiple ecoregions. For *N. viridescens*, in the unweighted-UniFrac, the Boston Mountains only differed from the Ouachita Mountains ($p < 0.01$), and all other ecoregions were significantly different from each other (Table 1). In the weighted-UniFrac, all ecoregions were significantly distinct, apart from the Arkansas Valley and Ozark Highlands comparison ($p = 0.16$, $r^2 = 0.02$). When comparing beta diversity across the three plethodontid species present in multiple ecoregions (*E. tynerensis*, *P. albagula*, and *P. angusticlavius*), there was no difference between the Boston Mountains and Ozark Highlands ecoregions in any comparison ($p > 0.1$, $r^2 < 0.05$).

We used Mantel and partial-Mantel tests to examine how beta diversity is shaped by differences in environmental conditions at a site. Relationships to environmental variation were similar with and without accounting for geographic distance (Tables 8 and 9), and all following values thus refer to partial Mantel results. Overall, the mean temperature of the month had the strongest effect on beta diversity (all: $r = 0.21$; newts: $r = 0.26$; plethodontids: $r = 0.17$; $p < 0.01$ in each case); the mean temperature of the season and mean annual temperature had significant but weaker positive relationships with beta diversity. Elevation had a significant effect on beta diversity across all groups but was not significant when considering salamander families separately after correcting for multiple comparisons (all: $r = 0.12$, $p < 0.01$; just newts: $r = 0.14$,

$p = 0.03$; plethodontids: $r = 0.11$, $p = 0.05$). In newts, there was a positive relationship between mean precipitation during the month of capture and beta diversity ($r = 0.30$, $p < 0.01$), although no other environmental variables impacted microbial community structure. In contrast, plethodontids had significant positive relationships with mean annual, seasonal, and monthly precipitation, with the latter being the strongest (annual: $r = 0.11$, $p = 0.01$; seasonal: $r = 0.17$, $p < 0.01$; monthly: $r = 0.26$, $p < 0.01$).

Role of host ecology

Microbiomes from hosts found in aquatic and terrestrial habitats differed significantly in both metrics ($p < 0.01$) when all samples were compared together. However, when testing only species that were sampled in both terrestrial and aquatic habitat types (*E. longicauda* and *E. tynerensis*), the significance of this result differed based on whether the UniFrac test was weighted (unweighted: $r^2 = 0.02$, $p = 0.02$; weighted: $r^2 = 0.02$, $p = 0.32$).

To evaluate how life stage impacted beta diversity, we only examined plethodontids, as all newts in this study were adults. Juvenile plethodontids were significantly different from terrestrial adults, paedomorphic adults, and larvae in the unweighted UniFrac but did not differ from any other life stages in the weighted UniFrac (Table 7). Adult plethodontids did not differ from paedomorphic adults but did differ from larvae in the unweighted UniFrac but not the weighted one (Table 7). Paedomorphic adults did not differ from larvae (Table 7).

Role of pathogen status

***Presences of the fungus* B. dendrobatidis**

Animals without *Bd* present on their skin ($n = 88$) had a skin microbial community composition made up of Proteobacteria (59.30%), Firmicutes (12.99%), Bacteroidetes (13.92%), and Actinobacteria (5.40%). In contrast, when evaluating the microbial community of animals with *Bd* present on their skin ($n = 59$), we found that it was made up of Proteobacteria (81.62%), Firmicutes (6.64%), Bacteroidetes (6.47%), and Actinobacteria (3.06%). There were six differentially abundant microbes between animals with *Bd* present on the skin and those without, all more abundant in animals without *Bd* (Supplementary Table 3). However, these results may be strongly driven by host species, as most animals with *Bd* present on their skin were *Notophthalmus viridescens*. For our dataset, the presence of *Bd* covaried with family-level

classifications, which are both strong predictors of alpha and beta diversity. This can be seen in the results from tests of both alpha and beta diversity: when we separated the samples by family and reran the analysis, none of the alpha diversity metric values were significantly different (Tables 3 and 4). However, when accounting for phylogeny and other predictors simultaneously using a PGLMM, the presence of *Bd* was associated with a slight decrease in alpha diversity.

Ranavirus

Ranavirus status yielded no significant results in any tests of alpha diversity at any rarefaction level ($p = 1.0$ for all metrics), even when looking at only plethodontids or only newts, and was not significant in either combined test of explanatory variables. Similarly, ranavirus status was not significant in either of the beta diversity analyses ($r^2 \leq 0.01$, $p \geq 0.34$ for both metrics).

DISCUSSION

In this study, we set out to determine the relative importance of four potential drivers of skin microbial diversity—phylogeny, host ecology, environment, and pathogen presence—in salamanders. Based on comparisons across two families, three genera, and six species we found that classification by family (i.e., differences between Salamandridae and Plethodontidae) was consistently one of the strongest factors shaping the skin microbiome. With further examination, we concluded that this was not due to phylogenetic signal, but that important differences at the level of family may be driving skin microbial diversity. Environmental and ecological factors play a secondary role in shaping both alpha diversity and community differentiation (i.e., beta diversity), with more evidence of structuring microbiomes among species. Although the presence of pathogens was not the most important factor determining microbiome structure or diversity, there was a slight, negative impact of *Bd* on the alpha diversity of salamander skin microbiomes. Together, these results provide further insight into the importance of multiple factors that simultaneously impact the skin microbiome in the presence of pathogens and underscore the importance of accounting for host family at this scale. Additionally, this research provides unique baseline data into salamander skin microbiomes, which could aid in conservation efforts as amphibian pathogens continue to spread.

Family-level differences, not phylogenetic history, significantly shapes microbiomes

When we examined multiple drivers simultaneously, or accounted for their impacts by subdividing our data, the most important and consistent factor influencing salamander skin microbiomes at this scale was the family of the host, with differences in host ecology and the environment becoming more important within species. Our results are similar to those of Buttimer et al. (2022), whose study evaluated the skin microbiomes of five salamander species (one from the family Salamandridae and four from the family Plethodontidae). In our study, family Salamandridae was distinct from Plethodontidae in both alpha and beta diversity metrics — with plethodontids having consistently higher alpha diversity than the newts — as well as overall microbial community membership (as indicated by beta diversity).

Differences in the skin microbiomes that correlate with phylogeny may be an example of phylosymbiosis, which theorizes that the microbes present in or on an organism are a result of long-term natural selection (Brucker & Bordenstein, 2012; Lim & Bordenstein, 2020; Yeoh et al., 2017). Families Plethodontidae and Salamandridae have been distinct for over 150 million years and the genera *Eurycea* and *Plethodon* diverged around 75 million years ago (Martin et al., 2016; Zhang & Wake, 2009). This could provide more than sufficient time for phylosymbiosis to develop, potentially driven by fitness consequences for animals that experience changes in the community composition of the skin microbiome (Becker et al., 2015; Bletz et al., 2018). However, while the strongest predictor of microbial abundance and diversity from most analyses in our study was host family there was an overall lack of phylogenetic signal overall – suggesting that either phylosymbiotic relationships are not occurring or that we are not able to identify them within our dataset. Univariate comparisons also support this conclusion; while some genera and species were different, these differences were not greater among genera than among species, as would expected under phylosymbiosis.

When comparing microbiomes within plethodontids we found instances of alpha or beta diversity of some species differing significantly from others. For example, beta diversity of species *Eurycea tynerensis* differed significantly from all other salamander species in the unweighted-UniFrac test (Table 5), indicating that the microbes present on the skin are phylogenetically similar, but different from the microbes present on other salamander species. In terms of alpha diversity, the CART analysis of Inverse Simpson metric values initially split *E.*

lucifuga and *P. angusticlavius* from all other species. With no evidence of phylogenetic signal, it is possible that unaccounted life history traits, behaviors, or diet are influencing these differences in microbial diversity. Microhabitat preference also suggest one possibility linking the two species: adult *E. lucifuga* and *P. angusticlavius* both dwell in shallow caves and rock ledges (Petranka, 1998). Identifying and understanding these unaccounted drivers of among-species differences may allow us to better predict how changes in food or habitat availability may drive microbiome shifts in the future. This will in turn allow us to identify the effects of these changes as they come.

Differences in environment & host ecology

Although environmental and ecological variables were a secondary factor in structuring microbial communities in our study, they still played a significant role in explaining microbiome diversity and composition. Increases in average monthly precipitation, average temperature, and site elevation were associated with increases in alpha diversity. Factors relating to host ecology, such as habitat and life stage, were significant in the PGLMMs, with juvenile plethodontids and terrestrial plethodontids exhibiting decreased alpha diversity in comparison to others. These results are similar to other studies' findings, which concluded that host ecology and environment are important drivers of skin microbial diversity (e.g., Bird et al., 2018; Bletz et al., 2017(b), Muletz Wolz et al., 2018), but differ in terms of the relative magnitude of predictors.

Although elevation, average precipitation, or average temperature of the month were secondary or tertiary drivers, all had positive relationships with alpha diversity. When considered jointly with phylogeny there was evidence that the environment surrounding an animal impacted alpha diversity; higher temperature or precipitation led to increases in the alpha diversity of microbial communities (Fig. 3). None of these environmental explanatory variables had a strong relationship to community membership alone, but after accounting for physical distance, partial Mantel tests indicated that microbial communities become more distinct between areas with different temperatures and those with different precipitation in each of the salamander families (Table 3). We also compared the effect of ecoregion on the skin microbiome; our expectation was that skin microbial diversity would vary across these regions. Our analyses show that while there was no difference in microbial alpha diversity across the ecoregions, each ecoregion significantly differed in community composition from one or more other ecoregions in analyses

of beta diversity, using weighted- and/or unweighted-UniFrac values. These results are qualitatively similar to those of other studies that looked only at salamander species within the same family or genus (e.g., Kueneman et al., 2014; Walker et al., 2019), further supporting the idea that environmental variables become more important in structuring the microbiomes of closely related hosts.

When considering explanatory variables relating to host ecology and habitat, we expected to find significant differences in skin microbial diversity between terrestrial and aquatic environments, as well as between different life stages, similar to previous work across habitat types (Xu et al., 2020). There are many differences between the life cycles of species in our study. Adult newts, the only life stage of newts included herein, are aquatic (Petranka, 1998). In contrast, *Plethodon* spp. lay eggs on land and spend their entire lives as terrestrial animals; the genus *Eurycea* lays eggs in water, which hatch into aquatic larvae (Petranka, 1998). Larval *Eurycea* either transform into terrestrial juvenile forms which become terrestrial adults or remain in the water to become aquatic adults (Petranka, 1998). Despite these varied life histories, the difference in alpha diversity between terrestrial and aquatic environments was significant only in the PGLMM, which treated host phylogeny as a continuous variable rather than a categorical one. The beta diversity of salamander microbiomes was not significantly different across different habitats when the phylogenetic distance of the microbes was considered (i.e., using the weighted UniFrac results), indicating that their microbiomes were mainly composed of different but closely related microbes. The difference in alpha diversity between life stages was also only evident in the PGLMM, in which being a juvenile plethodontid had a negative impact on the alpha diversity of the skin microbiome. While there were differences between microbiomes that related to life stages in tests of beta diversity, these were only evident in unweighted-UniFrac tests, signifying that, similar to the relationship between habitat and beta diversity, their microbes are closely genetically related even if the actual taxa differ.

