Microbiome Mediators: Elevation and Toxicity-Driven in Frog Skin Microbiomes

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INTRODUCTION

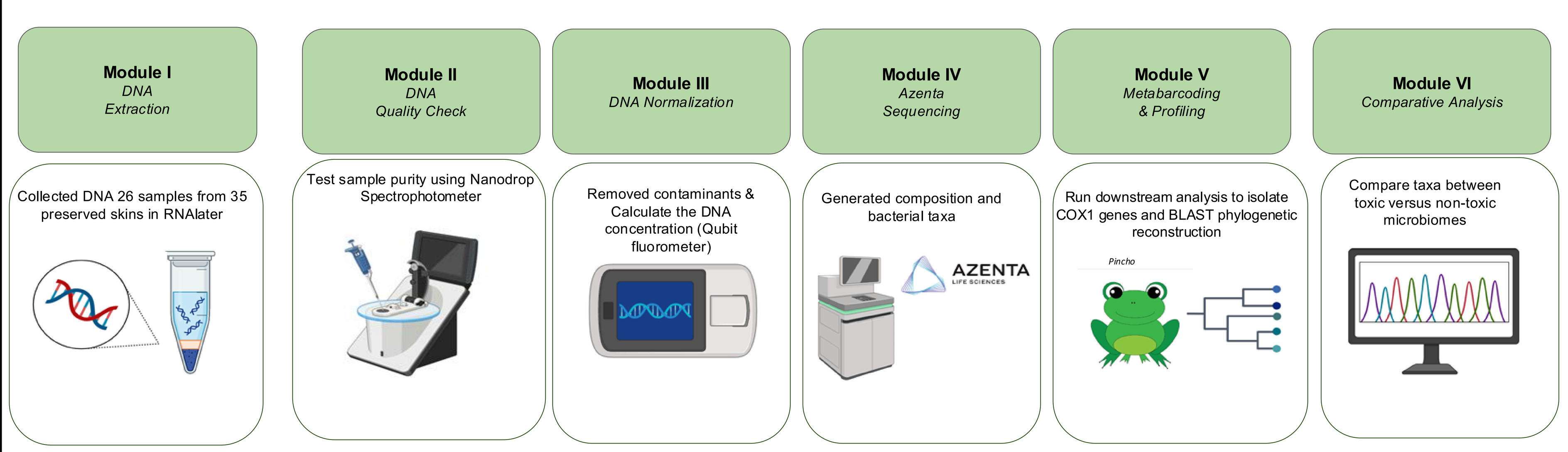
Abstract:

Amphibian skin microbiomes help defend against *Batrachochytrium dendrobatidis* (Bd), but their structure is shaped by elevation and host chemical defenses. Elevation imposes strong environmental filtering, while poison dart frogs (Dendrobatidae) sequester alkaloids that create a chemically distinct skin environment compared to non-toxic frogs (e.g., Hylidae). We compiled a database from >70 Neotropical species and found toxic frogs are enriched in antifungal/alkaloid-tolerant taxa (e.g., *Pseudomonas*, *Janthinobacterium*), while non-toxic frogs host more generalists, and highland species show more Bd-inhibitory, cold-adapted taxa than lowland frogs. We will test this using RNAlater-preserved skin samples across elevation (highland vs. lowland), toxicity, and Bd status with 16S rRNA sequencing (Azenta), ZymoBIOMICS extraction, and a Pincho pipeline, predicting elevation as the strongest driver and toxic hosts as having distinct alkaloid-associated microbiomes, with implications for Bd mitigation.

Introduction:

Amphibian skin microbiomes form complex and diverse bacterial communities influenced by both environmental gradients and host-associated traits. In Neotropical frogs, elevation represents a major ecological factor shaping microbial composition through changes in temperature, humidity, and habitat conditions, while host chemical ecology may further influence community structure through variation in skin-associated compounds. However, across many species, microbial assemblages are also strongly shaped by broad taxonomic patterns that include recurring dominance of bacterial groups such as Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria. This study focuses on several Neotropical frog species spanning lowland and highland environments. Lowland species such as *Epipedobates boulengeri* (western Colombia and northwestern Ecuador) and *Ameerega hahneli* (Amazon basin and Guiana Shield) host skin microbiota dominated by groups including Lactobacillales, Bacteroidales, Lactobacillus, and Salinispora, and are characterized by low alkaloid levels that do not significantly alter microbial community composition. *Epipedobates anthonyi,* found in southwestern Ecuador and northwestern Peru, contains microbial communities enriched in Pseudomonas, Acinetobacter, Chryseobacterium, Enterobacter, and Staphylococcus. In contrast, highland species such as *Colostethus pulchellus* (Andes of Ecuador and Colombia) show enrichment of Enterobacteriaceae, Pseudomonas, Aeromonas, and Staphylococcus, while *Hyloxalus nexipus* (Andean foothills of Ecuador and Peru) remains largely uncharacterized in terms of its skin microbiota. Across these species, microbial communities reflect both shared and variable taxonomic patterns across elevation and geography, providing a comparative framework to examine how environmental conditions and host-associated factors contribute to amphibian skin microbiome structure.

