"Custom Development of Molecular and Genetic Barcoding Tools for Predator Identification in Texas Horned Lizard (*Phrynosoma cornutum*) Populations"

Isabelle J. Galvan,[1] Kira N. Gangbin,[2] Dean A. Williams[1]

TCU Department of Biology, Departmental JVR Honors College Thesis

LOUISE DILWORTH DAVIS COLLEGE of SCIENCE & ENGINEERING

Abstract & Background

The Texas Horned Lizard (*Phrynosoma cornutum*) has undergone dramatic population declines across its native range due to habitat loss, invasive species, and predation.

To support reintroduction, graduate researchers from TCU's biology program glue harmonic tags onto the backs of hatchlings, (Figure 1) making them easier to locate in the wild and to identify the most viable reintroduction areas.

Hatchlings experience high predation after reintroduction, and we often find their tags in the scat of various predators. Identifying the primary predators of reintroduced populations is critical for improving hatchling survival and informing conservation strategies.

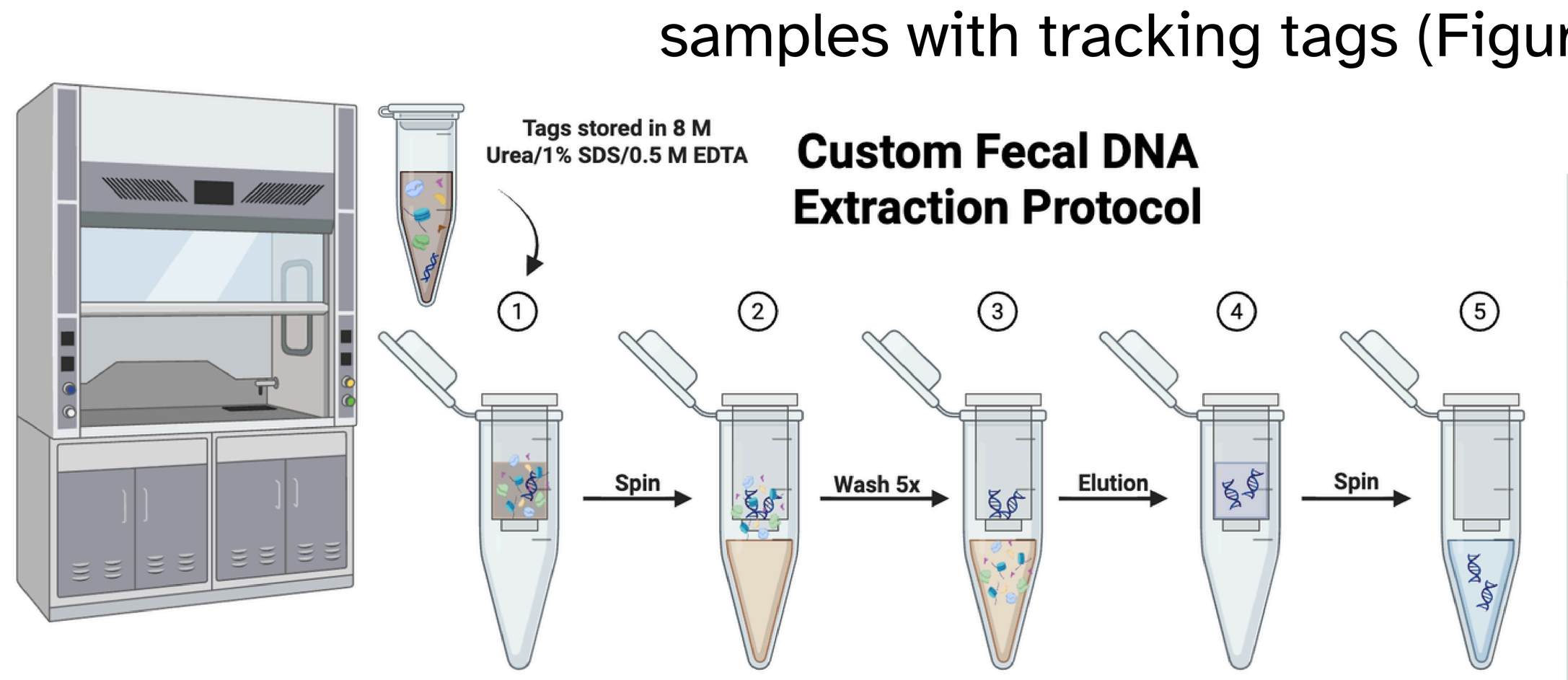
Previous studies suggest coachwhip snakes (*Masticophis flagellum*) are the major predators of these Texas Horned Lizards.