The presence of infection

We expected to find that animals with *Bd* present on their skin exhibited a significant difference in the skin microbiome's community composition, similar to the previous work on *Anaxyrus boreas* toads and *Plethodon cinereus* salamanders (Kueneman et al., 2016; Muletz Wolz et al., 2019). After taking phylogeny into account, we found that animals with *Bd* present

on the skin showed a slight decrease in alpha diversity (Figs. 3 & 5). Though only the PGLMM analyses provided statistical support for a significant decrease in alpha diversity in the presence of disease (e.g., Fig. 3), these results fit into our growing understanding of the interaction between chytridiomycosis and amphibian skin microbiomes (e.g., Bates et al., 2022; Harris et al., 2009).

Chytridiomycosis, the disease caused by *Bd*, infects the skin of amphibians and thereby disturbs osmoregulation and respiration in its host (Campbell et al., 2012; Martel et al., 2014; Rollins-Smith et al., 2011; Voyles et al., 2007, 2009). Recent studies have documented the significant prevalence of *Bd* and ranavirus in North America, including among populations of amphibians in Oklahoma (Davis et al., 2019; Marhanka et al., 2017; Smith et al., 2019; Watters et al., 2018; 2019). Though none of our animals exhibited visible symptoms of disease (e.g., lethargy, increased skin shedding, and skin lesions; Martel et al., 2013, 2014; Voyles et al., 2007, 2009), the presence of *Bd* on the animal's skin may indicate an ongoing or recent infection. Varying stages of infection have been shown to have very different impacts on amphibian skin microbiomes; while an overall decline in bacterial abundance has been observed related to *Bd* infections (Medina et al., 2017), this pattern involves the increase of some microbes and the decrease of others during and after infection (Jani & Briggs, 2014; Jani et al., 2021). Salamanders from our study may be at different stages of infection, which may minimize overall differences among "positive" animals if the microbiome's alpha and beta diversity change during and again after infection. Additionally, because we were not able to address changes in the function of the microbes present, there may be large differences in functional microbes but fewer differences in overall alpha or beta diversity (Jani et al., 2021).

Our results also indicate the presence of antifungal microbes in the skin microbiome, a feature that has been identified in multiple studies (Woodhams et al., 2015; Becker et al., 2015). Laboratory manipulation of antifungal microbes on amphibians' skin in has been linked to improved chytridiomycosis survival rates (Bletz et al., 2018; Harris et al., 2009; Kueneman et al., 2016), which suggests beneficial microbes may naturally increase in abundance during infection in animals that tolerate chytridiomycosis (Campbell et al., 2019). Although there were no significant differences in alpha or beta diversity of antifungal microbes between animals of varying *Bd* status in our study, there were ten differentially abundant antifungal microbes when contrasting animals with and without *Bd* (Supplementary Table 2). However, microbes with

antifungal properties may decline in number during infection, suggesting a more nuanced relationship between microbial function and microbiome composition (Medina et al., 2017). More studies about *Bd* and its effect on the amphibian skin microbiome are needed to aid in answering these questions.

In the case of ranavirus infections, we expected possible virus-driven variation in the composition of the skin microbiome, based on previous work that indicated amphibians with ranavirus infection exhibit changes in microbial community composition (Campbell et al., 2019). However, as infection with ranavirus takes place internally, it is possible that our approach for measuring ranavirus from toe clips is not representative of true infection prevalence (Gray & Chinchar, 2015). In our study, there was not a significant relationship between ranavirus presence and alpha or beta diversity in any tests. This does not necessarily indicate that there is no relationship between ranavirus infection and changes in the skin microbiome. Future studies on this topic using symptomatic animals, or animals confirmed to have ranavirus present in the tissue of internal organs, may be more indicative of the relationship (Gray & Chinchar, 2015).

CONCLUSIONS

Effective conservation planning requires an understanding of not just target species, but also its interactions with other species, including microbes (Hird et al., 2017). This study provides a snapshot of the skin microbiomes of six salamander species and how these microbiomes vary across different habitats, varying environments, and genetic distances. While the most important determinant of skin microbiome composition is the family of the host, the research herein suggests that the skin microbiomes are not becoming more distinct based on phylogenetic distance (as would be expected under phylosymbiosis at this scale). Secondarily, host ecology, host environment, and the presence of disease influence the diversity and community composition of microbiomes. Furthermore, the presence of differentially abundant microbes linked to antifungal properties on the skin of animals testing positive for *Bd* suggests a possible interaction occurring between pathogens and skin microbiomes.

Further questions remain about the drivers and impacts of skin microbial composition, such as whether other salamander families also show strong microbiome divergence. There are nine commonly accepted salamander families, but most of the research on salamander skin microbial diversity has focused either on salamanders within the same family (Bird et al., 2018;

Ellison et al., 2019; Muletz Wolz et al., 2017; Walker et al., 2020), or on the families Plethodontidae and Salamandridae (Buttimer et al., 2022). Including more families would allow a clearer picture of whether family-level differences are common, or whether other factors are driving differences between plethodontids and newts. Studies could also focus on evaluating the other salamander species which co-occur in these four ecoregions, as more research is needed to determine how the skin microbiome is structured across additional, unstudied genera, several of which are on watchlists as species of conservation concern. We could examine seasonal changes in microbial diversity, which may influence host ecology or provide greater insights into the importance of drivers of microbial diversity. This would also be an opportunity to study microbial diversity as pathogen presence waxes and wanes: ranavirus becomes more prevalent beginning in the spring as the breeding season begins, while *Bd* cannot tolerate the warmest temperatures of summer and so is most common in spring and fall (Gray & Chinchar, 2015; Kinney et al., 2011). As infectious diseases continue to decimate amphibian populations, characterizing host microbiomes and examining the function of antifungal microbes is more important than ever.

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Table 1. Sample distribution across species and the four level III ecoregions in Oklahoma. While newts (Family Salamandridae) were found across all four ecoregions, the other species (all family Plethodontidae) were each found in 1-2.

Species	Arkansas Valley	Boston Mountains	Ouachita Mountains	Ozark Highlands
Notophthalmus viridescens	13	10	18	55
Eurycea longicauda	–	–	–	40
E. lucifuga	–	–	–	24
E. tynerensis	–	12	–	52
Plethodon albagula	–	2	–	37
P. angusticlavius	–	9	–	8

Table 2. Differences in alpha diversity of salamander microbiomes by host phylogenetic group (i.e., families, genera, and species). Differences by family were assessed using the Kruskal-Wallis test, whereas those by genera and species were analyzed using a pairwise-Wilcoxon rank sum test with Holm *p*-value correction. The results indicate that microbial alpha diversity differs between the two salamander families, a pattern evident in all subsequent comparisons between *Notophthalmus* (family Salamandridae) and other genera and species (all family Plethodontidae) is significantly different.

Comparison		**ASVs**	**Shannon**	**Inverse Simpson**
Family	Plethodontidae vs. Salamandridae	**1.66E-07**	**1.09E-07**	**0.002**
	chi-squared	27.396	28.200	9.574
Genus	*Eurycea* vs. *Notophthalmus*	**5.60E-06**	**2.90E-07**	**1.50E-06**
	Effect size	0.387	0.433	0.408
	Plethodon vs. *Eurycea*	0.514	0.809	0.580
	Effect size	0.0632	0.024	0.054
	Plethodon vs. *Notophthalmus*	**0.0002**	**0.009**	**0.040**
	Effect size	0.388	0.286	0.236
Species	*E. longicauda* vs. *E. tynerensis*	1	1	1
	Effect size	0.137	0.115	0.089
	E. longicauda vs. *N. viridescens*	**0.003**	**0.0001**	**0.0005**
	Effect size	0.374	0.442	0.416
	E. longicauda vs. *P. albagula*	1	1	1
	Effect size	0.042	0.173	0.216
	E. longicauda vs. *P. angusticlavius*	1	1	1
	Effect size	0.205	0.201	0.271
	E. lucifuga vs. *E. longicauda*	1	1	1
	Effect size	0.076	0.021	0.128
	E. lucifuga vs. *N. viridescens*	**0.010**	**0.044**	**0.044**
	Effect size	0.369	0.319	0.319
	E. lucifuga vs. *P. albagula*	1	1	1
	Effect size	0.145	0.171	0.239
	E. lucifuga vs. *P. angusticlavius*	1	1	1
	Effect size	0.166	0.166	0.203
	E. tynerensis vs. *E. lucifuga*	1	1	1
	Effect size	0.176	0.163	0.197
	E. tynerensis vs. *N. viridescens*	**0.013**	**0.001**	**0.004**
	Effect size	0.313	0.376	0.345
	E. tynerensis vs. *P. albagula*	1	1	1
	Effect size	0.088	0.059	0.127
	E. tynerensis vs. *P. angusticlavius*	0.796	0.993	0.691
	Effect size	0.264	0.249	0.279
	N. viridescens vs. *P. albagula*	**0.028**	0.649	1
	Effect size	0.318	0.197	0.136
	N. viridescens vs. *P. angusticlavius*	**0.035**	**0.045**	**0.045**
	Effect size	0.337	0.331	0.331
	P. albagula vs. *P. angusticlavius*	1	0.993	0.691
	Effect size	0.225	0.314	0.359

Table 3. Role of pathogen status and ecoregion on alpha diversity within family Salamandridae (i.e., newts). The impact of ranavirus and *Batrachochytrium dendrobatidis* (*Bd)* status (i.e., presence/absence) was evaluated with Kruskal-Wallis tests; significance of ecoregion was assessed using a pairwise-Wilcoxon rank sum test with Holm *p*-value correction. Here we found no significant differences between disease status, or between ecoregions in any alpha diversity metric.

Family Salamandridae		**ASVs**	**Shannon**	**Inverse Simpson**
Ranavirus	yes vs. no	0.808	0.696	0.958
	chi-squared	0.059	0.153	0.003
Bd	yes vs. no	0.306	0.455	0.619
	chi-squared	1.048	0.558	0.248
Ecoregion	Arkansas Valley vs. Boston Mountains	0.417	1	1
	Effect size	0.395	0.139	0.139
	Arkansas Valley vs. Ozark Highlands	0.435	1	1
	Effect size	0.214	0.022	0.077
	Arkansas Valley vs. Ouachita Mountains	0.435	0.63	1
	Effect size	0.258	0.271	0.218
	Boston Mountains vs. Ozark Highlands	0.554	0.54	0.67
	Effect size	0.093	0.261	0.246
	Boston Mountains vs. Ouachita Mountains	0.227	0.54	0.67
	Effect size	0.415	0.344	0.318
	Ouachita Mountains vs. Ozark Highlands	0.086	0.63	1
	Effect size	0.338	0.196	0.139

Table 4. Role of pathogen status and ecoregion on alpha diversity within family Plethodontidae. The impact of ranavirus and *Batrachochytrium dendrobatidis* (*Bd)* status (i.e., presence/absence) was evaluated with Kruskal-Wallis tests; significance of ecoregion was assessed using a pairwise-Wilcoxon rank sum test with Holm *p*-value correction. Here we found no significant differences between pathogen status, habitat type, or between ecoregions in any of the alpha diversity metrics.

Family Plethodontidae		**ASVs**	**Shannon**	**Inverse Simpson**
Ranavirus	yes vs. no	0.47	0.32	0.3
	chi-squared	0.536	1	1.11
Bd	yes vs. no	0.94	0.724	0.696
	chi-squared	0.001	0.125	0.153
Habitat	aquatic vs. terrestrial	0.335	0.97	0.79
	chi squared	0.931	0.001	0.071
Ecoregion	Boston Mountains vs. Ozark Highlands	0.053	0.097	0.2
	chi squared	3.75	2.76	1.65

Table 5. Impact of phylogenetic differentiation on beta diversity. Tests of beta diversity revealed differences in skin microbiomes among salamander families and genera, but fewer differences at the level of comparisons among species. Beta diversity was evaluated using Permutational Analysis of Variance tests with Holm *p*-value correction and permutations set to 10,000.

