Abstract of proposed student project (1 page limit. This should mirror the aims page of a grant and CLEARLY indicate the student's role.)

Identification of parasites, especially from wild or exotic animals, can be difficult, especially if specimens are fragmented, decomposed and not handled or preserved properly. The lack of male worms with dioicous parasites can also make identification to species impossible for some species. The use of PCR and sequencing can aid in parasite identification, however, as with gross techniques (i.e. staining, clearing) preservation methods are important to ensure the best possible outcome.

All parasite identifications can benefit from proper handling and preservation, however one that is becoming increasingly valuable and important in veterinary medicine is that of pentastomes in native Florida snakes. The pentastome, *Raillietiella orientalis*, is an invasive pentastome that was introduced to Florida and the US through the Burmese pythons found in much of the southern region of the state. Our goal is 2 parts:

Part 1) Establish a qPCR for *Raillietiella orientalis* – this would be used to accurately assess the potential changes to DNA quality with parasite treatments.

Part 2) Assess changes in DNA quantity (by quantifying nucleic acid extract concentrations) and DNA quality (using the above qPCR) following different treatments (lactophenol, formalin fixation of different time lengths, etc.)

Parasites used would consist of those already in the laboratory, preserved in 70% ethanol, as well as those routinely sent into the laboratory for testing. Parasites would be exposed to varying concentrations of lactophenol, a reagent used to clear the worm to visualize internal organs, saline, 70% ethanol, isopropanol and formalin. Additionally, we would vary the time points at which specimens are left in each reagent.

The parasites would be exposed to saline, 10% buffered formalin, 70% ethanol and isopropanol at 1, 2, 14 and 30 days. Lactophenol would be at either 1) standard reagent formulation, or 2) 80:20 absolute ethanol and phenol at 1, 2, 6, 12 and 24 hours. Parasites are generally cleared between 1-24 hours depending on thickness and quality. All concentrations and time points will be run in triplicate. The parasites would then undergo DNA extraction and the concentration would be measured for each concentration/time point. This would be followed by qPCR and sequence analysis. This information would be used to establish a laboratory protocol for parasite (*R. orientalis*) handling and subsequent qPCR. These handling protocols could be used for additional species when PCR is necessary.

The student would be involved in carrying out all concentration experiments, DNA extraction and qPCR.