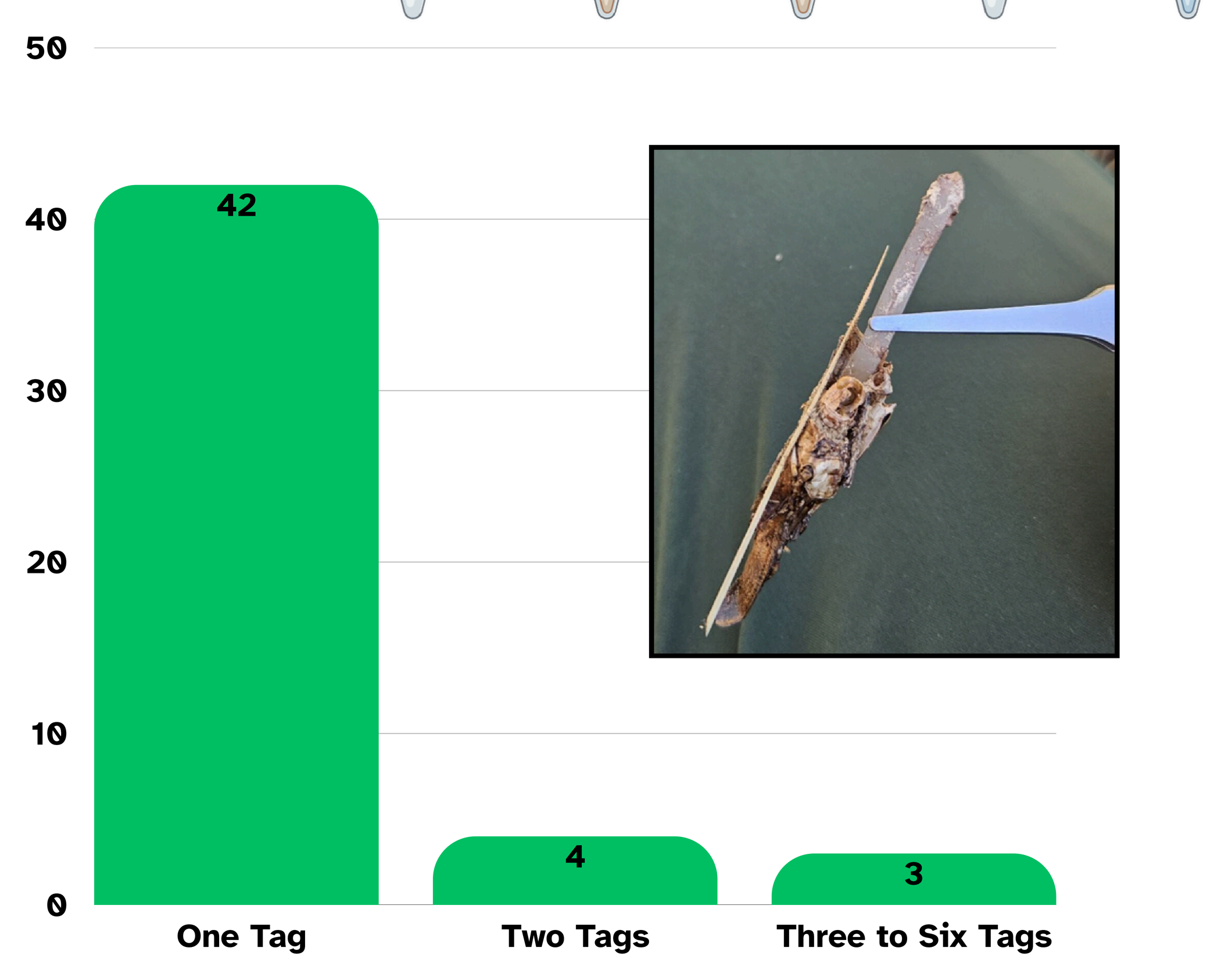
We extracted DNA from scat that contained tracker tags and used coachwhip specific primers to determine how common coachwhip predation is at the reintroduction sites.

