Identifying Patterns in the Culturable Skin Microbiome of Appalachian Salamanders

Bailey Burns, John Griffith, Gabrielle Russell, Fantasia Erdman, Megan Wharton, Donald Walker, and Aubree J. Hill

Department of Biology, Tennessee Technological University

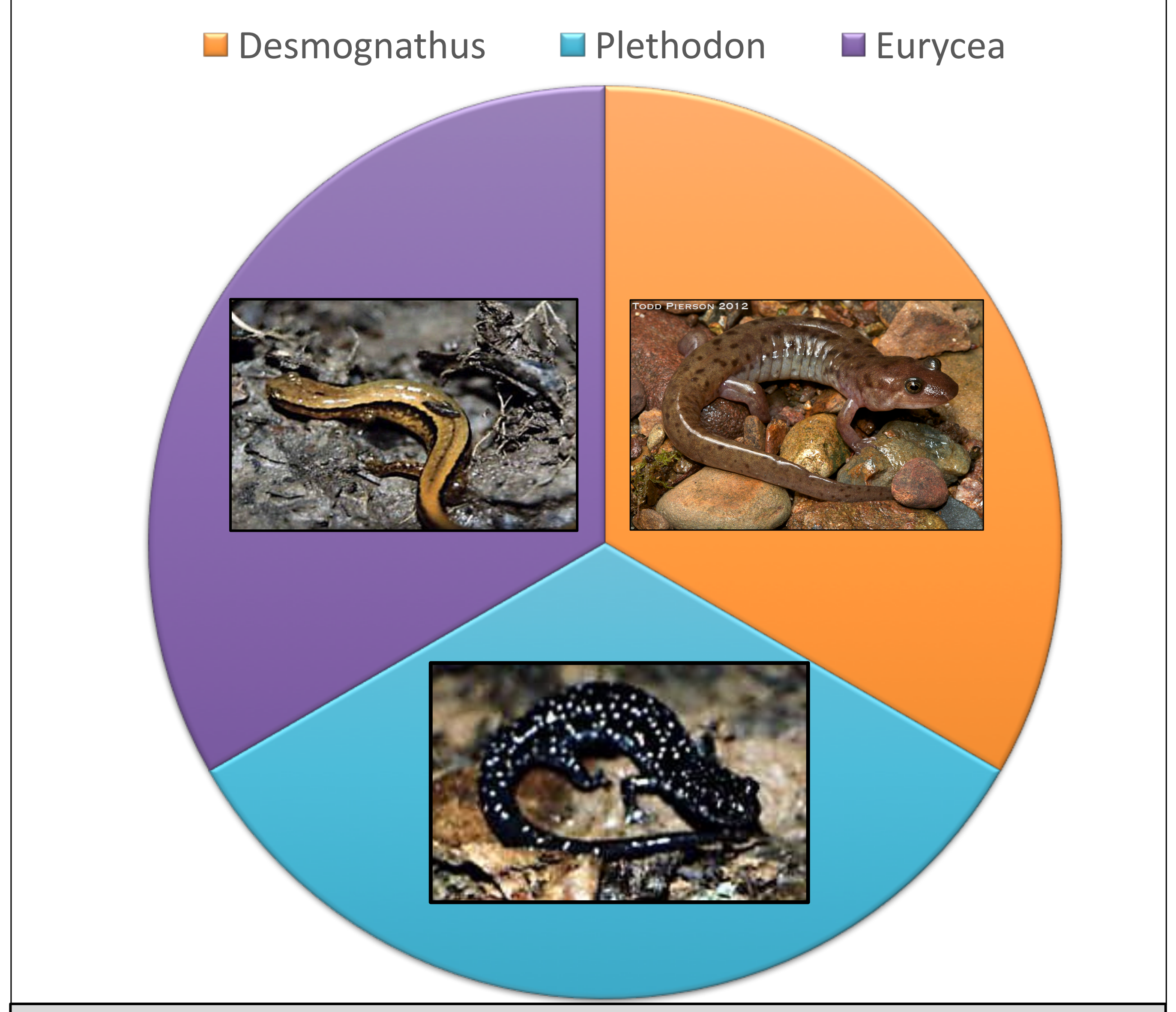

Figure 1. Salamander genera targeted for skin swab sampling (n=7 for each genus).

