Living on Poison: Skin Microbiota Profiling in Toxic (Dendrobatidae) versus Non-Toxic (Hylidae) Frogs

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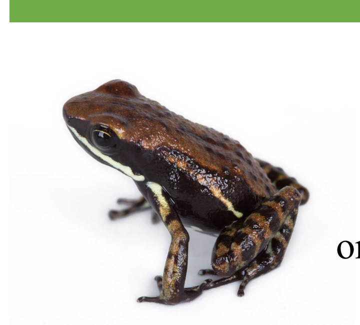
BACKGROUND

Figure 1: Images of poison dart frog and nontoxic frog



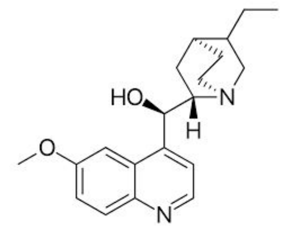
Epipedobates boulengeri **(toxic)**

Can branch out into organisms included in the diet (ants, mites, etc.)

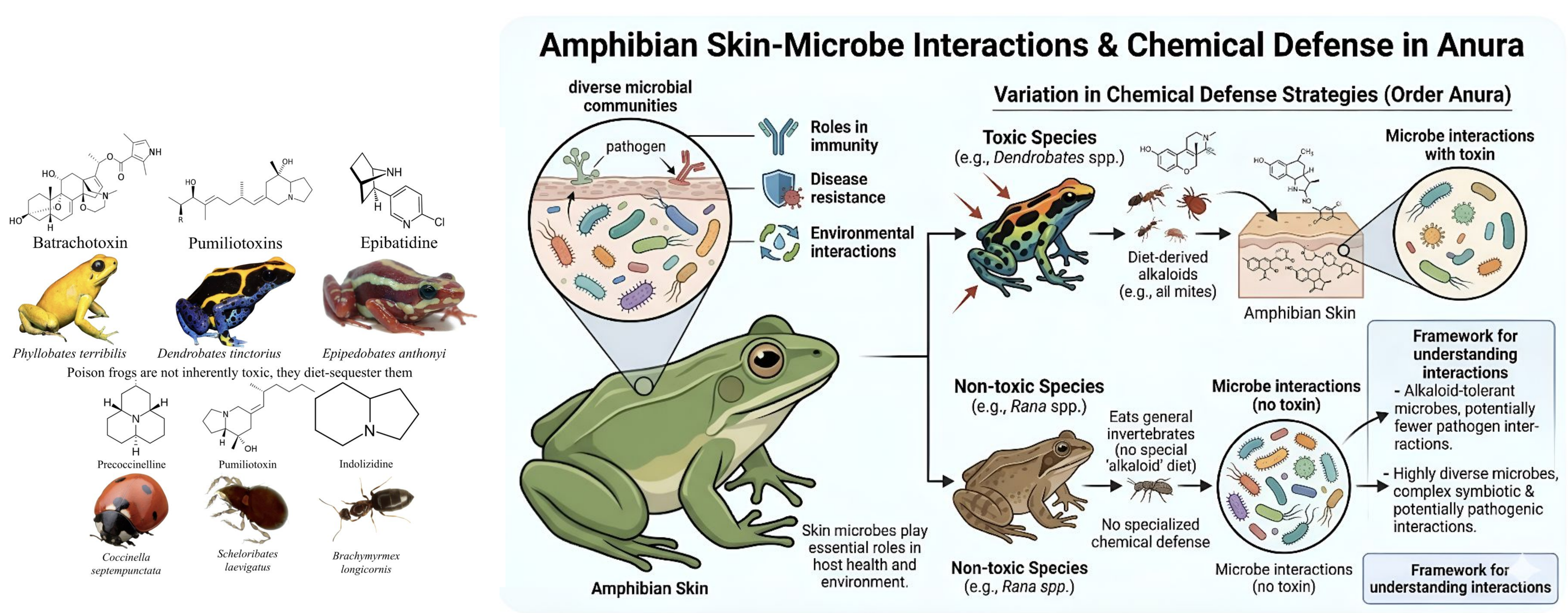
Hyloxalus vertebralis **(nontoxic)**

Can branch out into organisms included in the diet (ants, mites, etc.)