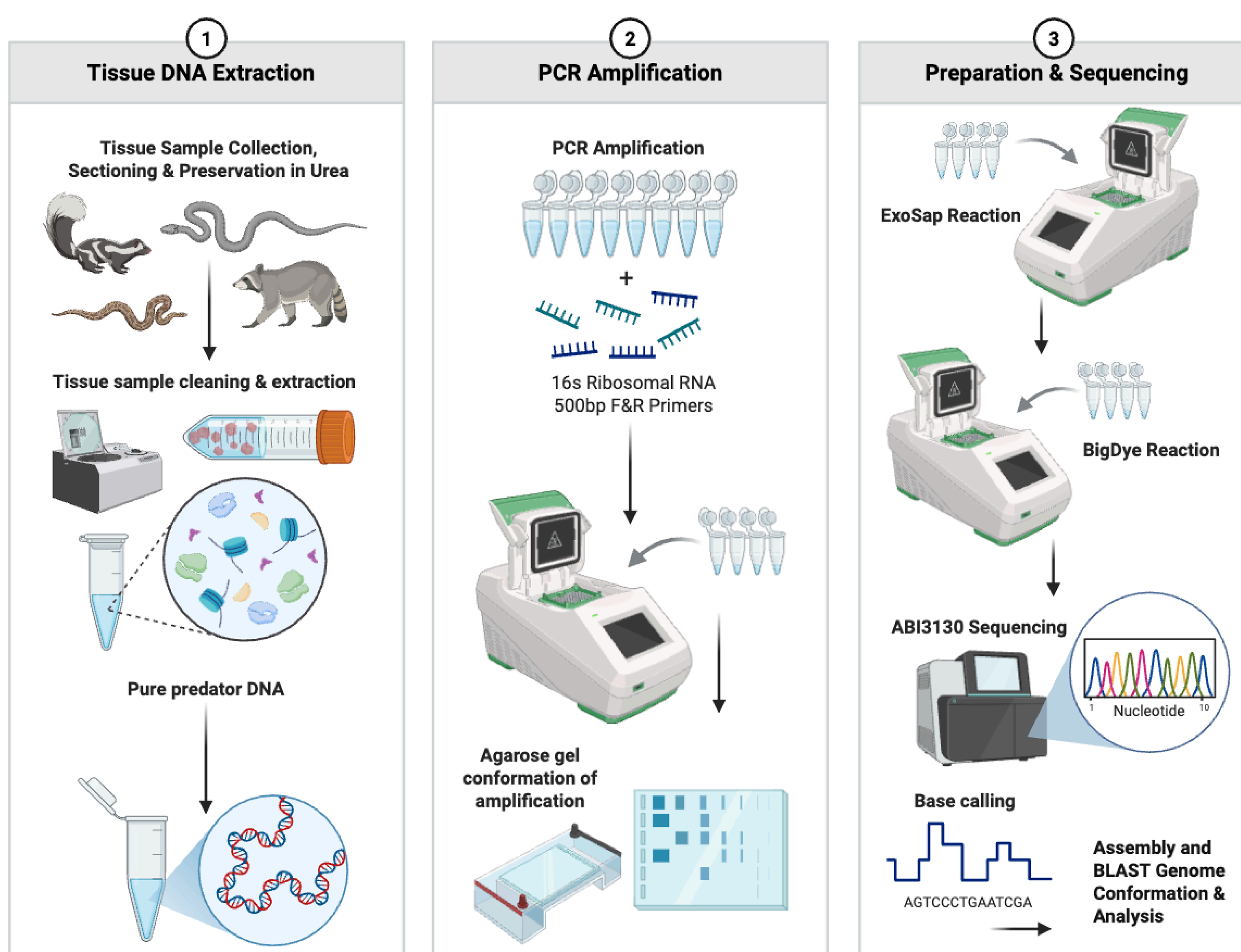
Figure 1: Hatchling THL with tracker tags

Methods

Figure 2: Methods and biological processes for predator tissue extraction, identification, and extraction of predator DNA from fecal samples found with hatchling tracker tags. We extracted DNA from the predator scat and developed a coachwhip-specific genetic marker and a horned lizard-specific primer to screen 75 fecal samples with tracking tags (Figure 3) from the 2024 fall season.







