Title - Characterization of the Peaks of Otter Salamander Microbiome as an Innate Defense against Emerging Infectious Diseases

Program of Study – Department of Biology and Chemistry

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Category – Basic

Abstract: Recent global amphibian population decreases have prompted inquiry into possible causes, and scientists have implicated the fungal pathogens *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*) as key causes. These fungi infect the skin of many amphibian species which can result in the pathogenesis of chytridiomycosis, a fatal disease. While some amphibian populations have been shown to be resistant to chytridiomycosis, others are susceptible to the disease; resistance against *Bd* infection has been linked to the production of anti-fungal biomolecules by symbiotic cutaneous bacteria. The Peaks of Otter salamander (*Plethodon hubrichti*) is a terrestrial species endemic to the Peaks of Otter within the Blue Ridge Mountains of Virginia. We sought to characterize the microbiome of the *P. hubrichti* to better understand host-symbiont-pathogen interactions. *P. hubrichti* individuals (N=15) were captured using gloves and rinsed with sterile water to remove transient microbes. The skin of each specimen was swabbed to collect microbial cells. Cells were transferred to R2A agar plates and incubated at room temperature. Bacterial and fungal colonies were examined for morphology and were isolated by streaking. The DNA of pure cultures was extracted and subsequent 16S rRNA Sanger sequencing will identify the species of each isolate. Co-culture
assays will be employed to test the \textit{in vitro} efficacy of each bacteria in inhibiting the growth of 
\textit{Bd}. Additionally, the presence of \textit{Bd} and \textit{Bsal} will be quantified using distinct skin swabs whose 
DNA will be extracted and quantified in species-specific qPCR assays. It is suspected that the 
healthy microbiome of \textit{P. hubrichti} will confer some anti-fungal properties, and this finding will 
prompt exploration into possible bioaugmentation strategies for conservation purposes.

**Christian Worldview Integration:** According to the Biblical Christian worldview, God created 
the earth and cosmos and filled them with living things. The pinnacle of the life He formed on 
earth was the human being, into whom He breathed life and implanted His own image. Within 
the creation account, the Creator grants humanity the authority to rule over the other creatures: 
“Then God said, ‘Let us make man in our image, after our likeness. And let them have dominion 
over the fish of the sea and over the birds of the heavens and over the livestock and over all the 
estate and over every creeping thing that creeps on the earth’” (Genesis 1:26, ESV). One of the 
first mandates given to humanity is to oversee the organisms of the earth, a responsibility 
demonstrated by Adam’s naming the animals. The 21st century Christian who seeks to follow 
the divine mandate of proper environmental stewardship will value goals including habitat 
preservation, the minimization of pollution, and protection for certain wildlife. Our research 
contributes to these efforts by seeking greater knowledge of the Peaks of Otter Salamander, a 
species endemic to a small area within the Blue Ridge Mountains. This species may be 
susceptible to certain cutaneous diseases and our intention is to gain information about the 
natural protection that may be afforded by symbiotic bacteria, a relationship that has been 
demonstrated in other amphibian species. Our research will increase our understanding of this 
species and may lead to bioaugmentation attempts to help protect and conserve this unique 
species. Pursuing knowledge about creation and using the information to develop strategies to
protect wildlife from disease is one way that we as scientists can demonstrate our love of God and His creation.