All Species		**unweighted UniFrac**	**weighted UniFrac**
Family	Plethodontidae vs. Salamandridae	**0.001**	**0.001**
	r-squared	0.050	0.165
Genus	*Plethodon* vs. *Notophthalmus*	**0.001**	**0.001**
	r-squared	0.065	0.179
	Plethodon vs. *Eurycea*	**0.013**	0.064
	r-squared	0.016	0.017
	Eurycea vs. *Notophthalmus*	**0.001**	**0.001**
	r-squared	0.050	0.185
Species	*E. longicauda* vs. *E. lucifuga*	0.348	0.229
	r-squared	0.025	0.031
	E. longicauda vs. *E. tynerensis*	**0.004**	**0.031**
	r-squared	0.027	0.032
	E. longicauda vs. *N. viridescens*	**0.001**	**0.001**
	r-squared	0.053	0.210
	E. longicauda vs. *P. albagula*	0.461	0.336
	r-squared	0.021	0.023
	E. longicauda vs. *P. angusticlavius*	0.543	0.939
	r-squared	0.028	0.013
	E. lucifuga vs. *N. viridescens*	**0.001**	**0.001**
	r-squared	0.052	0.196
	E. lucifuga vs. *E. tynerensis*	**0.005**	**0.045**
	r-squared	0.039	0.040
	E. lucifuga vs. *P. albagula*	0.44	0.101
	r-squared	0.029	0.049
	E. lucifuga vs. *P. angusticlavius*	0.354	0.815
	r-squared	0.054	0.030
	E. tynerensis vs. *N. viridescens*	**0.001**	**0.001**
	r-squared	0.050	0.182
	E. tynerensis vs. *P. albagula*	**0.001**	**0.006**
	r-squared	0.0385	0.048
	E. tynerensis vs. *P. angusticlavius*	**0.027**	0.349
	r-squared	0.034	0.024

N. viridescens vs. *P. albagula*	**0.001**	**0.001**
r-squared	0.057	0.160
N. viridescens vs. *P. angusticlavius*	**0.001**	**0.001**
r-squared	0.038	0.117
P. albagula vs. *P. angusticlavius*	0.837	0.658
r-squared	0.030	0.027

Table 6. Factors influencing beta diversity within the family Salamandridae. Significance assessed with a Permutational Analysis of Variance tests with Holm *p*-value correction and permutations set to 10,000. The weighted UniFrac test considers microbial phylogeny, while the unweighted test does not. We found there was no significant difference between disease statuses. We did find a significant difference in beta diversity between all ecoregions in at least one of the UniFrac tests.

Family Salamandridae		**Unweighted UniFrac**	**Weighted UniFrac**
RV	Yes vs. No	0.29	0.15
	r squared	0.024	0.033
Bd	Yes vs. No	0.57	0.29
	r squared	0.022	0.026
Ecoregion	Arkansas Valley vs. Boston Mountains	0.053	**0.045**
	r squared	0.063	0.097
	Arkansas Valley vs. Ouachita Mountains	**0.003**	**0.023**
	r squared	0.065	0.074
	Arkansas Valley vs. Ozark Highlands	**0.001**	0.163
	r squared	0.038	0.022
	Boston Mountains vs. Ouachita Mountains	**0.002**	**0.001**
	r squared	0.009	0.190
	Boston Mountains vs. Ozark Highlands	0.211	**0.01**
	r squared	0.018	0.050
	Ouachita Mountains vs. Ozark Highlands	**0.001**	**0.009**
	r squared	0.066	0.042

Table 7. Factors influencing beta diversity within the family Plethodontidae. Significance assessed with a Permutational Analysis of Variance tests with Holm *p*-value correction and permutations set to 10,000. The unweighted UniFrac test treats phylogeny as a categorical variable, while the weighted test treats it as a continuous variable. We found no significant difference in beta diversity between pathogen statuses or ecoregions. We did find significant differences between the beta diversity of the life stages and habitats in the weighted-UniFrac.

Family Plethodontidae		**Unweighted UniFrac**	**Weighted UniFrac**
RV	Yes vs. No	0.07	0.41
	r squared	0.017	0.013
Bd	Yes vs. No	0.18	0.47
	r squared	0.0148	0.012
Habitat	aquatic vs. terrestrial	**0.015**	0.232
	r squared	0.024	0.017
Ecoregion	Ozark Highlands vs. Boston Mountains	0.117	0.698
	r squared	0.020	0.051
Life Stage	juvenile vs. adult	**0.038**	0.382
	r squared	0.019	0.013
	juvenile vs. paedomorphic adult	**0.004**	0.082
	r squared	0.052	0.043
	juvenile vs. larva	**0.006**	0.094
	r squared	0.053	0.045
	adult vs. paedomorphic adult	0.085	0.375
	r squared	0.02	0.016
	adult vs. larva	**0.012**	0.217
	r squared	0.025	0.02
	paedomorphic adult vs. larva	0.468	**0.015**
	r squared	0.044	0.047

Table 8. Partial Mantel tests of the correlation between environmental conditions and beta diversity (based on Bray-Curtis distances). These results account for geographic distance, measured as the Euclidean distance between sampling localities.

Comparison	Variables	Mantel statistic *r*	significance
All Species	elevation | distance	0.122	**0.001**
	annual precipitation | distance	0.060	**0.005**
	seasonal precipitation | distance	0.060	**0.006**
	monthly precipitation | distance	0.039	0.133
	annual temperature | distance	0.195	**0.001**
	seasonal temperature | distance	0.179	**0.001**
	monthly temperature | distance	0.209	**0.001**
Salamandridae	elevation | distance	0.142	**0.025**
	annual precipitation | distance	0.010	0.402
	seasonal precipitation | distance	-0.026	0.745
	monthly precipitation | distance	0.297	**0.001**
	annual temperature | distance	0.104	**0.038**
	seasonal temperature | distance	0.115	**0.028**
	monthly temperature | distance	0.264	**0.001**
Plethodontidae	elevation | distance	0.111	0.052
	annual precipitation | distance	0.108	**0.012**
	seasonal precipitation | distance	0.166	**0.003**
	monthly precipitation | distance	0.214	**0.001**
	annual temp | distance	0.094	**0.004**
	seasonal temp | distance	0.145	**0.001**
	monthly temp | distance	0.166	**0.002**

Table 9. Mantel tests of the correlation between environmental conditions and beta diversity (based on Bray-Curtis distances) between microbiomes. These correlations, without accounting for geography, are similar to those from the partial mantel results (Table 8).

Variables	Mantel statistic r	r = 0	upper limit	lower limit
elevation	0.123	**0.001**	0.146	0.104
annual precipitation	0.063	**0.002**	0.077	0.050
seasonal precipitation	0.062	**0.009**	0.077	0.048
monthly precipitation	0.044	0.275	0.063	0.028
annual temperature	0.196	**0.001**	0.214	0.178
seasonal temperature	0.180	**0.001**	0.197	0.164
monthly temperature	0.209	**0.001**	0.230	0.189

Figure 1. Sampling locations and relative abundance of six salamander host species in this study. Sampling localities (small points) occur within the four level III ecoregions (Arkansas Valley, Boston Mountains, Ouachita Mountains, and Ozark Highlands) in eastern Oklahoma, USA. Pie charts refer to the proportion of salamander species found within each ecoregion.

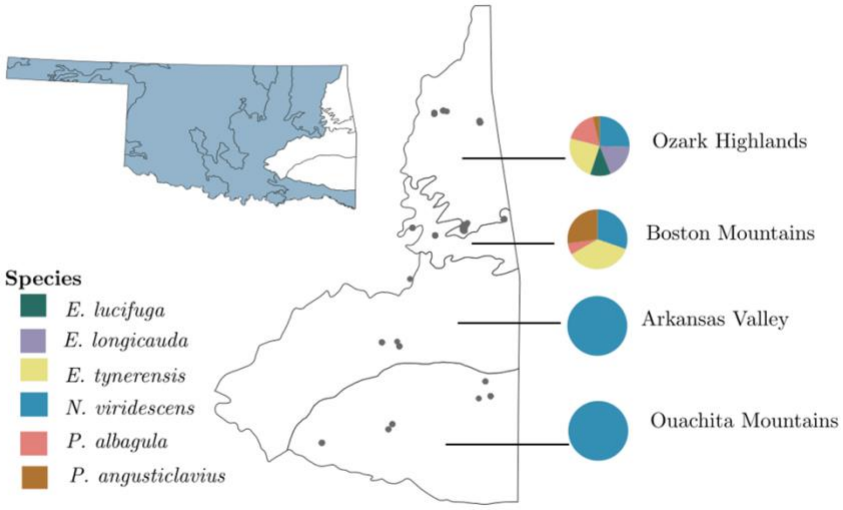


Figure 2. Relative compositional differences in skin microbiome communities based on host family. Plethodontids (left) had consistently higher alpha diversity than the Salamandridae (i.e., newts, right) and were distinct from the newts in analyses of beta diversity.

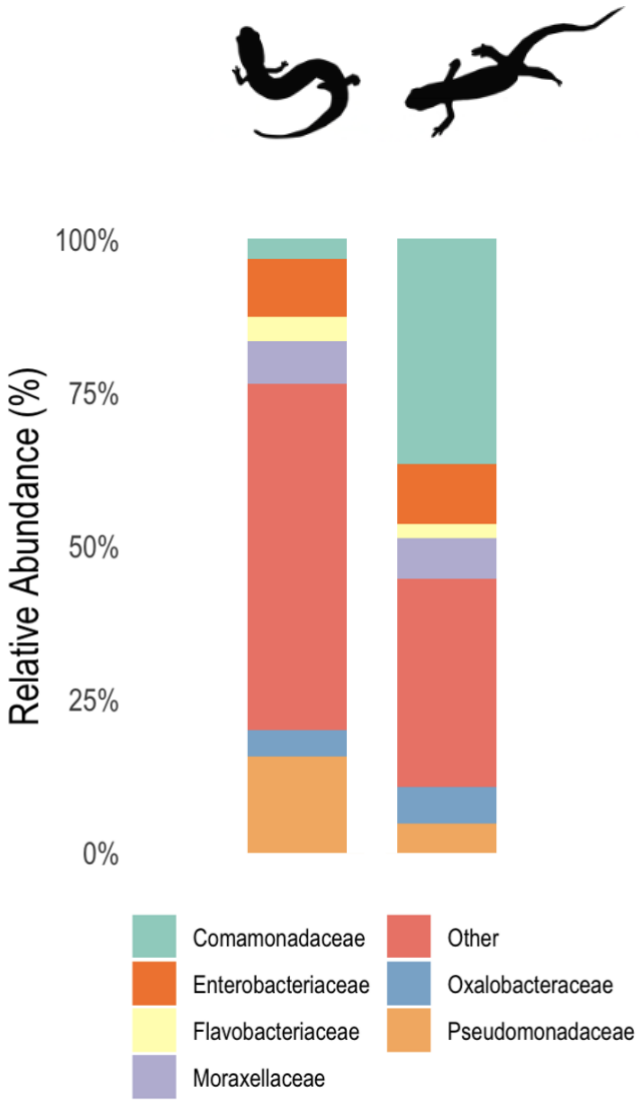


Figure 3. Relative contribution of explanatory variables to alpha diversity based on phylogenetic generalized linear mixed models. The figure contains posterior means and their 95% credible intervals. After accounting for phylogenetic non-independence, we found that ranavirus status has no impact on alpha diversity, but *Bd* presence has a slight negative impact. Warmer, wetter, and higher sampling sites had slight positive effects on alpha diversity, but terrestrial habitats and some life history stages reduced microbial diversity.

