**An evaluation of DNA extraction methods for Western black widows across developmental stages: minimizing cost and effort**

**Harrish Nithianandan**, **Gausiha Rathitharan**, **Kemavijojana Rajkumar**, **Charmaine Condy** and **Maydianne C. B. Andrade**

Integrative Behaviour & Neuroscience Group

Department of Ecology & Evolutionary Biology

University of Toronto Scarborough

Molecular data have revolutionized the cataloguing of biodiversity and inference of phylogenetic relationships, but require extraction of high quality, ‘clean’ DNA from samples that may be hard to obtain. Protocols that minimize the cost and effort involved can increase the range of species from which such data can be collected. Among arachnologists, common wisdom suggests that clean DNA is best obtained from muscle tissue extracted from legs (destructive sampling). However, it is challenging to obtain sufficient tissue from spiderlings in this way, and rearing spiders to adulthood in research laboratories is time consuming and expensive. Here we extracted DNA from newly emerged spiderlings, six-week old juveniles and mature females of the Western black widow species (*Lactrodectus hesperus,* Theridiidae) from British Columbia and California and compared destructive versus non-destructive (whole-body) methods. We assessed DNA quality by comparing the caliber of gel electrophoretic results, the success of sequence contig assembly, the confirmation of species identity using DNA barcoding (sequence of cytochrome *c* oxidase subunit I, COI), and neighbor-joining methods to support clustering of our samples with other *L. hesperus* populations among other *Latrodectus* species. DNA from all specimens was successfully extracted, amplified and sequenced.  Contig assembly was successful in most cases.  A neighbor-joining analysis provided support for our samples clustering with other *L. hesperus* populations.  These results show that high quality DNA can be extracted from the spiderlings using non-destructive methods. We discuss the utility of this reduced-effort method for analyzing genetic divergence among populations, which requires broad sampling.