Data & Discussion

Additional predators besides the coachwhip are found at reintroduction sites. Tissue samples were collected from various mammals and other native Texas snake species, and processed via DNA sequencing. (Figure 2)

The MfCOI primer was tested against all of them to ensure that only the coachwhip samples would amplify during PCR reactions. (Figure 4)

Figure 5: *Masticophis flagellum* eating a THL *Phrynosoma cornutum* adult

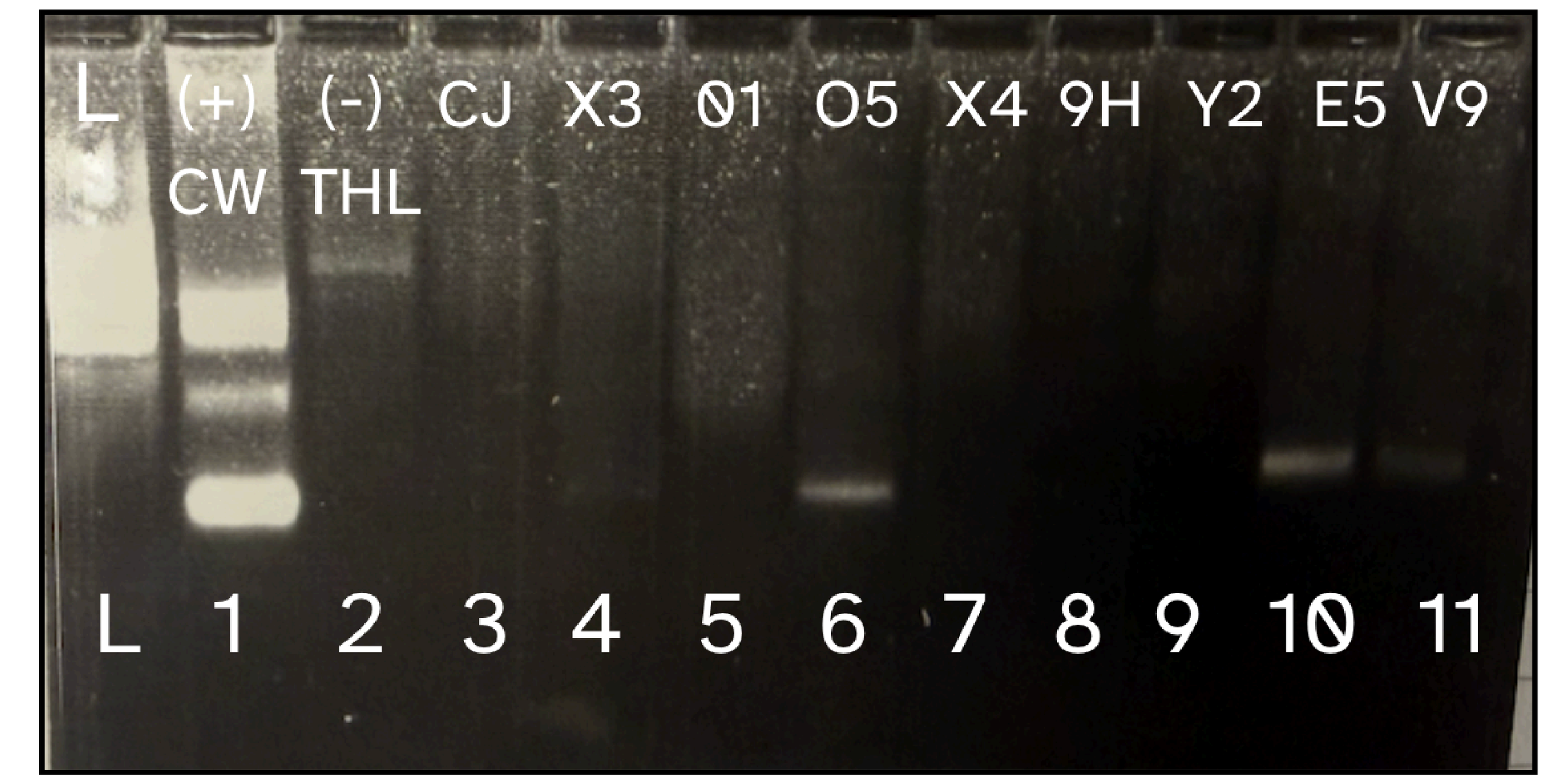


Figure 6: Example of Results from 8 samples tested with the custom MfCOI Primer. Samples with positive bands show the presence of coachwip DNA, located in wells 4, 6, 10, and 11