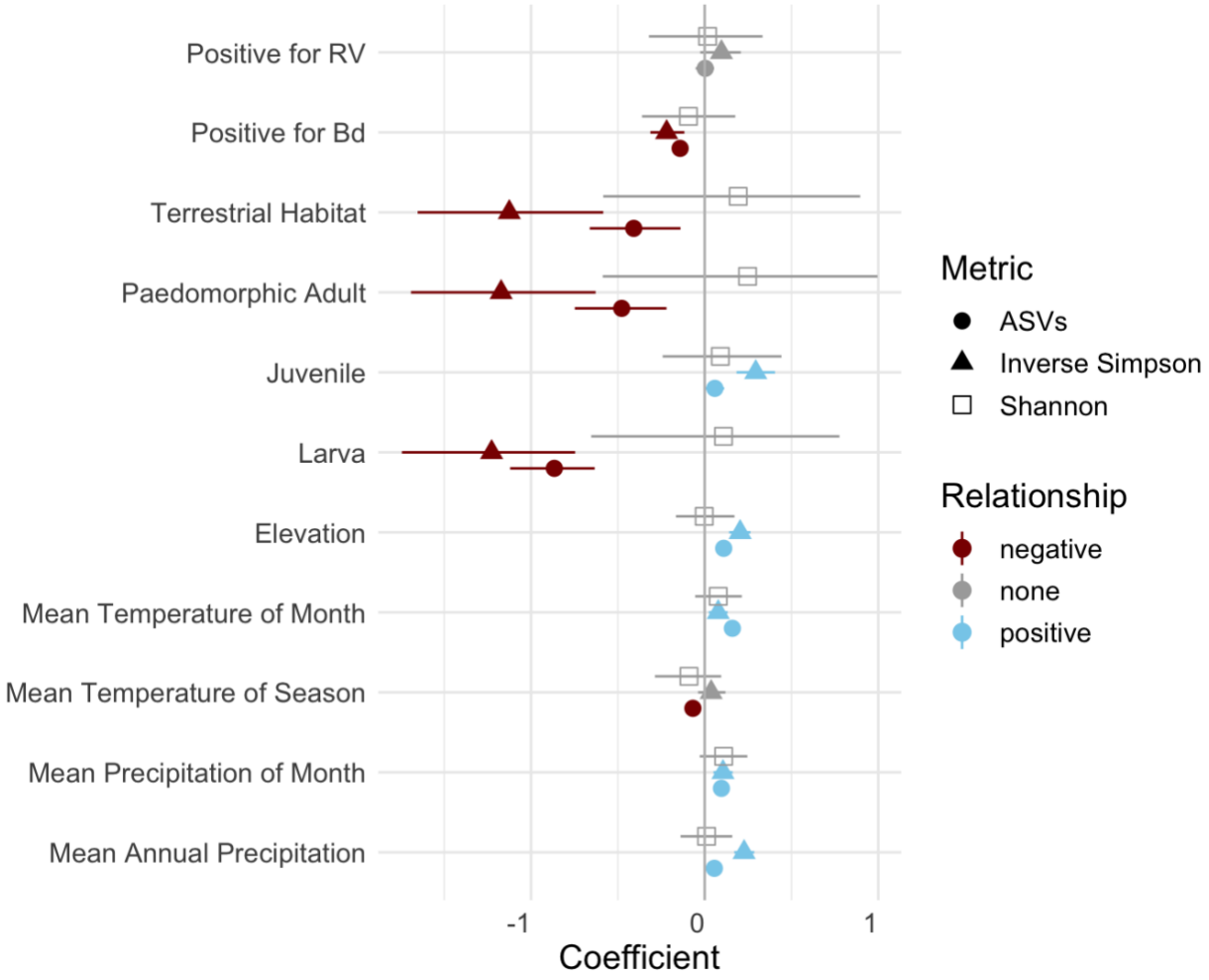


Figure 4. Results from the CART analysis of Observed ASVs. While family is the most important factor influencing the number of unique ASVs, environmental variables are important within family Salamandridae. Similar dominance of differentiation in microbial diversity based up on host phylogeny is observed in the other two metrics (Supplementary Figs. 2 & 3).

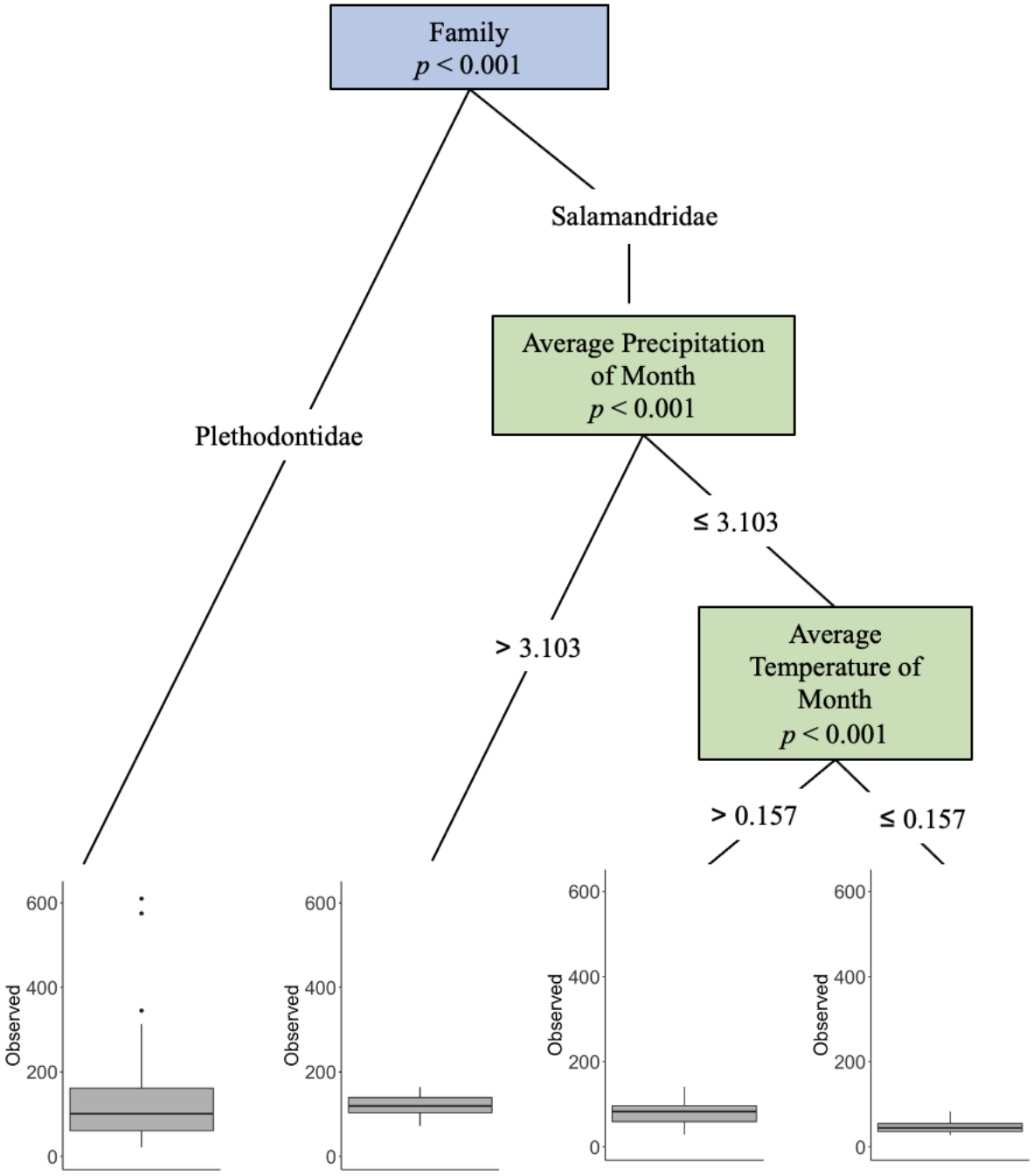
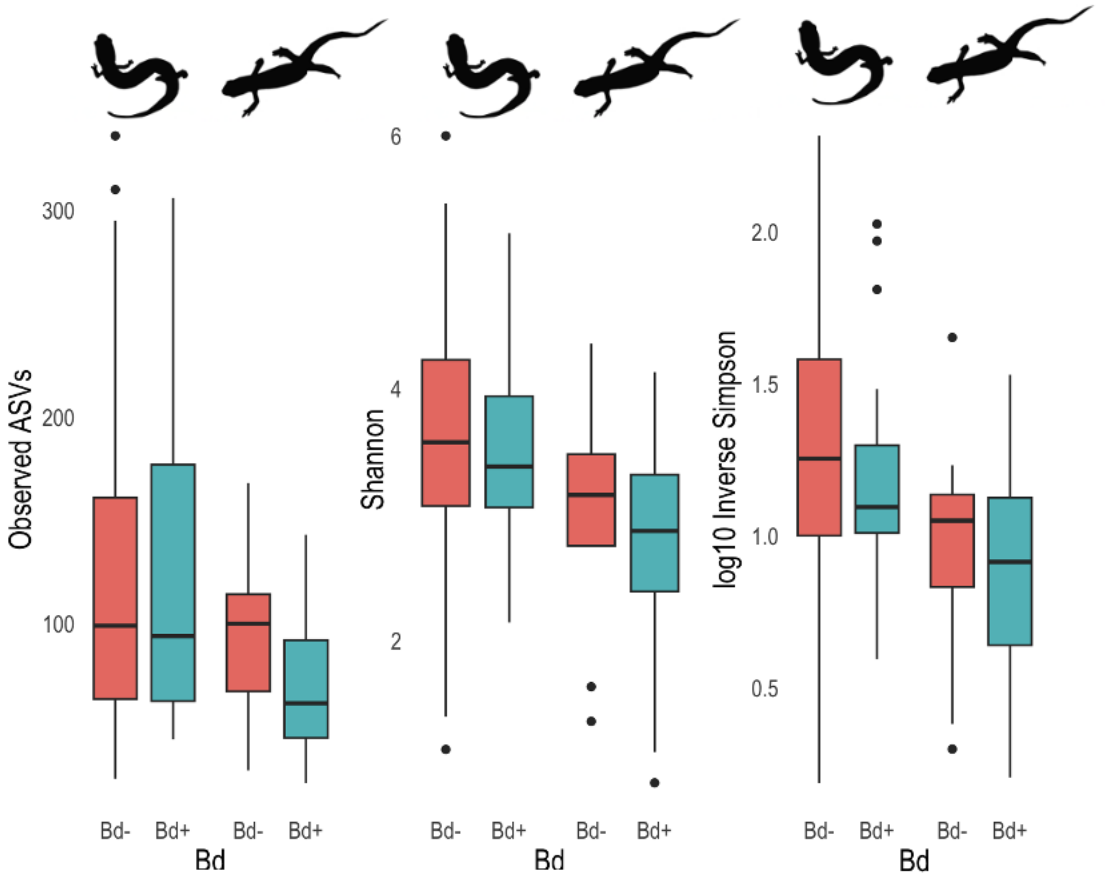
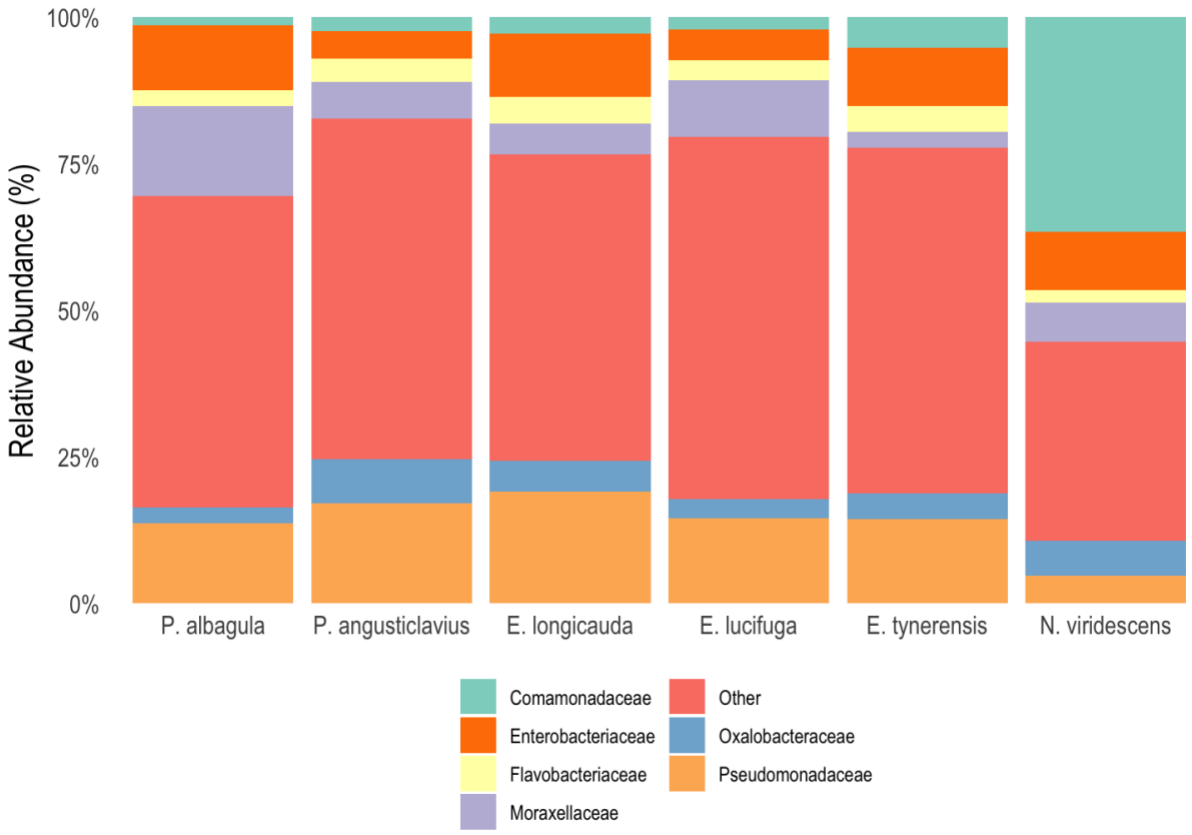


