

Asterophryinae Skin Microbiome Project

Alexis Shulga, Brianna Correa, Marguerite Butler

Project Description

The skin and its microbiome are the first layer between an organism and its environment. In moist-skinned animals such as frogs, the skin mucosa is particularly sensitive to external factors which may vary by habitat or site. The microbiome may play an important functional role in mediating the interaction between the host and its environment [11]. Another intriguing role that the microbial community may play is providing some level of protection against pathogens, particularly fungal pathogens, including chytridiomycosis which is linked to mass extinction of many amphibian species [1]. Although skin microbiomes are being characterized, we still know very little about the causes of microbiome diversity, whether bacterial composition provides some functional role, comes directly from the local environment, or is related to host diversity [10, 8, 4, 5].

In 2014, the Butler Lab conducted a field expedition to Papua New Guinea, one the world's biggest hotspots for frog diversity and sampled closely related frogs at three sites that vary in ecology (terrestrial, shrub, stream), and live together in complex tropical communities [6]. Fortunately, these frogs were carefully collected and individually stored in ethanol providing a unique dataset to explore factors which structure skin microbiome using NextGen sequencing metabarcoding methods. By comparing microbial community structure across sites, microhabitats, and species, I will test the hypotheses that microbiome composition is structured by microhabitat use [2], influenced by functional benefit such as protection against pathogens [3], is site specific perhaps resulting from differences in the source population of microbes (e.g., soil microbial diversity), or is host specific [12].

Significance

There are over 7000 species of frogs which are widely regarded as critical indicators of ecosystem health, as all amphibians have moist skin, are sensitive to environmental changes, and better to reveal complex skin microbiome dynamics.

Microbiome studies are rapidly growing in number yet we still know little about whether community composition is an accident of circumstance or whether it serves important functions.

Are the skin bacteria and fungi simply a sample of the soil where they happen to live, or do the particular combination of microbes confer protection, such as inhibiting or outcompeting pathogens, anti-fungal protection, immune boosts [1]? Given the devastating role of emerging fungal pathogens [9], understanding the relationships between microbial and fungal diversity are critical.

Alternatively do microbiomes mediate the absorption of water, nutrients, and functional exchanges with the environment [7]? Comparing microbiomes of frogs that live together at the same site, but are found in the leaf litter vs. on plants vs. in the stream can reveal these associations [4, 5], lessons which are applicable to all species.

Finally my study will provide an important baseline for understanding the relationship between skin and gut microbiome, and for future studies using museum specimens which have been formalin-fixed. While challenging to sequence, unlocking this “biobank” will provide access to thousands of important historical and geographical samples.

References

- [1] Kieran A. Bates, Ulf Sommer, Kevin P. Hopkins, Jennifer M. G. Shelton, Claudia Wierzbicki, Christopher Sergeant, Benjamin Tapley, Christopher J. Michaels, Dirk S. Schmeller, Adeline Loyau, Jaime Bosch, Mark R. Viant, Xavier A. Harrison, Trenton W. J. Garner, and Matthew C. Fisher. Microbiome function predicts amphibian chytridiomycosis disease dynamics. *MICROBIOME*, 10(1), MAR 10 2022. doi: 10.1186/s40168-021-01215-6.
- [2] K. C. Bierlich, Carolyn Miller, Emelia DeForce, Ari S. Friedlaender, David W. Johnston, and Amy Apprill. Temporal and regional variability in the skin microbiome of humpback whales along the western antarctic peninsula. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*, 84(5), MAR 2018. doi: 10.1128/AEM.02574-17.
- [3] Molly C. Bletz, R. G. Bina Perl, and Miguel Vences. Skin microbiota differs drastically between co-occurring frogs and newts. *ROYAL SOCIETY OPEN SCIENCE*, 4(4), APR 2017. doi: 10.1098/rsos.170107.
- [4] Zachary Gajewski, Leah R. Johnson, Daniel Medina, William W. Crainer, Christopher M. Nagy, and Lisa K. Belden. Skin bacterial community differences among three species of co-occurring ranid frogs. *PEERJ*, 11, JUL 14 2023. doi: 10.7717/peerj.15556.
- [5] Liza Garcia-Recinos, Patricia A. Burrowes, and Maria Dominguez-Bello. The skin microbiota of *Eleutherodactylus* frogs: Effects of host ecology, phylogeny, and local environment. *FRONTIERS IN MICROBIOLOGY*, 10, NOV 6 2019. doi: 10.3389/fmicb.2019.02571.
- [6] Ethan C. Hill, Claire J. Fraser, Diana F. Gao, Mary J. Jarman, Elizabeth R. Henry, Bulisa Iova, Allen Allison, and Marguerite A. Butler. Resolving the deep phylogeny: Implications for early adaptive radiation, cryptic, and present-day ecological diversity of

- Papuan microhylid frogs. *Molecular Phylogenetics and Evolution*, 177:107618, 2022. doi: <https://doi.org/10.1016/j.ympev.2022.107618>. URL <https://www.sciencedirect.com/science/article/pii/S1055790322002317>.
- [7] Myra C. Hughey, Janelle A. Pena, Roberto Reyes, Daniel Medina, Lisa K. Belden, and Patricia A. Burrowes. Skin bacterial microbiome of a generalist puerto rican frog varies along elevation and land use gradients. *PEERJ*, 5, AUG 29 2017. doi: 10.7717/peerj.3688.
 - [8] Ariel Kruger. Frog skin microbiota vary with host species and environment but not chytrid infection. *FRONTIERS IN MICROBIOLOGY*, 11, JUN 24 2020. doi: 10.3389/fmicb.2020.01330.
 - [9] Lionel Schilliger, Clement Paillusseau, Camille Francois, and Jesse Bonwitt. Major emerging fungal diseases of reptiles and amphibians. *PATHOGENS*, 12(3), MAR 2023. doi: 10.3390/pathogens12030429.
 - [10] Francois-Etienne Sylvain, Aleicia Holland, Sidki Bouslama, Emie Audet-Gilbert, Camille Lavoie, Adalberto Luis Val, and Nicolas Derome. Fish skin and gut microbiomes show contrasting signatures of host species and habitat. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*, 86(16), AUG 2020. doi: 10.1128/AEM.00789-20.
 - [11] T. S. Troitsky, V. N. Laine, and T. M. Lilley. When the host’s away, the pathogen will play: the protective role of the skin microbiome during hibernation. *ANIMAL MICROBIOME*, 5(1), DEC 21 2023. doi: 10.1186/s42523-023-00285-1.
 - [12] Yiluan Zheng, Jianlu Shi, Qi Chen, Chao Deng, Fan Yang, and Ying Wang. Identifying individual-specific microbial dna fingerprints from skin microbiomes. *FRONTIERS IN MICROBIOLOGY*, 13, OCT 6 2022. doi: 10.3389/fmicb.2022.960043.