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## Defining Species Concepts: Testing Species Boundaries of *Sceloporus Grammicus*.

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Species concepts are the methods biologists use to name and describe species. There are many important reasons for having an empirically based species concept. Species definitions are important to the fields of population biology and phylogenetics. Species are the fundamental unit of evolution. Operationally defining species is important because many biological disciplines depend on Species classifications. It is extremely important to use several methods when identifying a species. Operational species concepts are methods that apply certain criterion or measure in defining actual species groups in nature. These concepts generally fall into three basic groups: 1) concepts based on the phylogenetic and character patterns generated from evolutionary processes, 2) concepts based on evolutionary processes, and 3) concepts based on phenetic similarity. Three operational species concepts: the diagnostic phylogenetic species concept, monophyletic phylogenetic species concepts, and the cohesion species concept do fairly well in each category. Many studies have been done to test species boundaries. No studies have been done that compare the diagnostic, monophyletic and the cohesion species concepts together in a single organism. It has been unclear how each of these three current operational species concepts compares when tested within the same group of organisms. Do these three operational species concepts give the same results? In all the preliminary data no two methods (species concepts) gave the same results. This is a brief report of a study I was involved with that answers this question.

In this study we tested species boundaries in the chromosome races of the *Sceloporus grammicus* complex, based on two pattern based operational species concepts (diagnostic phylogenetic species concept, monophyletic phylogenetic species concept) and one process based concept (cohesion species concept). The demarcation of species was based on mitochondrial sequence data, nuclear sequence data, life-history traits and chromosomal numbers ( $2N=32-46$ ) for about 500-600 samples collected across central Mexico. The results were then compared between these three different analyses in order to assess similarities and differences as well as strengths and weakness of each concept. Comparing differences from the practical use of each concept has great value in investigating philosophical differences underlying each concept.

My main responsibilities in this project included extracting DNA from the collected samples, and amplifying specific genes by PCR. DNA was extracted using a standard protocol. DNA extractions and PCR amplifications were checked via agarose gel electrophoresis. Products were sequenced using the Perkin Elmer Big Dye cycle sequencing kit and analyzed on an ABI 377 automated sequencer. PCR and sequencing of two mitochondrial (cyt b and 12s) and two nuclear genes (fibrinogen and MYH2) were done.

My null hypothesis was that we would see a nested pattern of inclusiveness of species methods as we went from the diagnostic to the monophyletic to the cohesion method. We reasoned that once two populations became separated it would not be difficult for a fixation of a single character between the population to evolve (diagnostic species), as these population became

more separated, both temporally and by a reduction of gene flow that more and more genes would show a monophyletic relationship pattern (monophyletic species) and finally these population would evolve mechanisms that serve as barrier to all gene flow or niche competition (cohesion species). We have results from the diagnostic and monophyletic methods and these show that nature is often much more complicated than we anticipate. The diagnostic species were somewhat concordant with chromosome races but included several races in a single diagnostic species. Surprisingly these diagnostic species were not nested within the monophyletic species. Many of the monophyletic groups contained individuals from various diagnostic groups. There are a number of evolutionary explanations for this but all indicate that either the populations have not been separated for a significant length of time or there is recurring gene flow between the groups.

This project was not without its difficulties. The largest obstacle I dealt with was getting the extractions and PCR products to work. We learned that some genes were easier to amplify than others. Accordingly, we changed the genes we were using during the project. We also found that adjusting our protocols for the PCR helped improve our results. I learned that making small changes and adjustments during a project can help you get faster, more reliable results. Also, because this research is outside of my major, I had to learn a lot about phylogenetics. This experience will aid me in future research projects.

Overall, this was a great learning experience for me. I have learned lab techniques that I can use in future projects. I have learned much about phylogenetics and systematics. These are two relatively new and fast growing fields. I also learned the importance of using aseptic techniques when dealing with tissue samples. This valuable experience will help me in my future schooling and research to come.