

Jia Wern Pan
Internship Report for Summer 2010
Sewanee, TN
August 29, 2010

The evolutionary history of local land snails (*Anguispira* sp.) and the population structure of the eastern mud-snail (*Ilyanassa obsoleta*).

This project examines the evolutionary history and genetics of two kinds of snails, “snake-coiled forest snails” (*Anguispira* sp.) and the eastern mud-snail (*Ilyanassa obsoleta*). Snails from the genus *Anguispira* are endemic to southeastern Tennessee and I examined the evolutionary history of this genus by using DNA analysis to determine the phylogenetics of the genus leading to current snail diversity. I also used similar DNA analysis techniques to determine the population structure and genetic diversity of *Ilyanassa obsoleta* individuals collected from different parts of coastal America.

In the project involving *Anguispira* sp., I was primarily involved in extracting and amplifying DNA from *Anguispira* specimens collected from various different locations. To do so I would snip off a small piece of flesh from the tip of their tails using protocols developed during my previous work with *Anguispira* (developed specifically to avoid snail mortality while obtaining enough DNA material). This material was then processed using a standard DNA extraction kit to obtain DNA for amplification and sequencing purposes. Over the course of the summer I attempted to amplify, using polymerase chain reaction (PCR), 3 different mitochondrial genes from the specimens we obtained: 16S, cytochrome oxidase I (CO1) and cytochrome oxidase *b* (Cob). Upon successful amplification, the DNA was purified and sent to a DNA analysis facility to be sequenced. Over the course of the summer I managed to successfully obtain 16S and Cob gene sequences from a good number of the specimens we collected, and I also have been able to refine the DNA extraction and amplification procedures (determining optimum amplification temperatures, finding the best combination of primers used for DNA amplification, etc.) for use on future

Jia Wern Pan
Internship Report for Summer 2010
Sewanee, TN
August 29, 2010

specimens. However, despite various different attempts, I have not been able to obtain good CO1 sequences, and this remains a puzzle to be solved. From the 16S data we collected I have been able to run a preliminary analysis on the phylogeny of the specimens we collected, and the initial results seem to show a strong clustering of specimens by location and species (Figure 1). Our initial results are also interesting in that they indicate that one particular population of *A. picta* (an endangered species) seems to be different from the others, which could directly impact currently ongoing state wildlife conservation efforts.

Besides lab work, I was also involved in collecting specimens from the field. Together with Dr Haskell and a number of other students, we took field trips to many of the limestone outcrops in the region, documenting the presence or absence of the various different species of *Anguispira*. This will allow us to document the distribution and abundance of the various *Anguispira* species in the region, again with potential implications towards conservation efforts directed at *A. picta*.

As for the project involving the eastern mud-snail (*Ilyanassa obsoleta*), over the summer I analyzed the mitochondrial CO1 DNA sequences from various populations across both the East and West coasts that I had obtained in previous work. To do so I had to learn how to use a variety of DNA analysis programs such as Sequencher, MEGA, DNAsp, Arlequin, and TCS. The results of my analyses indicate that *Ilyanassa obsoleta* populations are not very structured, with most populations being genetically very similar to each other, including invasive populations on the West coast. This suggests that interbreeding between the snail populations occur all along the East coast, and that West coast populations have not been isolated long enough to accumulate genetic differences. The results also indicate a slight decrease

Jia Wern Pan
Internship Report for Summer 2010
Sewanee, TN
August 29, 2010

in genetic diversity from South to North, consistent with the theory that Northern populations are younger, becoming established in parallel with the Northern retreat of glaciers during the end of the Ice Ages. With the completion of data analyses in this project, I can now write it up for publication.

It should be noted that both of these projects are still ongoing. I will continue to work on both of these projects through the school year, in the hope that they will eventually be able to be published in scientific literature. As such, the Environmental Research fund provided an invaluable opportunity for me to get started on these projects and get them into full swing. Being able to work on these projects over the summer has enabled me to iron out many of the problems that inevitably plague such projects, and will make future work on these projects much easier.