METHODS



Research Aim

Objective:

Examine elevation-driven differences in frog skin microbiomes

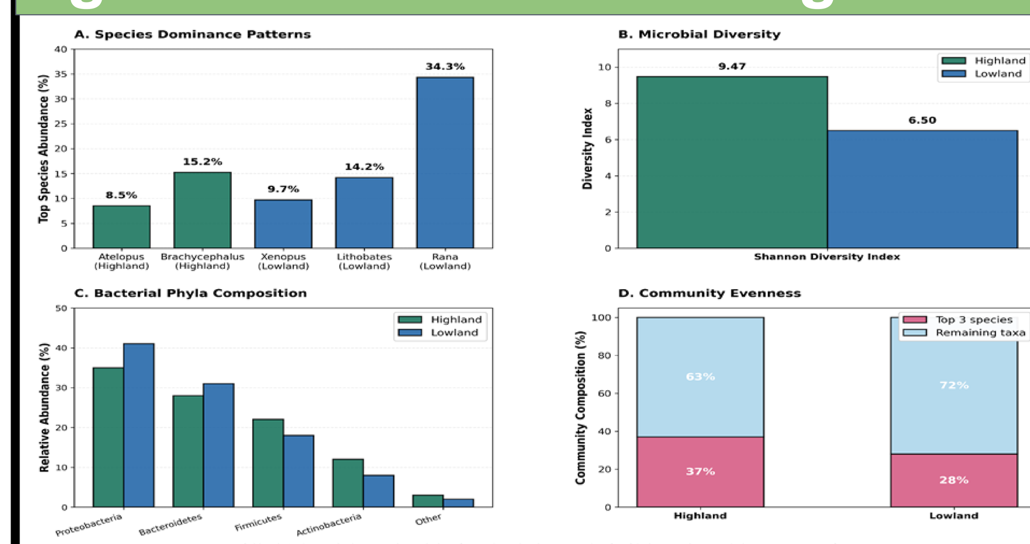
Why it matters:

Highland frogs may have better protection against chytridiomycosis (a deadly fungal disease)

What we are comparing:

Highland species (*Atelopus glyphus, Brachycephalus pitanga*) vs. Lowland species (*Xenopus laevis, Lithobates sphenocephalus*)

Highland VS Lowland Frog Skin Microbiome Comparison



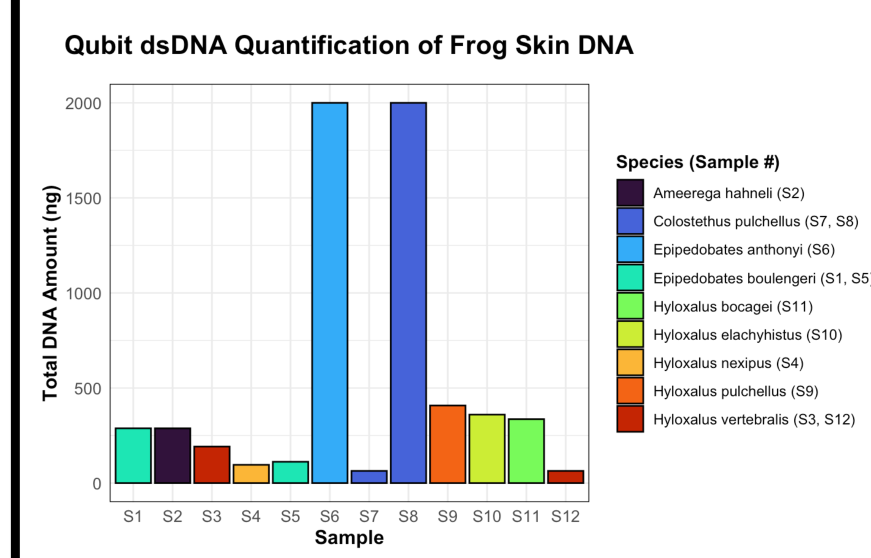
HIGHLAND ENVIRONMENTS (cool, dry, high UV):