Figure 2: Alkaloid chemical structure DHQ



High-alkaloid toxic frogs show greater microbial functional diversity (e.g., metabolism genes). Alkaloids, such as DHQ, may create niches that promote specialized microbial communities



1. Amphibian Skin Microbiomes & Chemical Defense

- Anurans possess permeable skin that supports diverse microbial communities integral to host immune defense. Their skin microbiome contributes to pathogen resistance, establishing anurans as effective model organisms for studying mucosal immunity [1].
- Toxic frogs (Dendrobatidae) sequester alkaloids from an ant-rich diet, associated with increased diversity and specialization of the skin microbiome compared to non-toxic species. These dietary differences are key drivers in the evolution of chemical defense and aposematic coloration
- Differences between toxic and non-toxic frogs create distinct skin chemical environments that likely shape microbial community composition and function.
- Alkaloid-based defenses can also produce broad antimicrobial effects, potentially influencing which bacteria can persist on amphibian skin.

2. Neotropical Habitat, Alkaloids, and Disease Resistance

- Neotropical environments intensify host-microbe interactions due to high biodiversity, humidity, and strong pathogen pressure. [5][7]
- Alkaloids in poison frogs may increase microbial diversity and select for rare or metabolically specialized taxa rather than simply inhibiting growth. [5]
- Skin-associated microbes (e.g., Pseudomonas, Acinetobacter) can inhibit Batrachochytrium dendrobatidis (Bd), contributing to disease resistance. [6][7]
- Some skin-associated bacteria may metabolize or tolerate alkaloid compounds, suggesting functional adaptation to chemically defended hosts. [5]

3. Gaps in Anuran Toxicity Research

- The influence of host chemical defenses on amphibian skin microbiome structure and function remains poorly understood. [8]
- It is unclear how alkaloid-rich environments in toxic frogs select for microbial diversity, tolerance, or metabolic adaptation. [3][4]
- This study tests whether toxic frogs act as ecological filters shaping alkaloid-tolerant microbial communities, with implications for evolution, disease resistance, and conservation. [8]
- There is limited integration of chemical ecology, microbiology, and phylogenetic analysis in understanding amphibian host-microbe interactions. [8]

