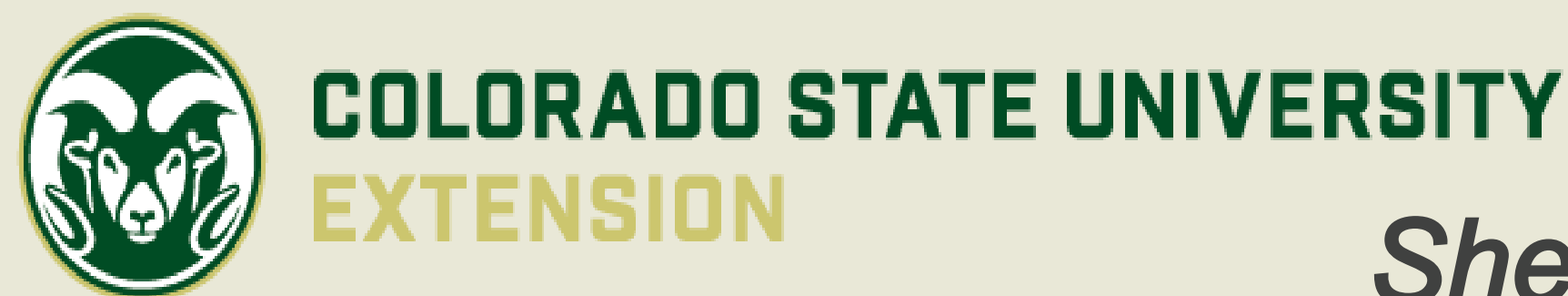


# MOLECULAR ANALYSIS OF MICROBIAL COMMUNITIES IN BIOCRUST LOCATED IN SWEETWATER, ARIZONA



Shekinah R. Johnson<sup>1</sup>, Talia J. Byrne-Haber<sup>1,2</sup>, Phillida A. Charley<sup>2</sup>, Caroline Havrilla<sup>3</sup>, Gilbert John<sup>1,2</sup>

1- College of Veterinary Medicine and Biomedical Sciences 2- Department of Microbiology, Immunology, and Pathology 3- Department of Forest and Rangeland Stewardship

## PROJECT INTRODUCTION

Biocrusts are communities of microorganisms living in the soil. They thrive in desert conditions where there is little to no physical disturbance and limited plant cover (Weber et al., 2022). The project for my summer internship focuses on the one health approach; this is the idea that human, animal, and environmental health are connected. Biocrust is mostly composed of cyanobacteria that bind soil particles together to reduce erosion and improve soil stability, perhaps allowing for better farming land. Based on this information, we hypothesized that biocrust soil thrives in areas where there is limited livestock presence with increased amounts of water which can play a role in ecosystem health. We conducted our project by collecting biocrust from Sweetwater, AZ using a grid divided into 100 squares. Overall, samples were collected from locations designated on a high livestock presence, medium livestock presence, and low livestock presence. The samples were processed at the CSU foothill's campus located in Fort Collins, CO. DNA was extracted from the soil samples. We then conducted PCR against the 16S rRNA and ITS genes before these samples were sent for sequencing. Metagenomics of these samples is ongoing to help us identify pathogenic and non-pathogenic communities within biocrust.

## INTERNSHIP GOALS

- **Goal #1:** Determine whether biocrust is linked to the health of humans, animals, and the environment by exploring the effects that livestock and wildlife have on biocrust soil stability
- **Goal #2:** Explore if microbial communities are defined by livestock populations
- **Goal #3:** Identify whether the proximity of uranium affect biocrust growth

## HOW DOES THIS APPLY TO YOUR EDUCATION

I am interested in becoming a veterinarian or to be involved in a career associated with animal science. Infectious disease is known to be associated with humans and water, but other environmental factors may also be associated when considering a One Health perspective, as these things are all connected. Since infectious diseases may go beyond water, animals, and humans, it's important to consider different sources and environments. For example, Sweetwater, AZ, a semi-arid region, is plaque with abandoned uranium mines that can affect livestock and wildlife watering holes through rain run-offs of past uranium tailings. Additionally, some livestock wells and springs have high levels of uranium and arsenic.