Introduction

Research interest in the cutaneous microbiome of amphibians has grown in recent years due to the emergence of the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) that has caused a worldwide disease epidemic.[1,2] Frog populations in the eastern United States exhibit a significantly higher rate of Bd infection compared to co-occurring salamander populations.[3] Our previous work focused on finding probiotic species of bacteria within the skin microbiome of salamanders that may explain their low rates of infection. We isolated hundreds of bacterial colonies from skin swabs collected from salamanders belonging to the genera *Plethodon, Desmognathus,* and *Eurycea* (Fig. 1). We found 16 isolates that possess anti-Bd properties. However, further investigation is needed to understand the feasibility of applying these probiotics as therapeutic treatments *in* vivo.[4]

The purpose of our current research was to identify patterns within the culturable skin microbiome across host taxa and habitats, and to pinpoint the best candidate probiotic species, specifically, those which occur on a variety of host taxa and can be consistently cultured in a laboratory setting.[3-5] Research objectives were to **(1)** identify all bacteria isolated from 21 skin swabs collected at two sites in the Great Smoky Mountains National Park, **(2)** identify potential effects of genus and/or site on culturable microbiota, and **(3)** determine what portion of the *total* microbiome is culturable in a laboratory setting. Data from a next-generation DNA sequencing (NGS) project were used as a proxy for the "total microbiome." We hypothesized we would detect differences in the culturable microbiome based on host taxon and geography, as these results were found during the NGS project.

Methods

- **Objective 1:**
  - Bacterial isolates were identified to genus level using direct-colony PCR amplification and Sanger sequencing of the 16S rRNA gene.
  - ChromasPro was used to edit sequences, which were compared to the National Center for Biotechnology Information Basic Local Alignment Search Tool (BLAST).
- **Objective 2:**
  - Isolate presence/absence data were transformed into a Bray-Curtis resemblance matrix using PRIMER ver. 7.0.9.
  - A two-way permuted multivariate analysis of variance (PERMANOVA) was used to identify potential effects of salamander genus or collection site.
  - Non-metric multidimensional scaling (NMDS) was used to plot communities separately for these two variables.
- **Objective 3:**
  - Total number of isolates per sample were compared to the NGS dataset (which included entire skin communities) to calculate the fraction of the community that was culturable.

Results

- **Objective 1:**
  - We collected a total of 110 unique isolates from the skin swabs, and obtained 102 genotypes representing 34 bacterial genera.
  - The top eight most commonly-isolated genera are listed in Table 1. Four of these genera exhibited inhibition of Bd during previous work.
- **Objective 2:** We found no statistically significant difference within the culturable microbiome when comparing different sites and salamander genera (Table 2; Fig. 2).
- **Objective 3:** On average, we isolated just under 0.04% (std. dev. = 0.23%) of the total number of bacteria found during the NGS project.