Figure 5. Alpha diversity differences based on *Bd* infection status. Alpha diversity of plethodontids and newts (left and right, respectively, within each set of plots) in the presence and absence of *Bd*. Overall, *Bd*+ salamanders exhibit a slight but non-significant (Tables 2–4) reduction in alpha diversity of the skin microbiome.

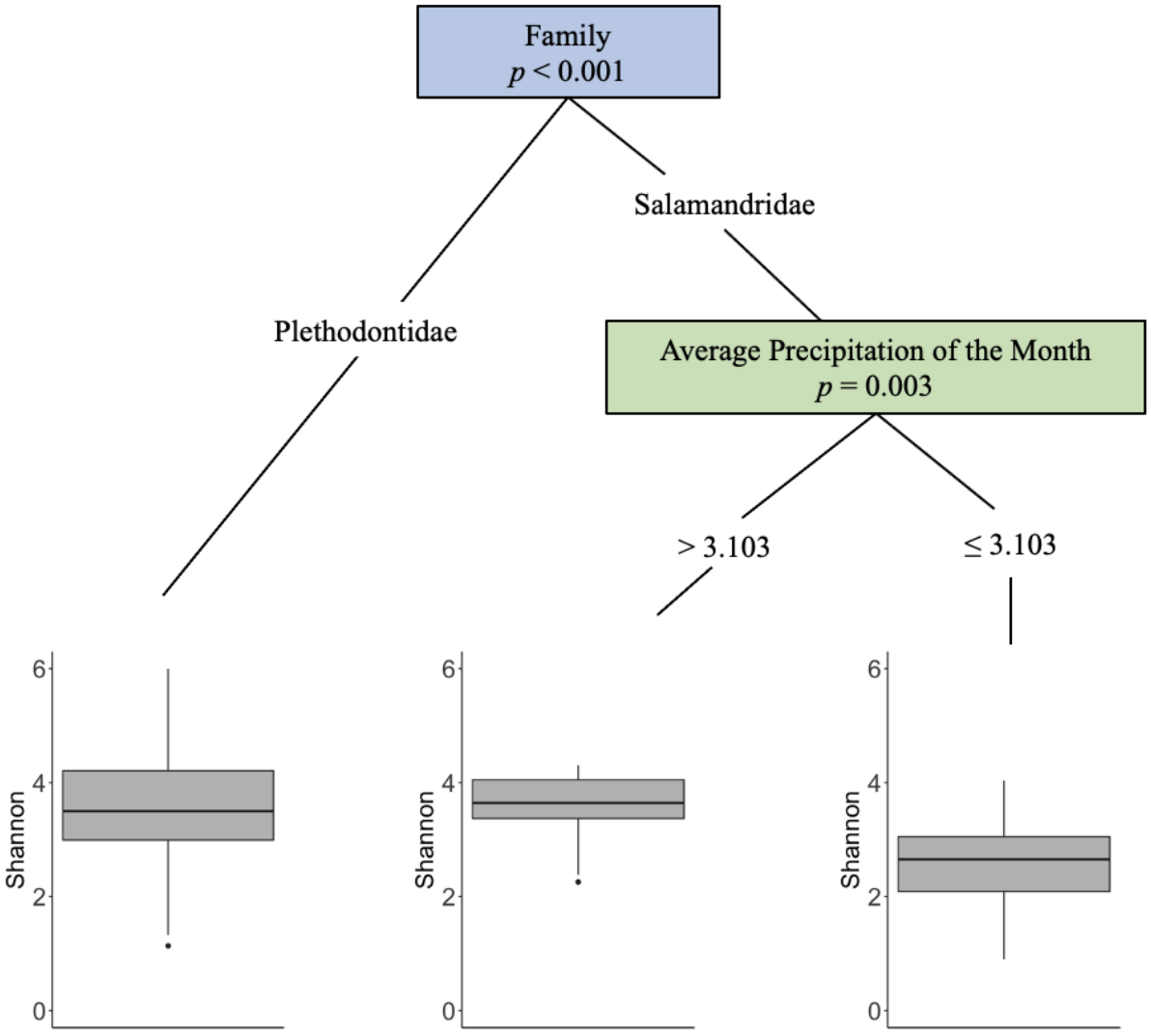


APPENDIX

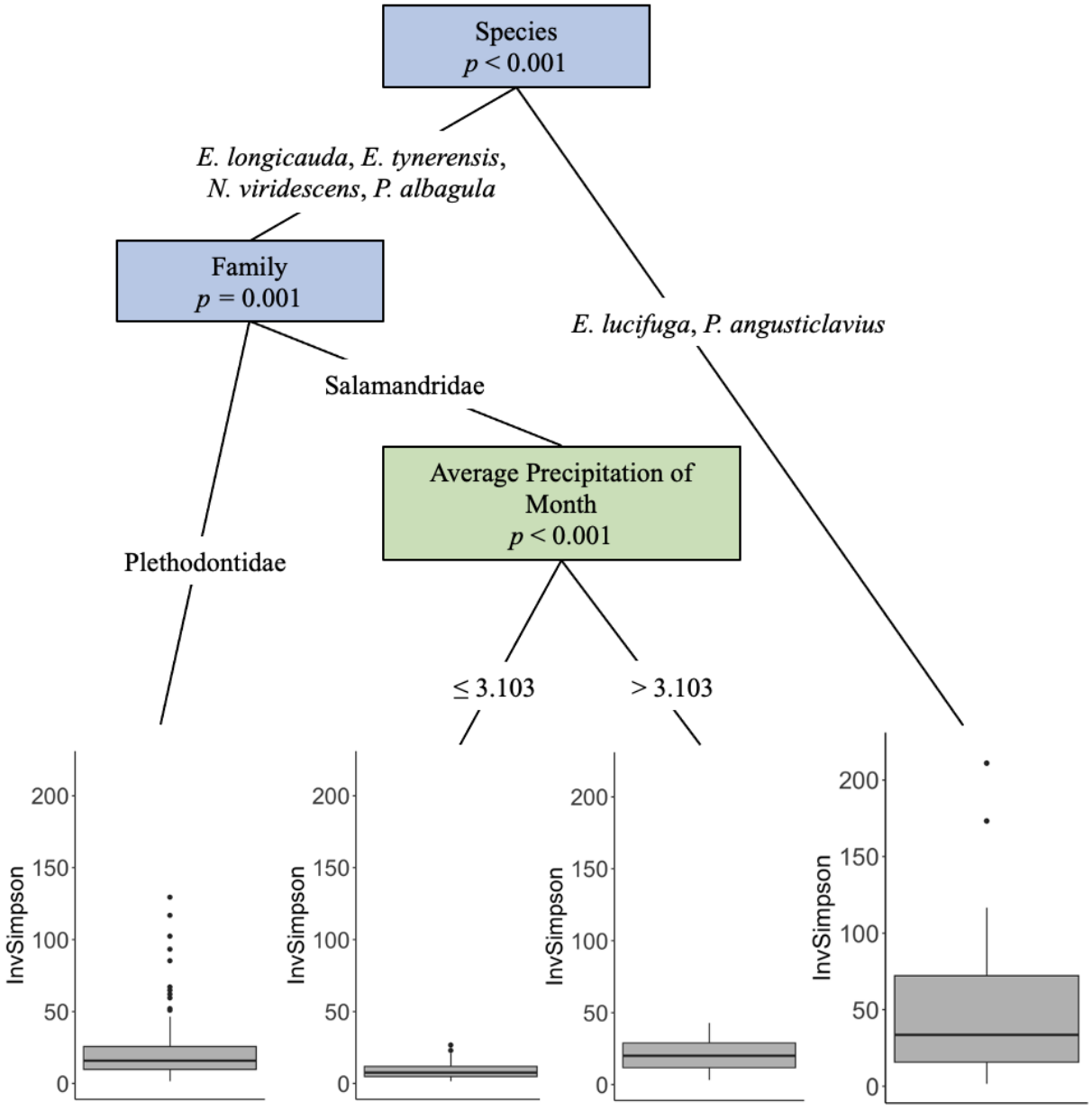
Supplementary Figure 1. Relative proportions of microbial families present in the skin microbiomes of salamanders across all six species.



Supplementary Figure 2. CART analysis of the drivers of difference in the Shannon-Wiener Diversity Index. Here, host family is the most important driver of microbial diversity, with the average precipitation of the month as a secondary driver of skin microbial diversity within the family Salamandridae.



Supplementary Figure 3. CART analysis of the drivers of differences in the Inverse Simpson Diversity Index. In the other two CART analyses of alpha diversity (Fig. 4, Supplementary Fig. 2), two species (*E. lucifuga* and *P. angusticlavius*) were the most different, with family as a secondary factor. Similar to analyses on the two other indices, average precipitation of the month is an important driver of skin microbial diversity within family Salamandridae.