Professionally, this internship has provided me with valuable experience that will be very useful in future endeavors. As my current goal is to become a research scientist in the field of evolutionary biology, this internship has allowed me to gain experience in many of the basic techniques and procedures used in that discipline. For example, as my internship consisted of both field and lab work, I was able to learn important field methods (data collection, describing the biogeography of the studied organism), as well as important lab techniques such as primer design, genetic data analysis and DNA barcoding. Also, if all goes well, I will have been able to contribute towards the publication of one or two scientific articles on my research subject (snail genetics and evolution), which will both improve my scientific credentials and allow me to learn the processes that lead towards the publication of such articles. Last but not least, I had to carry out these projects mostly on my own, and the experience has

taught me a lot about the importance of personal initiative and motivation and how to be independent.

Figure

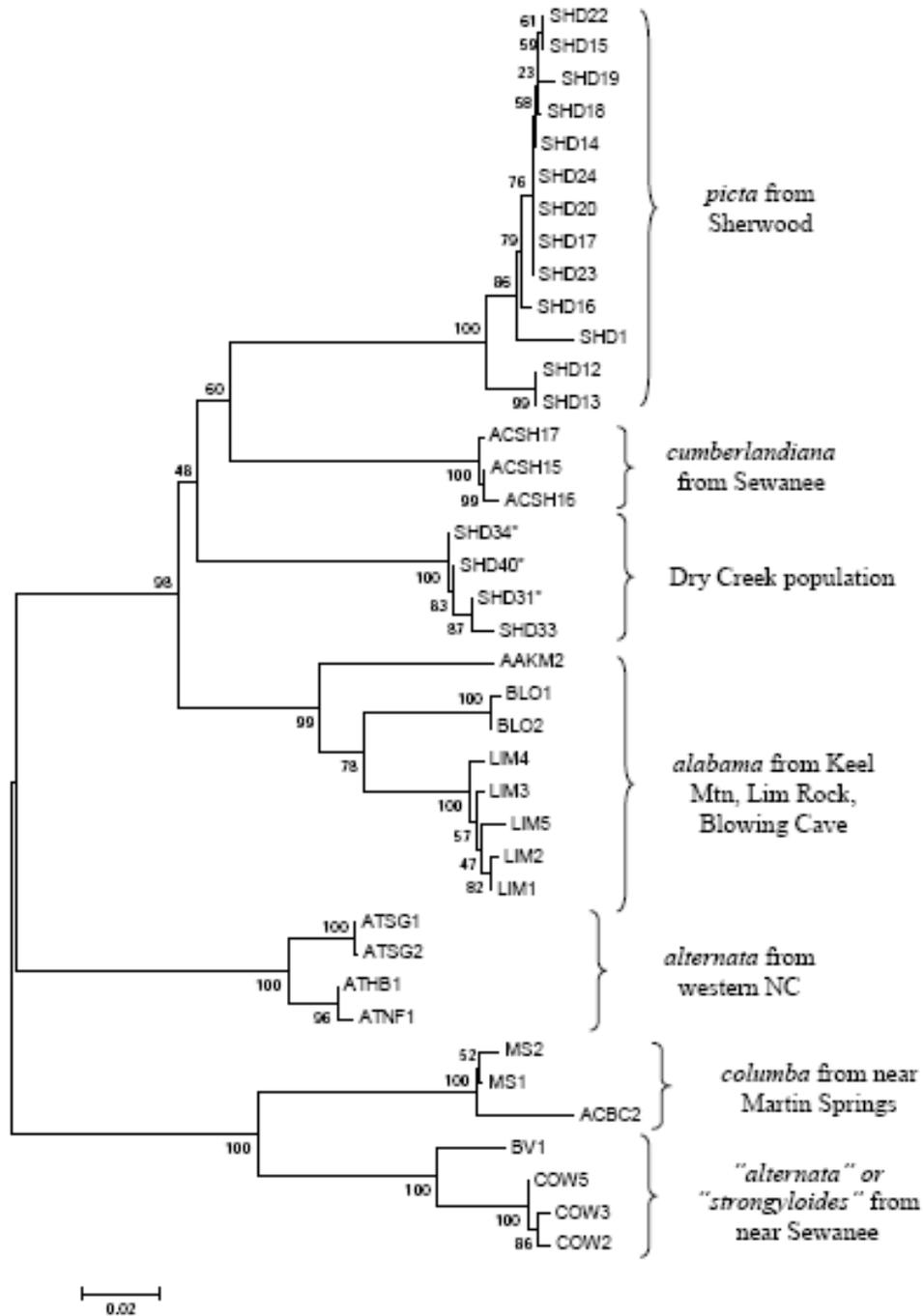


Figure 1: Preliminary analysis of *Anguispira* phylogeny.