RESEARCH AIM

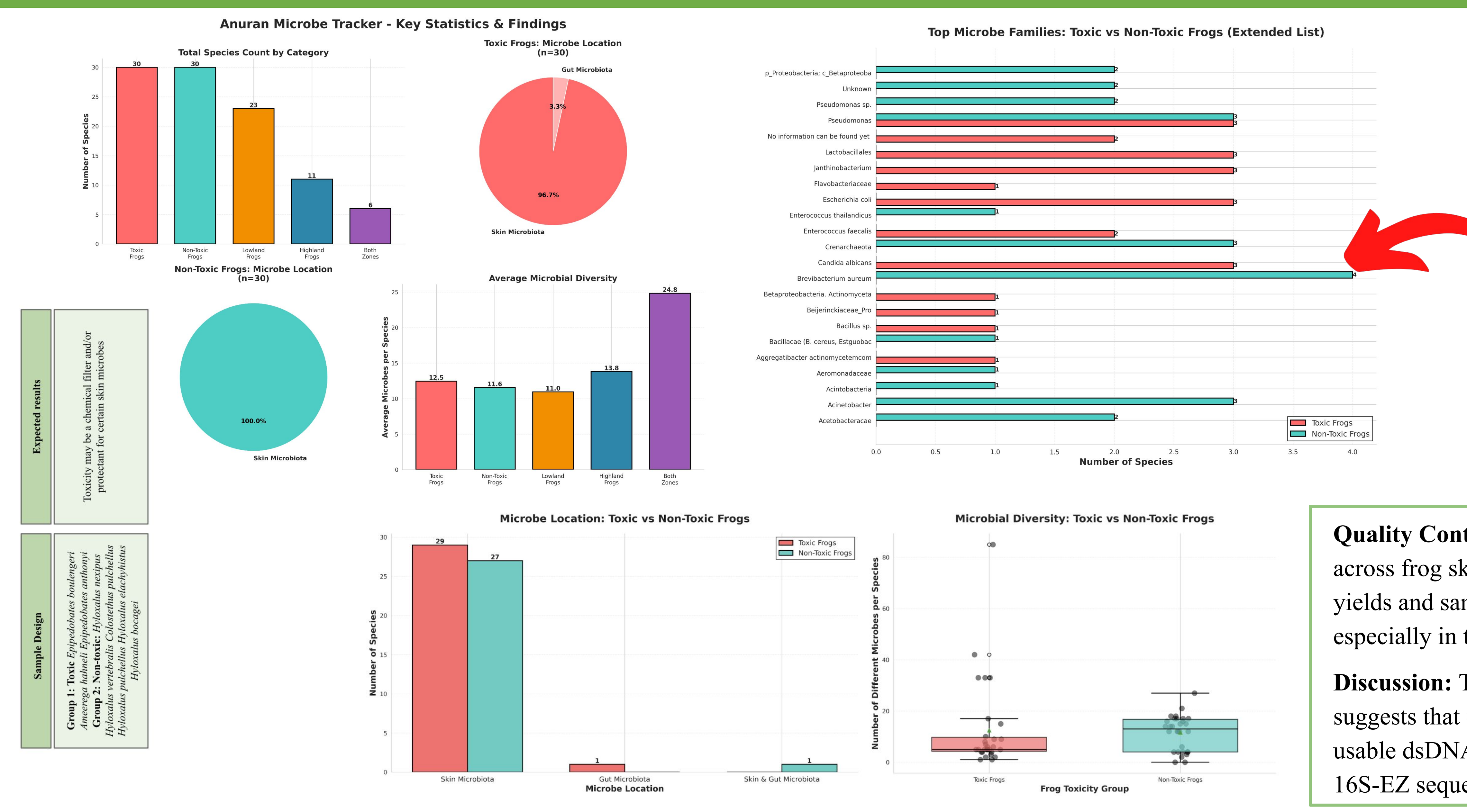
We ask: Do toxic, alkaloid-sequestering frogs have different skin-associated microbial communities than non-toxic or less chemically defended frogs?

Our aim: To compare the skin microbiomes of toxic and non-toxic frogs and determine whether alkaloid-based chemical defense is associated with differences in microbial diversity, composition, and bacterial community structure.

MATERIALS & METHODS

Module I *DNA Extraction*	Module II *DNA Quality Check*	Module III *DNA Normalization*	Module IV *Azenta Sequencing*	Module V *Metabarcoding & Profiling*	Module VI *Comparative Analysis*
Collected DNA 26 samples from preserved skins in RNAlater	Test sample purity using Nanodrop Spectrophotometer	Removed contaminants & Calculate the DNA concentration (Qubit fluorometer)	Generated composition and bacterial taxa	FASTQ parsing → quality control → taxonomic assignment → abundance quantification → visualization/statistical summary	Compare taxa between toxic versus non-toxic microbiomes