Supplementary Table 1. Collection and microbial diversity data for each sample. Information includes the SNOMNH voucher ID, the Oklahoma Department of Wildlife Conservation Field ID, the total number of microbes present, the number of microbe species present, and disease status (N = not present).

SNOMNH ID	*ODWC Field ID*	*Family*	*Genus*	*Species*	*Ecoregion*	*Habitat*	*Life Stage*	*Total ASVs*	*Observed ASVs*	*RV*	*Bd*	*Year*
43983	277	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	3777	54	N	N	2015
43984	278	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	5371	62	N	Y	2015
43986	281	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	705	54	N	Y	2015
43987	282	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	4149	44	N	N	2015
43991	288	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	1758	44	N	NA	2015
43935	329	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	5881	452	N	N	2015
43948	341	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	491	45	Y	N	2015
43936	346	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	2283	46	Y	N	2015
43972	350	Plethodontidae	*Plethodon*	*angusticlavius*	Ozark Highlands	terrestrial	juvenile	774	44	N	N	2015
43952	351	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult	12380	94	Y	N	2015
43953	355	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	1772	155	N	N	2015
43954	356	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	1297	90	Y	N	2015
43955	357	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	742	38	Y	N	2015
43944	358	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	6838	463	Y	Y	2015
43956	359	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	320	13	Y	N	2015
43957	361	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult	64965	663	N	N	2015
43903	378	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	1425	48	N	Y	2015
43945	399	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	paedomorphic adult	12820	609	N	Y	2015
43908	402	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	1163	38	NA	NA	2015

43909	403	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	2091	63	N	Y	2015
43910	404	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	5413	263	N	N	2015
43911	405	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	605	45	N	N	2015
43912	407	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	1092	54	N	Y	2015
43913	408	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	3976	168	NA	NA	2015
43914	409	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	1302	56	NA	NA	2015
44758	412	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	745	46	Y	N	2015
44750	413	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	aquatic	larvae	1205	87	N	Y	2015
44753	414	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	larvae	8474	127	N	N	2015
43976	416	Plethodontidae	*Plethodon*	*angusticlavius*	Ozark Highlands	terrestrial	juvenile	583	37	N	N	2015
44759	417	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	1322	59	NA	NA	2015
44751	418	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	aquatic	larvae	10979	239	NA	NA	2015
43916	421	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	juvenile	5064	449	N	Y	2015
43917	423	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	2372	147	NA	NA	2015
43962	427	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult	518	46	Y	N	2015
43938	428	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	2179	197	Y	N	2015
43920	441	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	201	17	Y	N	2015
43921	442	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	230	19	Y	N	2015
43922	443	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	149	12	Y	N	2015
43923	444	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	juvenile	146	12	Y	Y	2015
43963	449	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult	73	12	N	Y	2015
43964	450	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult	111	12	NA	NA	2015

43965	451	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	350	45	N	N	2015
43966	452	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult	227	33	N	N	2015
43967	453	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult	237	28	N	Y	2015
43968	454	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult	137	49	N	N	2015
43977	455	Plethodontidae	*Plethodon*	*angusticlavius*	Ozark Highlands	terrestrial	juvenile	204	6	N	N	2015
43978	456	Plethodontidae	*Plethodon*	*angusticlavius*	Ozark Highlands	terrestrial	juvenile	129	4	N	Y	2015
43928	457	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	193	3	N	N	2015
43929	458	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	92	3	N	N	2015
43930	459	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	juvenile	175	7	NA	NA	2015
43931	460	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	juvenile	79	9	N	Y	2015
43969	476	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult	52	7	N	N	2015
43970	480	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	6684	518	Y	N	2015
43996	481	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	277	24	N	Y	2015
43997	482	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	459	21	N	Y	2015
44000	485	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	2500	37	N	Y	2015
44001	486	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	1086	48	NA	NA	2015
44002	487	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	532	34	N	Y	2015
44003	488	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	1322	61	N	Y	2015
43946	520	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	paedomorphic adult	2893	99	N	N	2015
44023	538	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	1201	33	N	Y	2015
44024	539	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	427	12	N	Y	2015
44025	540	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	194	20	NA	NA	2015

44026	541	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	233	14	NA	NA	2015
44027	542	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	318	24	NA	NA	2015
44028	543	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	493	24	NA	NA	2015
43971	560	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	2268	153	Y	Y	2015
43932	590	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	400	33	N	Y	2015
43934	592	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	9673	256	N	N	2015
44031	627	Salamandridae	*Notophthalmus*	*viridescens*	Arkansas Valley	aquatic	adult	1480	87	Y	Y	2015
44032	628	Salamandridae	*Notophthalmus*	*viridescens*	Arkansas Valley	aquatic	adult	2979	133	N	Y	2015
44033	629	Salamandridae	*Notophthalmus*	*viridescens*	Arkansas Valley	aquatic	adult	12074	111	Y	N	2015
44034	630	Salamandridae	*Notophthalmus*	*viridescens*	Arkansas Valley	aquatic	adult	4042	181	Y	Y	2015
44042	633	Salamandridae	*Notophthalmus*	*viridescens*	Arkansas Valley	aquatic	adult	6642	99	Y	Y	2015
44047	638	Salamandridae	*Notophthalmus*	*viridescens*	Arkansas Valley	aquatic	adult	690	29	N	NA	2015
44050	641	Salamandridae	*Notophthalmus*	*viridescens*	Arkansas Valley	aquatic	adult	1289	55	N	NA	2015
44051	642	Salamandridae	*Notophthalmus*	*viridescens*	Arkansas Valley	aquatic	adult	22818	134	Y	NA	2015
44037	683	Salamandridae	*Notophthalmus*	*viridescens*	Arkansas Valley	aquatic	adult	863	39	N	Y	2015
44038	684	Salamandridae	*Notophthalmus*	*viridescens*	Arkansas Valley	aquatic	adult	20940	95	Y	Y	2015
44039	685	Salamandridae	*Notophthalmus*	*viridescens*	Arkansas Valley	aquatic	adult	39218	127	N	Y	2015
44040	692	Salamandridae	*Notophthalmus*	*viridescens*	Arkansas Valley	aquatic	adult	4395	101	Y	Y	2015
44041	693	Salamandridae	*Notophthalmus*	*viridescens*	Arkansas Valley	aquatic	adult	2010	77	N	Y	2015
44070	1007	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	25960	146	Y	Y	2015
44071	1008	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	41991	292	Y	Y	2015
44073	1040	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	50618	386	N	N	2015

44074	1041	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	13153	181	Y	Y	2015
44075	1042	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	429627	703	N	NA	2015
44076	1043	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	53121	284	N	N	2015
44085	1052	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	34547	243	N	Y	2015
44056	1136	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	15470	244	N	Y	2015
44057	1137	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	9580	185	N	Y	2015
44059	1176	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	10639	223	N	Y	2015
44060	1177	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	24368	308	N	Y	2015
44062	1179	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	99506	246	N	Y	2015
46000	1570	Plethodontidae	*Eurycea*	*tynerensis*	Boston Mountains	terrestrial	adult	2549	79	NA	NA	2016
46001	1573	Plethodontidae	*Eurycea*	*tynerensis*	Boston Mountains	terrestrial	adult	357	42	N	N	2016
46002	1574	Plethodontidae	*Eurycea*	*tynerensis*	Boston Mountains	aquatic	paedomorphic adult	488	15	N	N	2016
46003	1575	Plethodontidae	*Eurycea*	*tynerensis*	Boston Mountains	aquatic	paedomorphic adult	870	10	N	N	2016
46004	1576	Plethodontidae	*Eurycea*	*tynerensis*	Boston Mountains	aquatic	paedomorphic adult	946	19	N	N	2016
46005	1577	Plethodontidae	*Eurycea*	*tynerensis*	Boston Mountains	terrestrial	adult	1170	26	N	N	2016
46006	1578	Plethodontidae	*Eurycea*	*tynerensis*	Boston Mountains	terrestrial	adult	3330	81	N	Y	2016
46007	1579	Plethodontidae	*Eurycea*	*tynerensis*	Boston Mountains	aquatic	paedomorphic adult	1867	43	N	N	2016
46008	1580	Plethodontidae	*Eurycea*	*tynerensis*	Boston Mountains	aquatic	paedomorphic adult	15099	70	N	N	2016
46009	1581	Plethodontidae	*Eurycea*	*tynerensis*	Boston Mountains	aquatic	paedomorphic adult	870	26	N	N	2016
46010	1582	Plethodontidae	*Eurycea*	*tynerensis*	Boston Mountains	aquatic	paedomorphic adult	1273	26	N	N	2016
46011	1583	Plethodontidae	*Eurycea*	*tynerensis*	Boston Mountains	terrestrial	adult	2809	97	N	N	2016
46095	1590	Salamandridae	*Notophthalmus*	*viridescens*	Boston Mountains	aquatic	adult	2663	46	N	Y	2016

46096	1597	Salamandridae	*Notophthalmus*	*viridescens*	Boston Mountains	aquatic	adult	865	23	N	Y	2016
46097	1598	Salamandridae	*Notophthalmus*	*viridescens*	Boston Mountains	aquatic	adult	37877	77	N	Y	2016
46098	1599	Salamandridae	*Notophthalmus*	*viridescens*	Boston Mountains	aquatic	adult	6924	88	N	Y	2016
46099	1600	Salamandridae	*Notophthalmus*	*viridescens*	Boston Mountains	aquatic	adult	639	14	N	Y	2016
46100	1601	Salamandridae	*Notophthalmus*	*viridescens*	Boston Mountains	aquatic	adult	7147	97	N	Y	2016
46101	1602	Salamandridae	*Notophthalmus*	*viridescens*	Boston Mountains	aquatic	adult	22773	110	N	Y	2016
46102	1603	Salamandridae	*Notophthalmus*	*viridescens*	Boston Mountains	aquatic	adult	4575	82	N	Y	2016
46103	1604	Salamandridae	*Notophthalmus*	*viridescens*	Boston Mountains	aquatic	adult	516	22	N	Y	2016
46104	1615	Salamandridae	*Notophthalmus*	*viridescens*	Boston Mountains	aquatic	adult	5298	66	N	Y	2016
45983	1639	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	309744	4062	N	N	2016
46206	1640	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	1670	54	N	N	2016
46012	1641	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	10736	146	N	N	2016
46013	1642	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	juvenile	20732	227	N	N	2016
46014	1643	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	paedomorphic adult	20467	203	N	Y	2016
46015	1644	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	4722	127	N	N	2016
46016	1645	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	paedomorphic adult	12983	214	N	N	2016
46017	1646	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	paedomorphic adult	13815	171	N	N	2016
46050	1650	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	11	10	N	Y	2016
46051	1651	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	844	24	N	Y	2016
46052	1652	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	1025	32	Y	Y	2016
46053	1653	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	4000	70	N	Y	2016
46054	1654	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	9886	87	N	Y	2016

46055	1655	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	2221	47	Y	Y	2016
46056	1656	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	9474	87	N	Y	2016
46057	1657	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	7027	100	N	Y	2016
46058	1658	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	3621	66	N	Y	2016
46059	1659	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	2378	51	Y	Y	2016
46060	1660	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	11025	49	NA	NA	2016
46061	1661	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	84	16	NA	NA	2016
46062	1662	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	384	15	NA	NA	2016
46063	1663	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	1446	63	NA	NA	2016
46064	1664	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	7548	95	NA	NA	2016
46065	1665	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	285	16	NA	NA	2016
46066	1666	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	1413	34	NA	NA	2016
46067	1667	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	498	32	NA	NA	2016
46068	1668	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	656	40	NA	NA	2016
46069	1669	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	190	7	NA	NA	2016
46070	1670	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	1705	53	NA	NA	2016
46071	1671	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	1187	45	NA	NA	2016
46072	1672	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	5662	112	NA	NA	2016
46073	1673	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	1103	38	NA	NA	2016
46074	1674	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	23063	74	NA	NA	2016
46075	1675	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	2239	39	NA	NA	2016
46076	1676	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	2423	50	NA	NA	2016