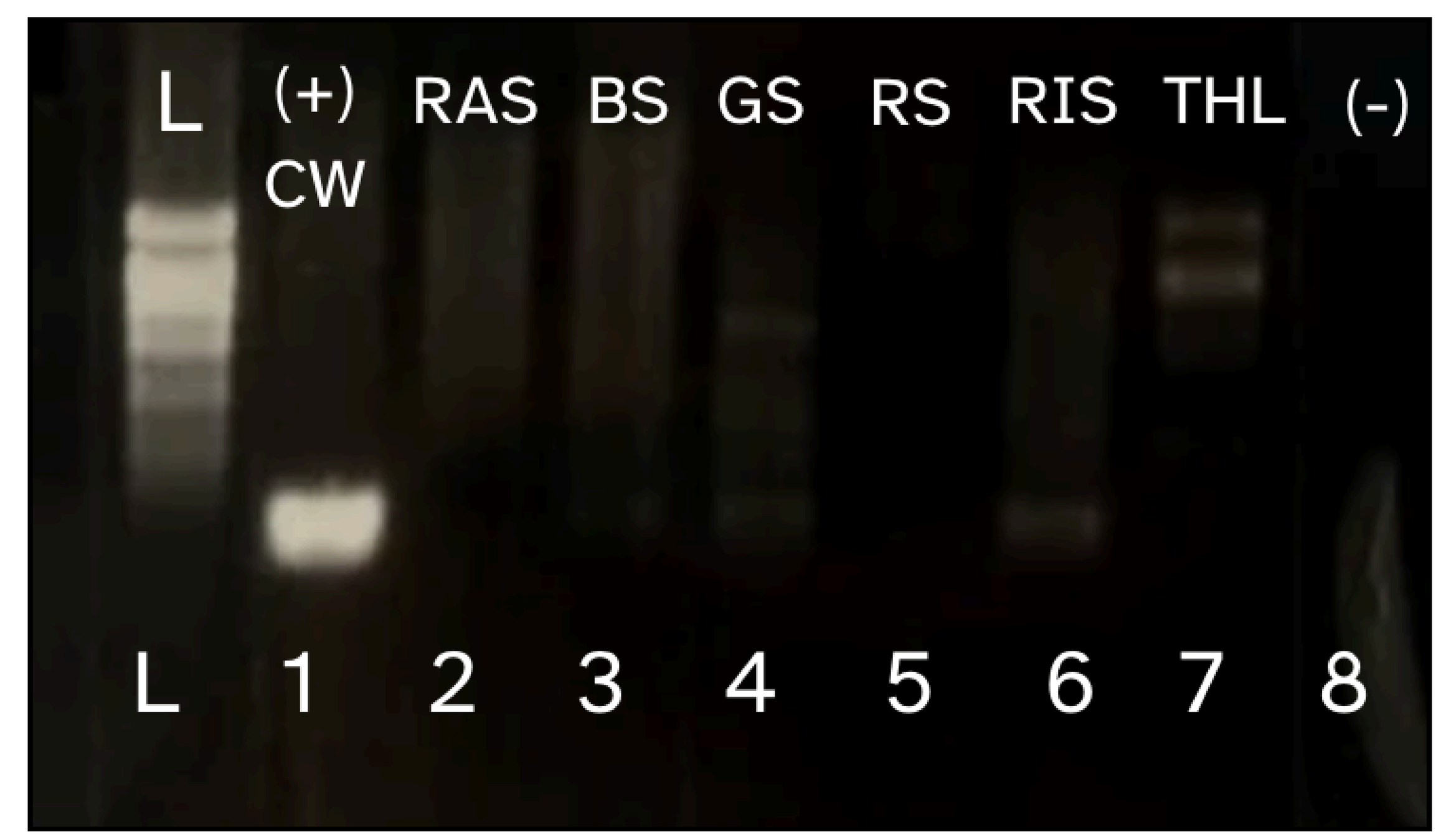
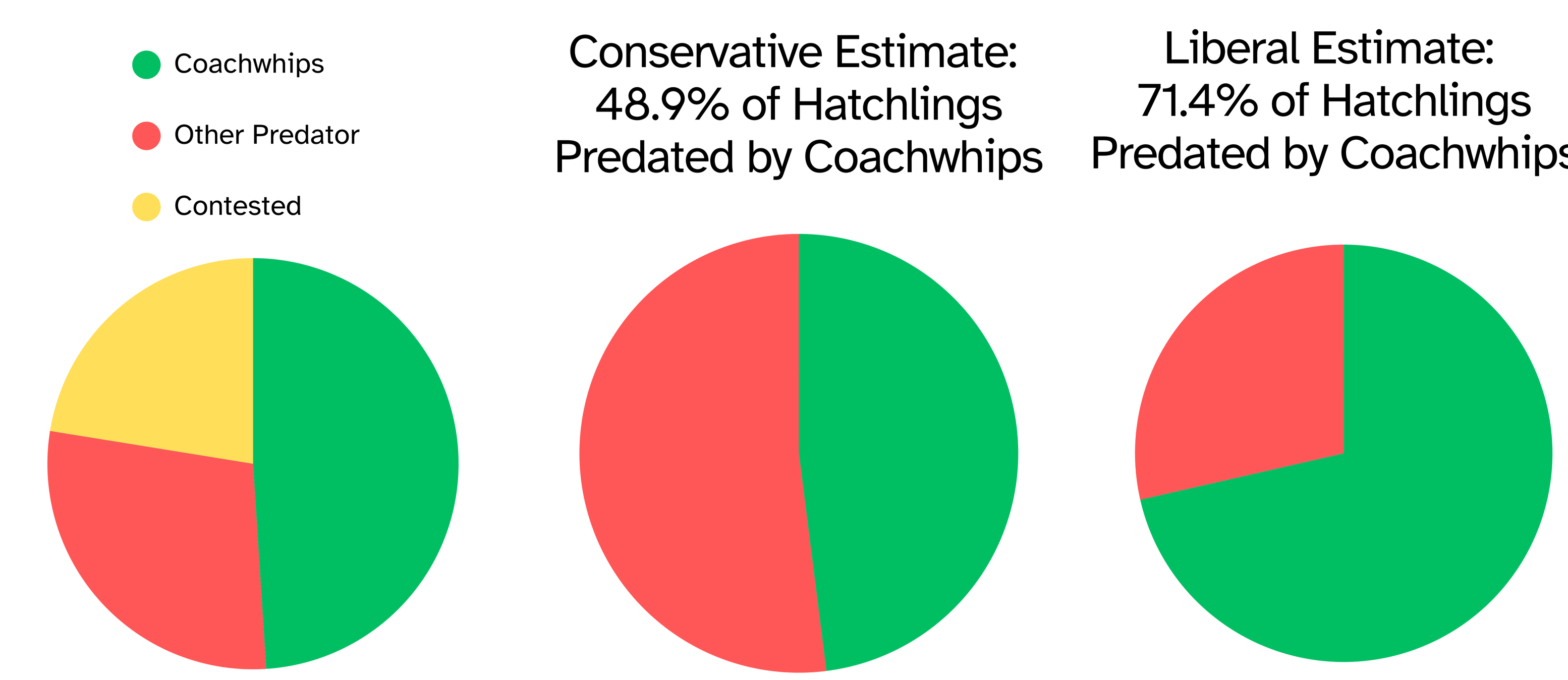


Figure 4: PCR Amplification Confirmation for MfCOI Primer

Rat Snake: RAS (*Pantherophis obsoletus*)
Bullsnake: BS (*Pituophis catenifer*)
Western Diamondback Rattlesnake: RS (*Crotalus atrox*)
Green Snake: GS (*Opheodrys aestivus*)
Ribbon Snake: RIS (*Thamnophis proximus*)



Out of 49 fecal samples with tracker tags that were tested, the Coachwhip (*Masticophis flagellum*) accounted for aproximetely 50-71% of all predation events.

Aknowledgements & References

I'd like to thank Dr. Williams for trusting in me to give good results and for giving me a shot at departmental honors with him after my first research position. Notably, I would like to recognize my lab mates Lexi and Kira for all their assistance and advice on the project. I would also like to thank my friends and my family for helping me see this project through to the end.

HONORS COLLEGE AT TEXAS CHRISTIAN UNIVERSITY

HORNED LIZARD CONSERVATION SOCIETY

TEXAS PARKS & WILDLIFE

fort worth zoo