High-elevation habitats are colder and drier, which slows down the growth of many harmful microbes. Lower humidity also makes it harder for fungi to spread, while stronger UV radiation selects for microbes that can tolerate stress. Together, these conditions tend to support more stable and balanced microbial communities. These environments may also favor bacteria like Actinobacteria that are known for producing antimicrobial compounds, adding an extra layer of protection on the skin.

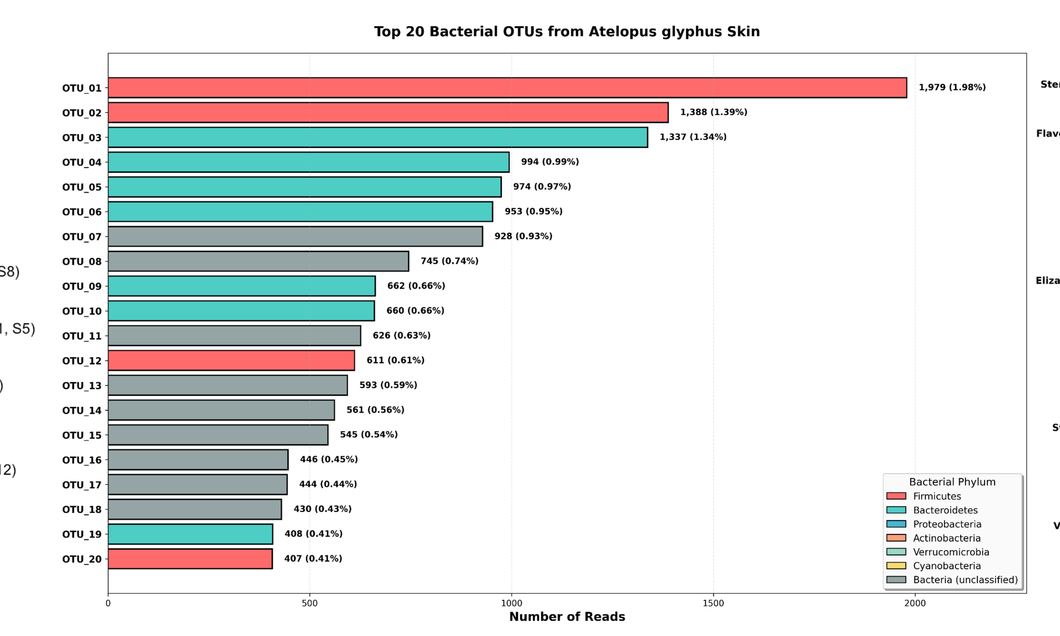
LOWLAND ENVIRONMENTS (warm, wet, lower UV):

Lowland habitats are warm and humid, which creates ideal conditions for fast microbial growth, including many opportunistic and environmental microbes. High moisture supports fungal growth and increases exposure to microbes from soil and water sources, making the community more variable and less stable. Because of this constant influx of microbes, lowland frog skin communities often show more fluctuation and lower overall stability, which can reduce how well the microbiome resists disturbance.