46077	1677	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	10326	114	NA	NA	2016
46078	1686	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	10832	64	NA	NA	2016
46079	1687	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	1885	59	NA	NA	2016
46080	1688	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	1424	56	NA	NA	2016
46081	1689	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	2811	56	NA	NA	2016
46082	1690	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	686	43	NA	NA	2016
46018	1692	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	2142	89	N	N	2016
46019	1693	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	924	56	N	N	2016
46020	1695	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	539	66	N	N	2016
46021	1696	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	639	120	NA	NA	2016
46022	1697	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	1717	61	N	N	2016
46025	1698	Plethodontidae	*Plethodon*	*albagula*	Boston Mountains	terrestrial	juvenile	15152	180	N	Y	2016
46041	1707	Plethodontidae	*Plethodon*	*angusticlavius*	Boston Mountains	terrestrial	juvenile	239	20	N	N	2016
46042	1708	Plethodontidae	*Plethodon*	*angusticlavius*	Boston Mountains	terrestrial	juvenile	1524	231	N	N	2016
46043	1709	Plethodontidae	*Plethodon*	*angusticlavius*	Boston Mountains	terrestrial	juvenile	3449	424	N	N	2016
46044	1712	Plethodontidae	*Plethodon*	*angusticlavius*	Boston Mountains	terrestrial	juvenile	6836	155	NA	NA	2016
45984	1728	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	183	18	N	N	2016
45986	1730	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	juvenile	3643	242	N	N	2016
46027	1732	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	631	28	N	N	2016
46028	1733	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	3448	60	N	N	2016
45987	1736	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	juvenile	830	64	N	N	2016
45988	1737	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	880	72	N	N	2016

45989	1741	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	11585	152	N	N	2016
46023	1745	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	paedomorphic adult	11834	111	N	Y	2016
46029	1751	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	39164	787	N	N	2016
46024	1755	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	6185	193	N	N	2016
46045	1763	Plethodontidae	*Plethodon*	*angusticlavius*	Boston Mountains	terrestrial	juvenile	2	2	N	N	2016
46046	1764	Plethodontidae	*Plethodon*	*angusticlavius*	Boston Mountains	terrestrial	juvenile	6	4	N	N	2016
46047	1765	Plethodontidae	*Plethodon*	*angusticlavius*	Boston Mountains	terrestrial	juvenile	4	3	N	N	2016
46048	1766	Plethodontidae	*Plethodon*	*angusticlavius*	Boston Mountains	terrestrial	adult	1	1	N	N	2016
46049	1767	Plethodontidae	*Plethodon*	*angusticlavius*	Boston Mountains	terrestrial	adult	13	8	N	N	2016
46026	1768	Plethodontidae	*Plethodon*	*albagula*	Boston Mountains	terrestrial	juvenile	17	11	N	N	2016
46083	1774	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	10	6	NA	NA	2016
45990	1818	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	46	29	N	N	2016
45991	1819	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	3	3	N	N	2016
45992	1825	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	9	7	N	N	2016
45993	1826	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	9	9	N	Y	2016
46040	1828	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	202	28	NA	NA	2016
46030	2230	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	58978	2776	NA	NA	2016
46031	2231	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	4607	55	N	N	2016
46032	2232	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	13346	318	N	N	2016
46033	2233	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult	2802	83	N	N	2016
46034	2234	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	8678	179	N	N	2016
46035	2235	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	103626	388	N	N	2016

45994	2236	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	68964	582	Y	N	2016
45995	2237	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	51476	200	N	N	2016
45996	2238	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	61090	411	N	N	2016
45997	2239	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	14306	142	N	Y	2016
45998	2240	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	74216	267	N	N	2016
45977	2258	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	juvenile	12129	149	N	Y	2016
46084	2265	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	18540	256	N	Y	2016
46085	2266	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	567	9	N	N	2016
46086	2267	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	6477	111	N	Y	2016
45978	2277	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	13851	602	N	N	2016
45979	2278	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	6532	214	N	N	2016
45980	2279	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	juvenile	24715	574	N	Y	2016
46036	2296	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	2254	125	N	N	2016
46037	2297	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	3112	84	N	N	2016
46038	2298	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	7458	331	N	N	2016
46039	2299	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	1589	45	N	N	2016
45982	2300	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	15186	545	N	N	2016
46087	2301	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	18131	173	N	N	2016
46088	2302	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	6494	128	N	Y	2016
46089	2303	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	11035	151	N	Y	2016
46092	2306	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	98639	374	N	N	2016
46093	2307	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	10907	157	N	N	2016

46094	2309	Salamandridae	*Notophthalmus*	*viridescens*	Ozark Highlands	aquatic	adult	3301	79	NA	NA	2016
47329	2994	Salamandridae	*Notophthalmus*	*viridescens*	Ouachita Mountains	aquatic	adult	2347	37	NA	NA	NA
47906	3540	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	juvenile	1289	56	N	N	2018
47885	3541	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	45222	509	N	N	2018
47886	3542	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	13283	362	N	N	2018
47887	3547	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	paedomorphic adult	54351	478	N	Y	2018
47888	3548	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	paedomorphic adult	57314	300	N	N	2018
47889	3549	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	paedomorphic adult	66865	280	N	Y	2018
47890	3550	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	7449	176	N	N	2018
47891	3551	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	1129	88	N	N	2018
47892	3552	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	2146	149	N	N	2018
47872	3556	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	1024	86	N	N	2018
47873	3557	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	2217	104	N	N	2018
47893	3561	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	283	37	N	Y	2018
47902	3565	Plethodontidae	*Plethodon*	*angusticlavius*	Ozark Highlands	terrestrial	adult	455	41	N	N	2018
47903	3566	Plethodontidae	*Plethodon*	*angusticlavius*	Ozark Highlands	terrestrial	adult	9974	643	N	N	2018
47904	3567	Plethodontidae	*Plethodon*	*angusticlavius*	Ozark Highlands	terrestrial	adult	2404	150	N	N	2018
47905	3568	Plethodontidae	*Plethodon*	*angusticlavius*	Ozark Highlands	terrestrial	adult	1788	68	N	N	2018
47898	3569	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult	502	31	N	N	2018
47899	3570	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult	315	19	N	N	2018
47900	3571	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult	1112	67	N	N	2018
47901	3572	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	90292	487	N	N	2018

47883	3573	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	1409	75	N	N	2018
47884	3574	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	61565	670	N	N	2018
47896	3580	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	5997	226	N	N	2018
47874	3582	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	7523	337	N	N	2018
47875	3583	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	14635	377	N	N	2018
47876	3584	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	5561	85	N	N	2018
47877	3585	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	2293	99	N	N	2018
47878	3586	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	7900	177	N	N	2018
47879	3587	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	69581	529	N	N	2018
47880	3588	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	6772	162	N	N	2018
47881	3589	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	4851	340	N	N	2018
47882	3590	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	juvenile	2476	60	N	N	2018
47897	3594	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	69532	523	N	N	2018
	4173	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	juvenile	3	2	N	N	2021
	4177	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult or juvenile	1	1	N	N	2021
	4178	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	316	15	N	Y	2021
	4179	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult	482	10	N	N	2021
	4180	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	183	9	N	Y	2021
	4181	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	4524	213	N	Y	2021
	4194	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	727	56	N	N	2021
	4196	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	351	8	N	N	2021
	4197	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	5323	122	N	N	2021

4198	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	1112	62	N	Y	2021
4199	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	13	12	N	N	2021
4202	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	478	25	N	N	2021
4203	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	161	38	N	N	2021
4205	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	12478	71	N	N	2021
4207	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	152	22	N	N	2021
4208	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	201	23	N	N	2021
4209	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	186	33	N	N	2021
4210	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	terrestrial	adult or juvenile	700	45	N	N	2021
4273	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	515	28	N	N	2021
4274	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	276	20	N	N	2021
4275	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	54591	169	N	N	2021
4276	Plethodontidae	*Eurycea*	*tynerensis*	Ozark Highlands	aquatic	larvae	239	13	N	N	2021
4277	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	juvenile	173	17	N	N	2021
4278	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult	168	17	N	N	2021
4281	Plethodontidae	*Eurycea*	*lucifuga*	Ozark Highlands	terrestrial	adult	903	31	N	N	2021
4289	Plethodontidae	*Plethodon*	*albagula*	Ozark Highlands	terrestrial	adult or juvenile	347	12	N	N	2021
4290	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult or juvenile	489	30	N	N	2021
4291	Plethodontidae	*Eurycea*	*longicauda*	Ozark Highlands	terrestrial	adult or juvenile	1597	94	N	N	2021

Supplementary Table 2. Differentially abundant antifungal microbes between animals based on *Bd* status. Negative log_2 fold change values indicate a greater abundance of that microbe in animals without *Bd*, and positive ones indicate greater abundance in animals with *Bd*.

Name	Family	Genus	Species	Base Mean	log_2 Fold Change	Standard Error	Test Statistic	*p*-value	Adjusted *p*-value
Craugastorcrassidigitus-inhibitory_91	Pseudomonadaceae	*Pseudomonas*	unknown	2.502	-2.471	0.676	12.747	0.0003	0.006
Dendrobatesauratus-inhibitory_2	Pseudomonadaceae	*Pseudomonas*	*fragi*	8.740	-2.465	0.539	17.368	3.08E-05	0.0009
Smiliscasordida-inhibitory_34	Pseudomonadaceae	*Pseudomonas*	unknown	1.770	-2.862	0.868	10.926	0.0009	0.010
Atelopuselegans-ns_15	Oxalobacteraceae	*Cupriavidus*	unknown	5.307	-3.415	0.697	19.406	1.06E-05	0.0005
Colostethuspanamensis-inhibitory_8	Moraxellaceae	*Acinetobacter*	unknown	0.977	3.418	1.211	11.786	0.0006	0.008
Smiliscasordida-inhibitory_4	Oxalobacteraceae	unknown	unknown	14.528	1.923	0.677	8.685	0.003	0.028
Hemidactylumscutatum-inhibitory_18	Oxalobacteraceae	*Janthinobacterium*	unknown	2.329	-2.308	0.760	8.925	0.003	0.027
Ranamuscosa-inhibitory_37	Comamonadaceae	unknown	unknown	2.973	3.991	0.672	36.694	1.38E-09	1.22E-07
Litorianannotis-inhibitory_54	Enterobacteriaceae	unknown	unknown	7.742	2.089	0.628	12.094	0.0005	0.007
Hemidactylumscutatum-inhibitory_15	Micrococcaceae	*Arthrobacter*	unknown	4.068	-3.337	0.754	16.423	5.07E-05	0.001

Supplementary Table 3. Differentially abundant microbes without antifungal properties related to on *Bd*-status. Microbes with a negative log_2 fold change are more abundant in animals without *Bd* present, and ones with a positive log_2 fold change are more abundant in animals with *Bd* found in the sample.