RESULTS & DISCUSSION

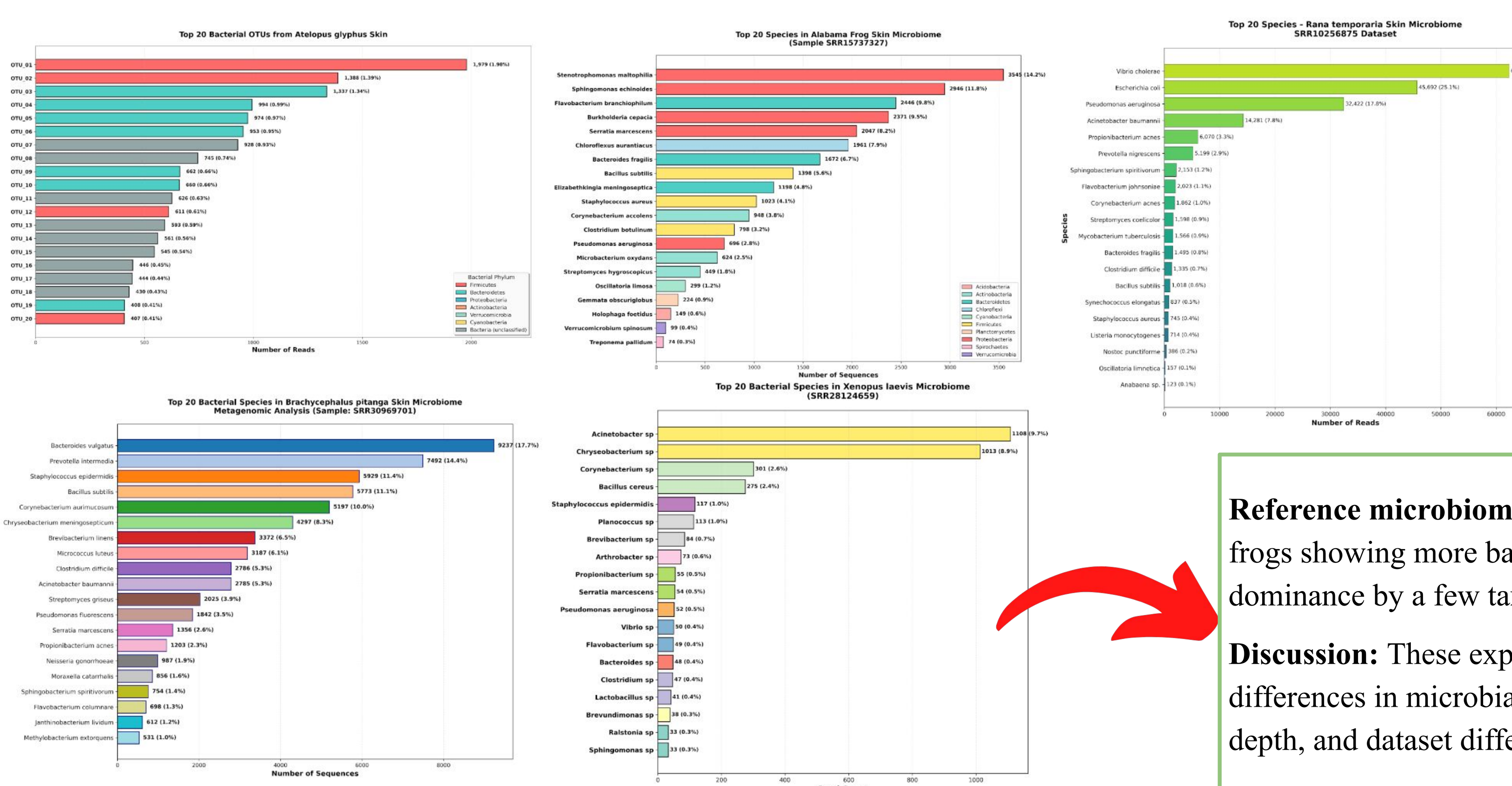


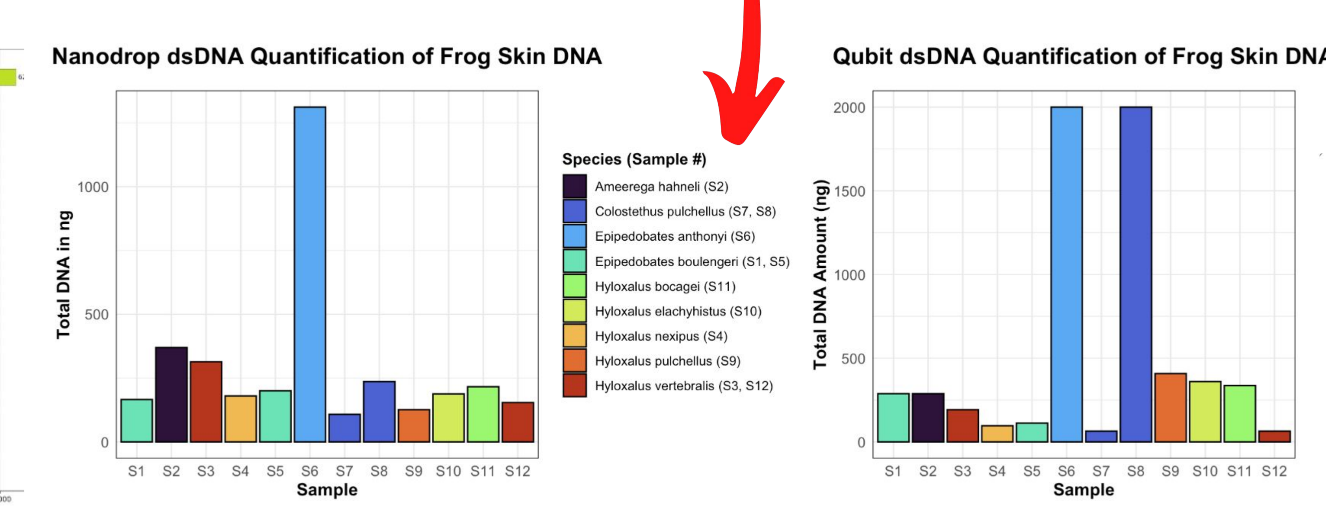
Anuran Microbe Tracker: Toxic and non-toxic frogs were both dominated by skin-associated microbiota, but microbial diversity and taxonomic composition varied across toxicity and habitat categories, with some non-toxic groups showing stronger dominance

Discussion: These preliminary comparisons suggest that frog skin microbiomes may be shaped by toxicity status, habitat, and host ecology, but the patterns remain exploratory until the submitted sequencing results are analyzed.

Quality Control: DNA quantification showed variable DNA recovery across frog skin samples, with some samples producing moderate yields and samples S6 and S8 showing the highest total DNA amounts, especially in the Qubit measurements.