Figure 1: Photo of myself collecting 3 samples from a medium livestock presence area.

## WHAT YOU DID

The summer project included one trip to Sweetwater, AZ to collect over 120 biocrust soil samples from three areas- high livestock, medium livestock, and low/no livestock. We obtained Navajo Nation Chapter approval to collect biocrust soil samples. For each area, we collected 3 soil samples along a diagonal strung grid (Figure 2). Samples were collected using a 1cm diameter conical tube by 2cm in length.

- We extracted genomic DNA from all biocrust soil samples using a commercial extraction kit.
- We performed three separate PCR amplifications on the extracted DNA to generate a 16s amplicon. Their correct amplicon sizes (1500bp) was visualized using gel electrophoresis.

1. Polymerase Chain Reaction (PCR): amplified 16s rRNA or ITS genes



Figure 2: The 3 conical tubes show where the samples were collected.

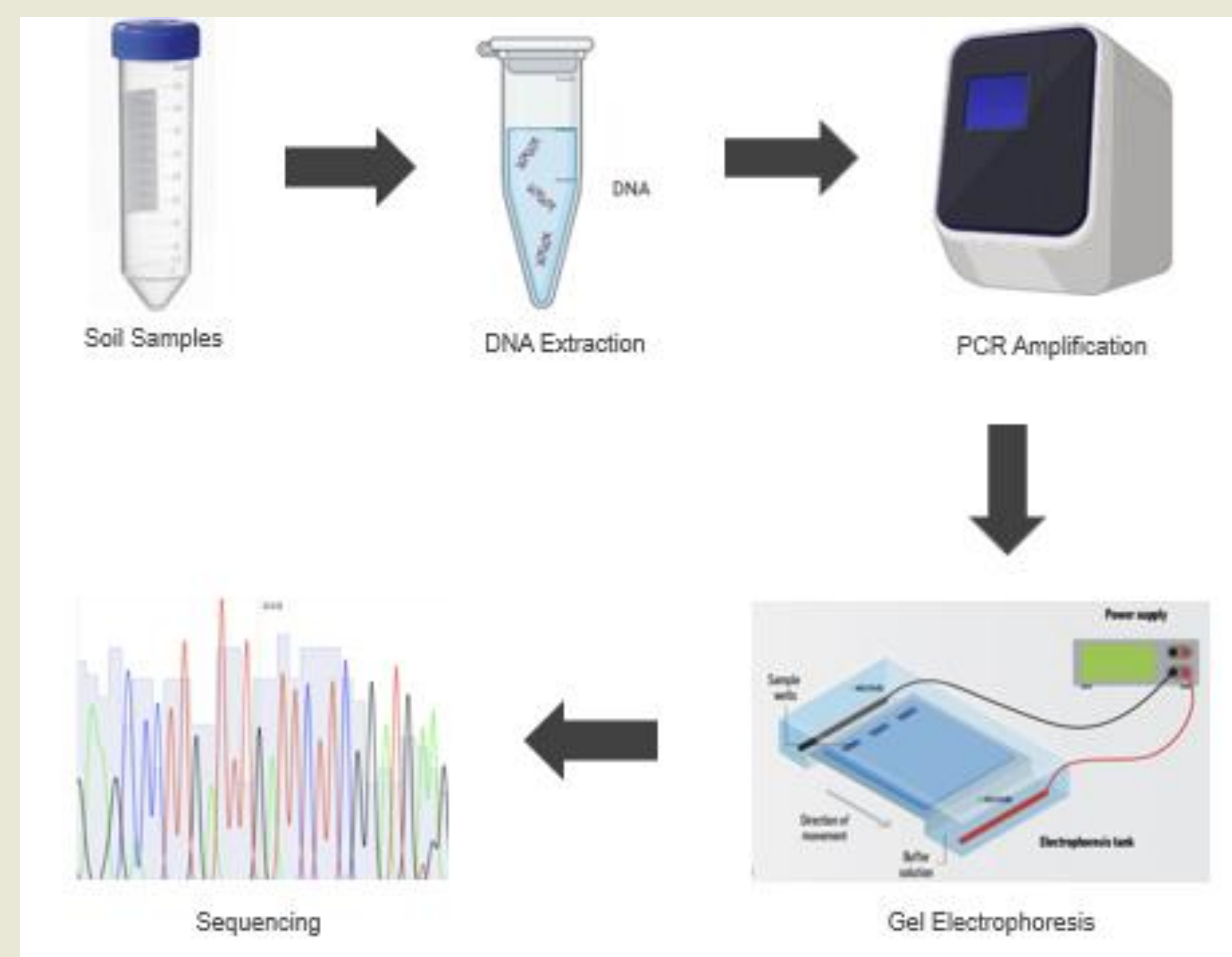


Figure 3: Flowchart showing the methodology of using PCR amplification, gel electrophoresis, and sequencing to identify pathogenic and non-pathogenic material present in soil samples.

## WHAT YOU LEARNED

- From the three areas tested (9A= Semi livestock and wildlife area; 21A= located near game trail; 15C= No note of livestock presence), the genus *Rubrobacter* is the most prominent bacteria in two (9A and 21A) of the three results.
- We found nine samples produced no PCR amplicons (Figure 4)
- There are pathogenic bacteria present in three of our samples (Samples 9A, 21A, and 15C)
- Recent results from the CSU diagnostic lab's sequencing core suggest that the lack of amplification may have been due to issues with the primers and not the soil and/or DNA samples