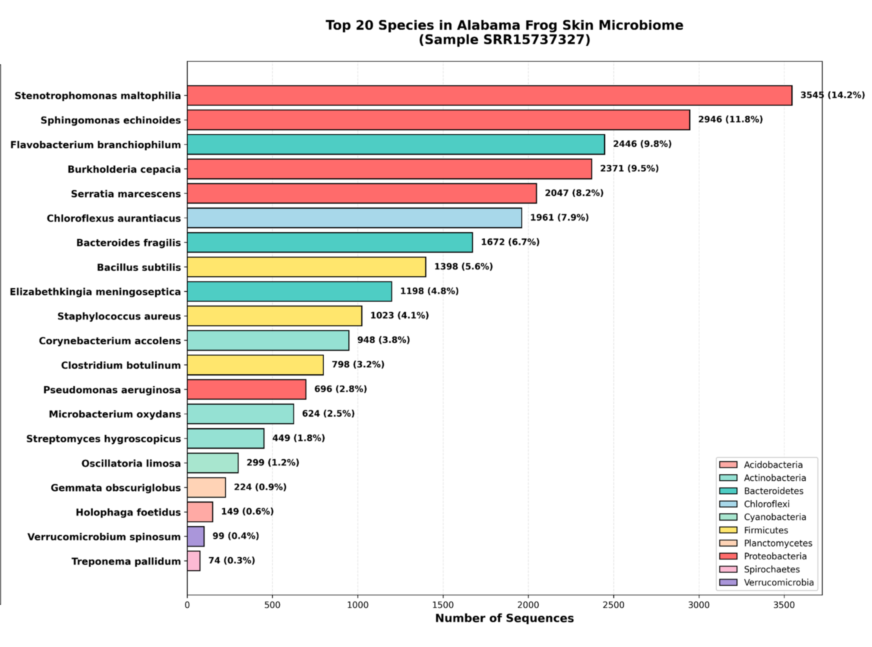
RESULTS



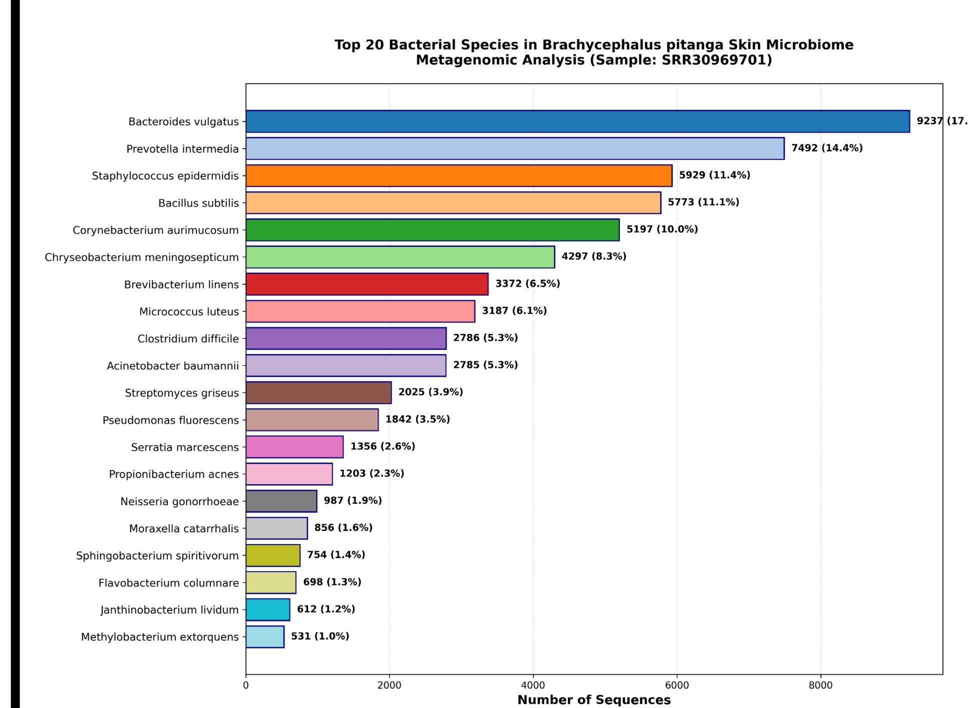
Qubit results



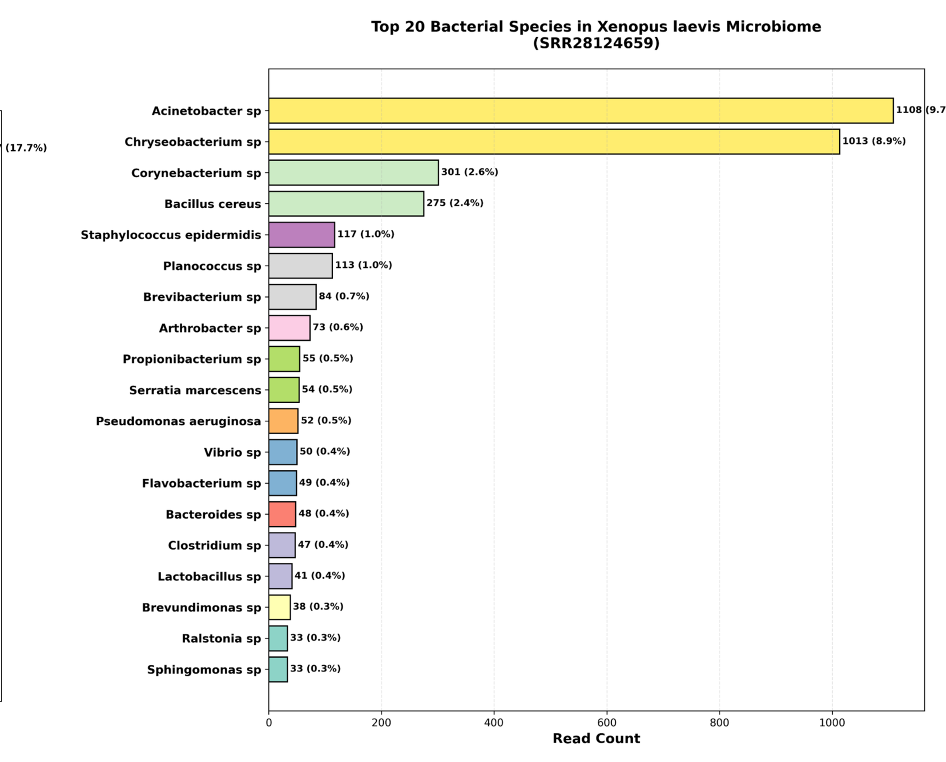
Atelopus glyphus skin microbiome



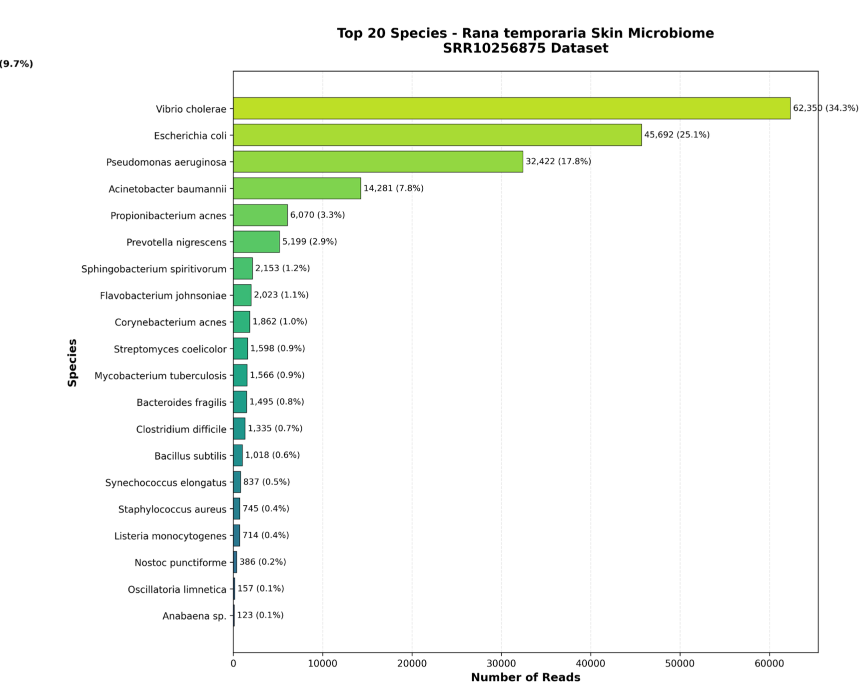
Lithobates sphenocephalus skin microbiome



Brachycephalus pitanga skin microbiome



Rana temporaria skin microbiome



Xenopus_laevis skin microbiome

DISCUSSION

Elevation significantly shapes frog skin microbiome composition and stability. Highland frogs maintain diverse, stable communities dominated by Actinobacteria (12%), which produce antimicrobial compounds against fungal pathogens, while lowland frogs exhibit variable microbiomes rich in Proteobacteria (41%) with weak pathogenic buffering. These findings suggest that cool, dry high-elevation environments select for protective bacterial communities, whereas warm, humid lowland habitats favor dynamic but vulnerable microbiomes. As climate change and habitat modification alter environmental conditions, frogs may lose the microbial defenses that protect against chytridiomycosis- a deadly fungal disease. Understanding these elevation-driven microbiome differences is critical for developing conservation strategies for amphibian populations threatened by disease and environmental change.

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