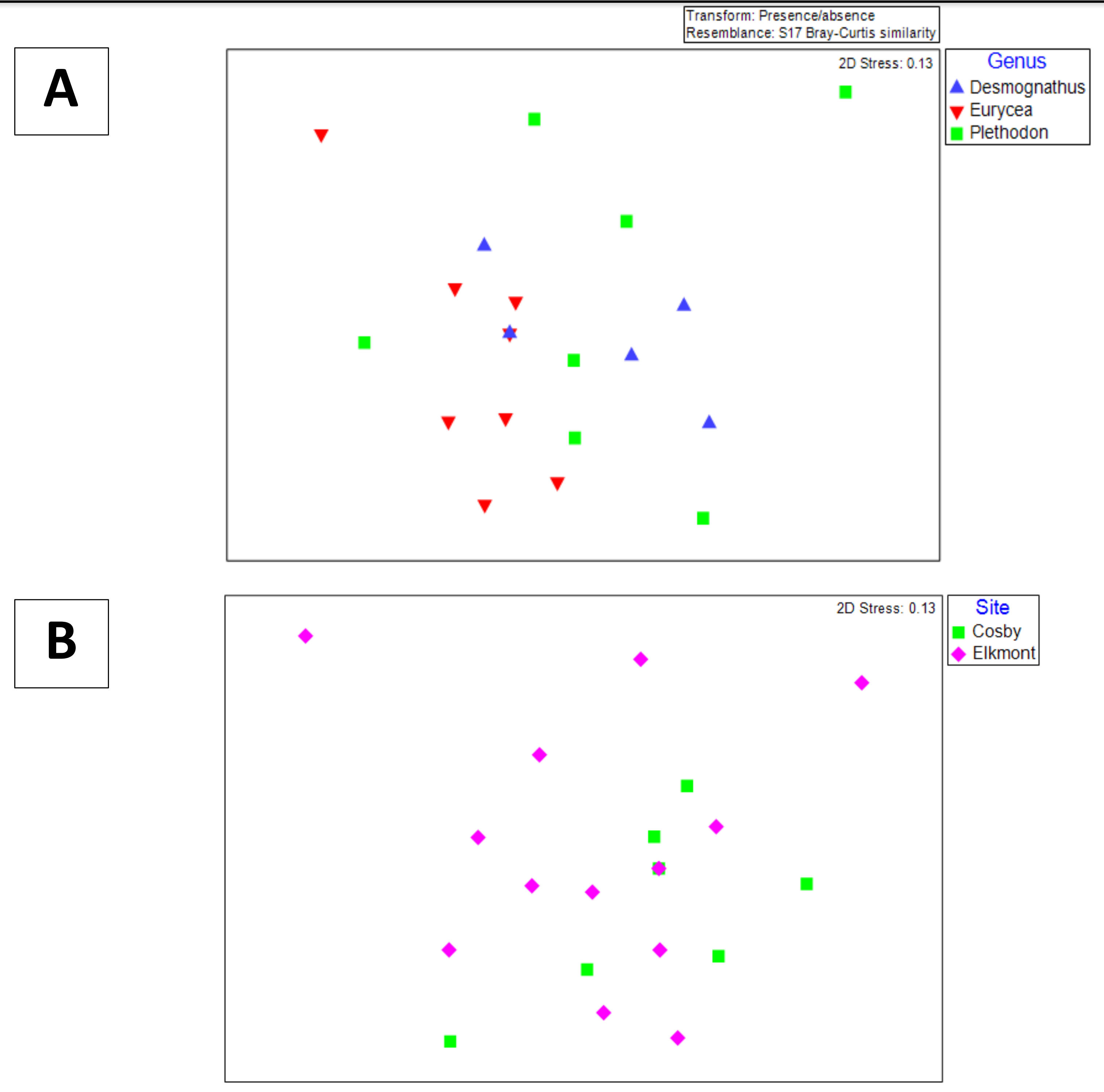

Figure 2. Two-dimensional NMDS ordinations of culturable skin communities, plotted separately by a factor of **(A)** salamander genus and **(B)** collection site.

Table 1. Most common bacterial genera recovered from swabs, with number of isolates (n) in parentheses. Genera marked with asterisks exhibited inhibition against Bd.

Paenibacillus sp.	(n = 20)
Bacillus sp. *	(n = 17)
Methylobacterium sp.	(n = 8)
Burkholderia sp. *	(n = 7)
Streptomyces sp.	(n = 6)
Rhodococcus sp.*	(n = 5)
Luteibacter sp.	(n = 5)
*Collimonas sp.**	(n = 3)

Table 2. Statistical test results. A p(perm) value of < 0.05 indicates a significant effect.

Two-way PERMANOVA tests	**pseudo-F statistic**	**p (perm)-value**
Test for interaction between factors (Genus X Site)	1.743	0.060
Main effects: Genus	0.950	0.546
Main effects: Site	1.063	0.397

Discussion

Probiotic treatments are only feasible if they are normal, commensal symbionts of a broad range of amphibian taxa and are culturable in a lab setting. Our results indicate that regimes of culturable microbiota are similar across salamander species and habitats. Although only a fraction of a percent of the total community was recoverable, we were able to consistently isolate four bacterial genera that previously demonstrated anti-Bd properties, including *Burkholderia* spp., common residents of frogs and other distantly-related amphibians.[5] At a time when much attention and conservation efforts are aimed at finding viable probiotic treatment options for susceptible amphibian taxa, we hope our work has helped pinpoint the best candidate probiotic species. Further research will be needed to determine ecological roles of these bacteria within skin communities, and efficacy of probiotic treatments *in vivo.*

References

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