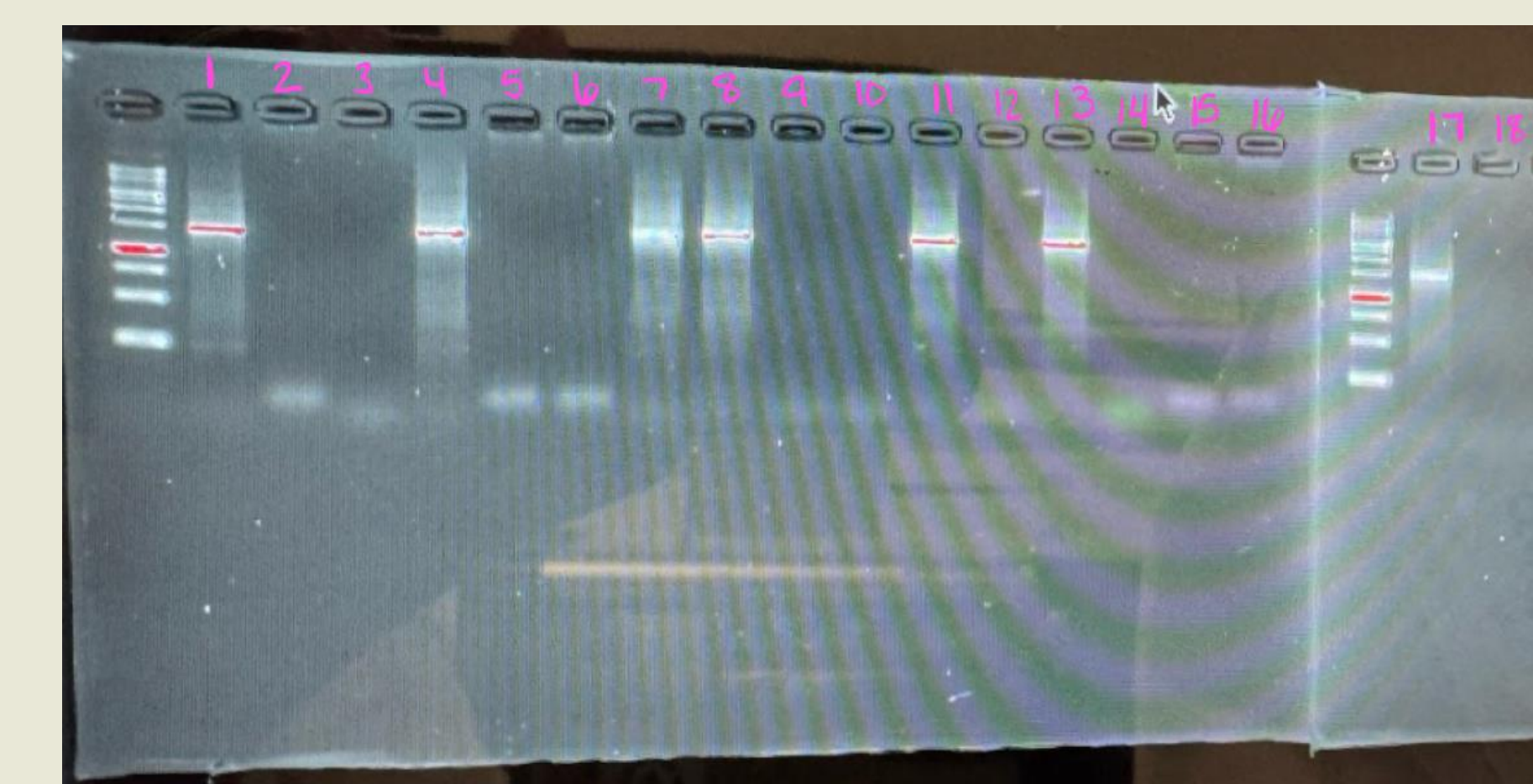


Figure 4: 2% agarose electrophoresis gel results from samples 25A, 27A, 16B, 16A, 8B, 8A1, 3B, 4A, 1C, 2C, 6B, 19A, 27B, 18A, 15B, 30B, 20B, and 20C. The red markings indicate the base ladder and corresponding 1500 bp amplicon. The 500 bp amplicon is not shown.

## NEXT STEPS AND CONCLUSION

- The data discussed is preliminary, and we are continuing to characterize the remaining biocrust soil samples.
- Analyze the extent of pathogenic microorganisms in the soil and to confirm the type of non-pathogenic microorganism present which can vary depending on the environmental and ecological factors (heavy metals, drought, etc.).
- We observed that Sweetwater, AZ contained numerous biocrust-rich communities, with the densest populations occurring in areas with minimal or no livestock activity.
- In conclusion, biocrust likely plays a key role in fostering vegetation growth and maintaining productive farmland. Further research into the interaction between biocrust and livestock interaction could offer new insights for balancing agricultural use with ecological preservation.

## REFERENCES

- Duan, Mingzheng, and Tolgor Bau. "IGufwan, L. A., Gufwan, N. M., Abdulraheem, M. I., & Jaafar, A. A. (2025). Biocrust ecology and functionality for regenerative agriculture: A review of the opportunities and challenges facing microbial agroecosystem restoration. *Archives of Agronomy and Soil Science*, 71(1), 1–28. <https://doi.org/10.1080/03650340.2025.2508763>
- Initial Sample Processing Can Influence the Soil Microbial Metabarcoding Surveys, Revealed by *Leucocalocybe Mongolica* Fairy Ring Ecosystem." *Biotechnology & Biotechnological Equipment*, vol. 35, no. 1, Jan. 2021, pp. 1427–38. *EBSCOhost*, <https://doi-org.ezproxy2.library.colostate.edu/10.1080/13102818.2021.1996272>.
- Weber, Bettina, et al. "What Is a Biocrust? A Refined, Contemporary Definition for a Broadening Research Community." *Biological Reviews*, vol. 97, no. 5, Oct. 2022, pp. 1768–85. *EBSCOhost*, <https://doi-org.ezproxy2.library.colostate.edu/10.1111/brv.12862>.

Acknowledgements: Funded by the CSU One Health Institute