Family	**Genus**	**Species**	**Base Mean**	**log_2 FoldChange**	**Standard Error**	**Test Statistic**	***p*-value**	**Adjusted *p*-value**
Neisseriales	*Vogesella*	*fluminis*	7.736	4.566	0.953	19.419	1.05E-05	0.016393
Comamonadaceae	*Ideonella*	*paludis*	2.086	4.277	1.034	22.504	2.10E-06	0.003931
Comamonadaceae	*Rhizobacter*	unknown	67.862	3.745	0.696	28.732	8.31E-08	0.000195
Comamonadaceae	*Rhizobacter*	unknown	317.617	6.075	0.635	78.146	9.57E-19	8.97E-15
Comamonadaceae	*Rhizobacter*	unknown	3.617	4.090	0.774	29.246	6.37E-08	0.000195
Peptostreptococcaceae	*Romboutsia*	*sedimentorum*	18.197	4.387	0.768	32.632	1.11E-08	5.22E-05

Supplementary Table 4. Differentially abundant microbes between salamander families Plethodontidae and Salamandridae. Microbes identified to species or strain when available. Microbes with a negative log_2 fold change are more abundant in Plethodontidae, and vice versa.

Family	Genus	Species	Base Mean	log_2 Fold Change	Standard Error	Test Statistic	*p*-value	Adjusted *p*-value
Moraxellaceae	*Acinetobacter*	NEGJ	18.527	18.527	-4.254	0.582	3.93E-09	1.51E-07
Ralstonia	*Polynucleobacter*	*sphagniphilus*	6.08277858	6.083	3.965	0.720	5.32E-09	1.82E-07
Bacteroidaceae	*Bacteroides*	unknown	2.27424302	2.274	-4.672	1.307	0.001	0.011
Desulfovibrionaceae	*Desulfovibrio*	unknown	1.58544499	1.585	-4.107	1.378	0.004	0.037
Bacteroidaceae	*Bacteroides*	unknown	1.89690959	1.897	-4.427	1.229	0.0007	0.008
Moraxellaceae	*Acinetobacter*	LSZI	17.0917601	17.092	3.431	0.660	1.63E-07	5.07E-06
Pseudomonadaceae	*Pseudomonas*	*graminis*	6.57241577	6.572	-4.529	0.736	2.85E-07	8.08E-06
Pseudomonadaceae	*Pseudomonas*	*peli*	3.7832676	3.783	-5.056	1.064	3.77E-05	0.0007
Pseudomonadaceae	*Pseudomonas*	*thivervalensis*	4.69798566	4.698	-3.941	0.757	7.01E-06	0.0001
Micrococcaceae	*Pseudarthrobacter*	*oxydans*	3.39662443	3.397	-2.845	0.820	0.002	0.018
Alcaligenaceae	*Bordetella*	unknown	3.03726309	3.037	-5.012	1.251	0.0004	0.006
Flavobacteriaceae	*Flavobacterium*	PEFE	3.76047914	3.760	-2.889	0.804	0.002	0.018
Pseudomonadaceae	*Pseudomonas*	*batumici*	14.8093713	14.809	-2.364	0.534	8.06E-05	0.001
Porphyromonadaceae	*Parabacteroides*	unknown	1.87843545	1.878	-4.418	1.088	0.0001	0.002
Comamonadaceae	*JN679217*	DQ520187	3.66465562	3.665	5.456	0.875	9.22E-12	5.46E-10
Oxalobacteraceae	*Undibacterium*	HQ111154	39.2082065	39.208	4.891	0.578	1.05E-16	1.14E-14
Comamonadaceae	AF418942	AF418942	3.41695755	3.417	1.821	0.708	0.005	0.044
Comamonadaceae	AF418942	AF418942	2.06184352	2.062	-3.670	1.083	0.003	0.027
Pseudomonadaceae	*Pseudomonas*	*coleopterorum*	3.39058618	3.391	-3.252	0.766	0.0001	0.002

Fusobacteriaceae	*Cetobacterium*	*somerae*	2.37278294	2.373	4.162	1.194	6.30E-05	0.001
Comamonadaceae	*Rhizobacter*	unknown	87.9024838	87.902	8.830	0.404	1.88E-75	6.13E-73
Peptostreptococcaceae	*Romboutsia*	*sedimentorum*	17.6017191	17.602	4.105	0.624	2.81E-11	1.41E-09
Oxalobacteraceae	*Duganella*	FODC	2.86788606	2.868	-4.810	0.794	7.28E-08	2.37E-06
Alishewanella	*Rheinheimera*	LXWK	1.77183201	1.772	-4.324	1.060	0.0001	0.002
Flavobacteriaceae	*Flavobacterium*	*fluvii*	1.34230097	1.342	-3.925	1.088	0.0005	0.006
Oxalobacteraceae	*Herbaspirillum*	*rhizosphaerae*	2.3329191	2.333	2.969	0.892	0.00013816	0.00225206
Neisseriaceae	*Vogesella*	*mureinivorans*	14.8678164	14.868	5.944	0.664	9.45E-18	1.23E-15
Neisseriaceae	*Deefgea*	*chitinilytica*	6.12877553	6.129	-3.979	0.956	0.0005	0.007
Comamonadaceae	*Sphaerotilus*	*hippei*	0.99696528	0.997	2.862	1.304	0.005	0.044
Oxalobacteraceae	*Massilia*	*eurypsychrophila*	2.95001834	2.950	-4.281	0.868	1.01E-05	0.0002
Oxalobacteraceae	*Undibacterium*	*aquatile*	12.4692314	12.469	4.422	0.615	4.09E-13	3.81E-11
Flavobacteriaceae	*Flavobacterium*	EU431729	1.11151719	1.112	-3.683	1.087	0.0007	0.009
Comamonadaceae	*Acidovorax*	DF238959	1.30893655	1.309	-3.399	0.920	0.0003	0.004
Comamonadaceae	*Albidiferax*	NOXW	1.98207903	1.982	1.983	0.817	0.005	0.042
Comamonadaceae	*Acidovorax*	*defluvii*	2.89791811	2.898	3.145	0.763	4.36E-06	0.0001
Pseudomonadaceae	*Pseudomonas*	*jessenii*	22.9283762	22.928	-2.130	0.563	0.0007	0.008
Clostridiaceae	*Clostridium*	unknown	3.47435959	3.474	4.814	0.907	4.48E-09	1.62E-07
Pseudomonadaceae	*Pseudomonas*	LMOA	1.81293501	1.813	-2.911	0.992	0.005	0.044
Comamonadaceae	*Rhizobacter*	unknown	40.6450917	40.645	8.413	0.579	3.36E-35	7.31E-33
Comamonadaceae	*Rhodoferax*	CP019236	2.47601431	2.476	2.666	0.780	9.80E-05	0.002
Comamonadaceae	*Acidovorax*	*valerianellae*	1.32046423	1.320	-3.264	1.016	0.002	0.018
Peptostreptococcaceae	*Paraclostridium*	*benzoelyticum*	2.01696681	2.017	3.900	0.722	1.03E-09	4.22E-08
Fusobacteriaceae	GQ850557	GQ850557	1.80243482	1.802	4.217	1.029	1.12E-06	3.03E-05

Flavobacteriaceae	*Flavobacterium*	*buctense*	2.6001516	2.600	4.334	1.083	5.18E-06	0.0001
Moraxellaceae	*Acinetobacter*	NEFZ	9.03817308	9.038	1.733	0.579	0.002	0.017
Moraxellaceae	*Acinetobacter*	NGCT	12.3437496	12.344	4.149	0.897	6.74E-06	0.0001
Bacillaceae	*Bacillus*	*muralis*	1.13294281	1.133	-2.875	0.950	0.002	0.023
Moraxellaceae	*Acinetobacter*	*celticus*	2.17792052	2.178	5.347	1.490	0.004	0.038
Moraxellaceae	*Acinetobacter*	NHRO	3.21961319	3.220	5.969	1.397	0.00113855	0.012
Pseudomonadaceae	*Pseudomonas*	*granadensis*	25.3014969	25.301	-2.473	0.481	5.59E-06	0.0001
Comamonadaceae	*Variovorax*	OCMW	1.17627763	1.176	-3.274	1.068	0.002	0.021
Lachnospiraceae	*Epulopiscium*	unknown	1.164105	1.164	3.802	1.367	0.0004	0.006
Comamonadaceae	*Comamonas*	*thiooxydans*	1.55640094	1.556	3.438	1.462	0.005	0.041
Erwiniaceae	*Erwinia*	*endophytica*	2.53640765	2.536	-2.986	0.914	0.003	0.031
Pseudomonadaceae	*Pseudomonas*	PDJN	18.2294931	18.229	-2.379	0.618	0.0006	0.008
Methylophilaceae	HQ827934	LN794158	0.8088923	0.809	3.315	1.213	0.0003	0.004
Pseudomonadaceae	*Pseudomonas*	*lurida*	23.535501	23.536	-2.171	0.564	0.0006	0.007
Methylophilaceae	JCKJ	FJ665204	6.28565559	6.286	6.806	1.304	1.35E-05	0.0003
Comamonadaceae	*Sphaerotilus*	*hippei*	1.1982487	1.198	3.658	1.640	0.006	0.050
Comamonadaceae	*Comamonas*	BAEC	1.52614334	1.526	3.594	1.398	0.002	0.019
Neisseriaceae	*Aquitalea*	*pelogenes*	6.0863584	6.086	5.841	1.015	1.16E-06	3.03E-05
Neisseriaceae	*Vogesella*	*fluminis*	14.7336597	14.734	4.840	0.727	2.70E-11	1.41E-09
Flavobacteriaceae	*Flavobacterium*	*succinicans*	7.7378491	7.738	-5.467	0.722	7.49E-10	3.25E-08
Neisseriaceae	*Aquitalea*	*denitrificans*	7.63580201	7.636	9.207	0.859	2.58E-12	2.10E-10
Pseudomonadaceae	*Pseudomonas*	*endophytica*	13.5556679	13.556	-3.213	0.569	1.48E-06	3.72E-05
Comamonadaceae	*Limnohabitans*	CP011834	1.12065639	1.121	3.804	1.308	0.0002	0.003
Microbacteriaceae	*Rathayibacter*	unknown	3.75162597	3.752	-5.170	1.254	0.0005	0.007

Flavobacteriaceae	*Flavobacterium*	*terrigena*	2.80948788	2.809	-4.874	0.825	2.47E-07	7.31E-06
Comamonadaceae	*Ideonella*	*paludis*	3.3694293	3.369	4.924	0.846	1.80E-10	8.40E-09
Bacteroidaceae	*Bacteroides*	unknown	1.57275744	1.573	-4.171	1.301	0.002	0.019
Alcaligenaceae	*Orrella*	unknown	0.59123314	0.591	2.894	1.315	0.002	0.023
Pseudomonadaceae	*Pseudomonas*	*umsongensis*	8.66532344	8.665	-2.695	0.690	0.0006	0.008
Peptostreptococcaceae	*Romboutsia*	EU089965	12.0860824	12.086	6.436	0.587	1.34E-26	2.18E-24
Porphyromonadaceae	*Parabacteroides*	unknown	2.15633226	2.156	-4.606	1.153	0.0002	0.003
Comamonadaceae	*Curvibacter*	*gracilis*	6.14789315	6.148	5.293	0.787	3.98E-12	2.89E-10
Comamonadaceae	*Leptothrix*	*discophora*	8.32534066	8.325	5.200	0.784	8.11E-12	5.29E-10
Flavobacteriaceae	*Flavobacterium*	*fluminis*	1.46359032	1.464	-3.352	0.987	0.0009	0.011
Comamonadaceae	*Rhizobacter*	unknown	361.06383	361.064	9.432	0.400	2.35E-84	1.53E-81
Bacteroidaceae	*Bacteroides*	unknown	1.89784262	1.898	-4.257	1.205	0.0008	0.010