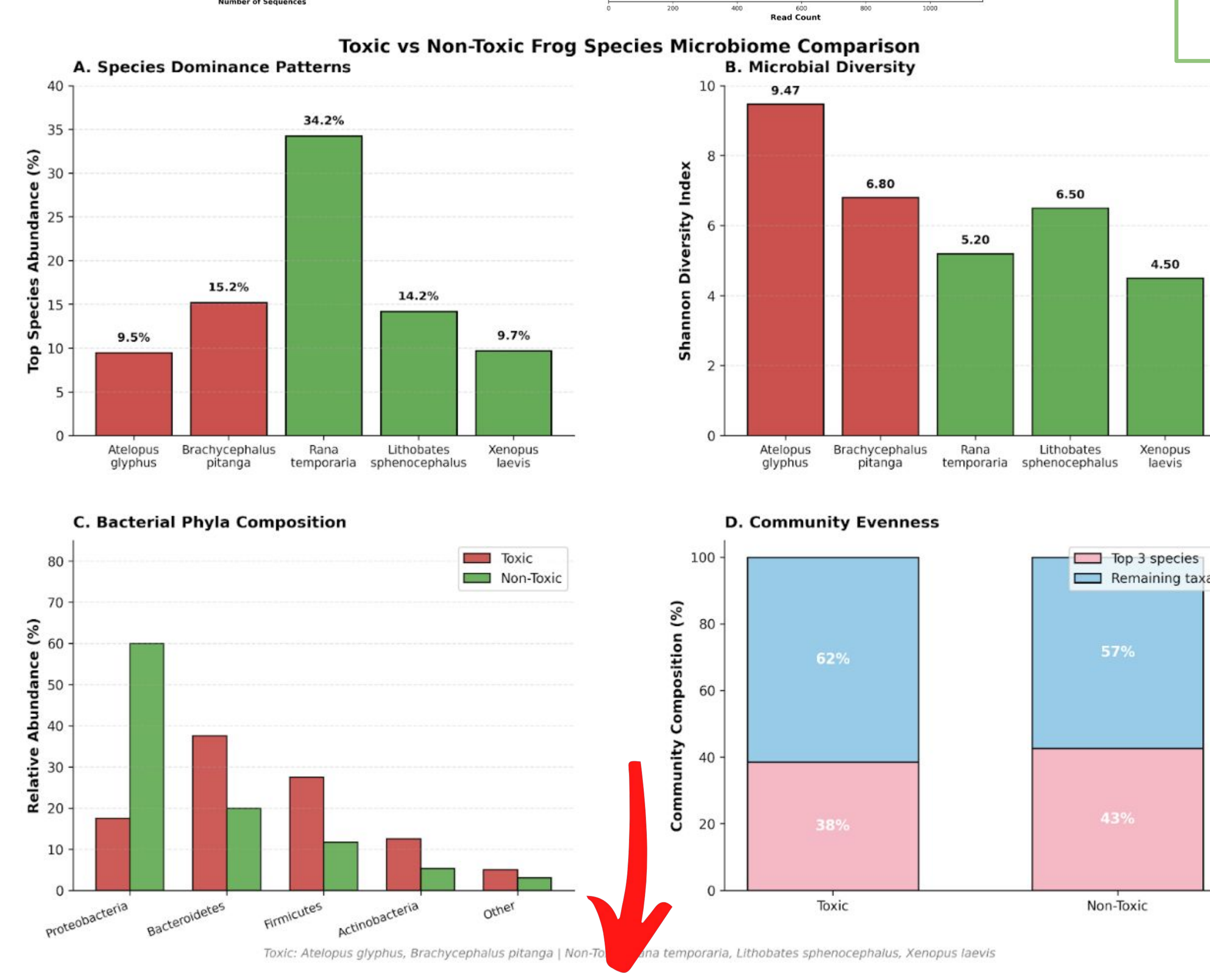
Discussion: The variation between NanoDrop and Qubit results suggests that Qubit provided a more sequencing-relevant estimate of usable dsDNA, supporting sample selection and normalization before 16S-EZ sequencing.





Reference microbiome profiles: showed distinct dominant bacterial taxa across frog species, with toxic frogs showing more balanced top-taxon distributions and some non-toxic frogs showing stronger dominance by a few taxa, especially Rana temporaria.

Discussion: These exploratory reference comparisons suggest that toxicity status may be associated with differences in microbial community evenness and composition, but species identity, habitat, sequencing depth, and dataset differences may also influence the observed patterns.



Summary profiles: Toxic reference frogs showed lower top-species dominance and higher Shannon diversity than most non-toxic reference frogs, while non-toxic frogs showed stronger Proteobacteria dominance and slightly higher top-three species contribution.

Discussion: These patterns suggest that toxic frog skin microbiomes may be more even and compositionally distinct, but the comparison remains exploratory because species identity, habitat, and dataset differences may also explain microbial variation.

FUTURE STEPS

- Alkaloid-microbiome interactions remain poorly understood across amphibians and other taxa, despite extensive study of alkaloids in pharmacology.
- Further research can be done to answer the question of whether alkaloids inhibit, promote, or are metabolized by specific microbial taxa, shaping community assembly.
- Microbial shifts may reflect direct antimicrobial effects or ecological restructuring via new metabolic niches for alkaloid-tolerant/degrading bacteria.
- Findings may extend to human microbiomes and drug development, especially for compounds with alkaloid-like activity.
- Identifying alkaloid-adapted microbes could reveal novel enzymes/pathways relevant to detoxification, pharmacokinetics, and therapeutics.

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