Systematics of *Desmognathus monticola*

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Abstract and/or Background

In 1951. Richard Hoffman described a new subspecies of the Seal Salamander, Desmognathus monticola from Virginia. His recognition of a new subspecies was based on his perception that the color pattern of animals from the eastern part of the species range was different from those farther west. Petranka (1998) did not accept the subspecific designation because the two forms overlapped in range to some extent. Nevertheless, it would seem prudent to investigate the molecular genetics of any salamander species which shows morphological differences since such differences are rare within a single salamander species. The goal of our research was to examine the molecular genetics of Desmognathus monticola from throughout its range, at least in Virginia, looking for any genetic discontinuities that could indicate a restriction in gene flow and the presence of multiple species. DNA was extracted from salamander tail clips and used to amplify specific genes using PCR technology. These DNA sequences were compared and a phylogenetic tree of genetic relationships was developed to assess the extent of genetic differences among these different populations. No significant genetic differences were found between the two morphological forms, or between eastern and western populations of the Seal Salamander. We did not find any justification, from a genetic standpoint, for the recognition of *Desmognathus monticola jeffersoni* at any taxonomic level.

Introduction and/or Research Question

The species concept is one of the most important in biology. A species is typically defined as a group of plants or animals which are capable of freely interbreeding. If a population, usually on the periphery of the species range, becomes geographically isolated from the remainder of the populations, any genetic differences which arise in that population, could be passed to the descendants of that population, but not others from which they are isolated. Over time, if enough genetic differences become established, individuals from that population may not be able to produce viable fertile offspring when crossed to individuals from other populations. Thus, the population would become a separate species. Individuals from different species may not react similarly if used in experiments involving physiology, ecology, genetics, embryology, or other areas because of their different genetic backgrounds. Therefore, it is important to know the number and range of species in an area to ensure that experiments using wild animals have similar genetic backgrounds. One group of animals particularly subject to hidden, or cryptic, genetic differences, is salamanders. Their morphology is conserved so there are few anatomical differences, particularly among closely related species. Many "species" of salamanders have been split when new molecular techniques are used to examine different "populations". When these "populations" show discrete genetic differences, they are described as new species since gene flow between them would have prevented the accumulation of such differences if all the populations represented one species. Our goal was to examine the genetic structure of Seal salamander populations from throughout Virginia, with particular interest in eastern and western populations in northcentral Virginia, to investigate whether there was a genetic basis for recognizing *Desmognathus monticola jeffersoni* at any taxonomic level.

Methods

- Tail clips were obtained from animals in the wild and stored at 20°C until use.
- Genomic DNA was extracted from tail clips following the protocol of the Qiagen DNeasy® Isolation Kit.
- After extraction, sequences were amplified by polymerase chain reaction (PCR). PCR was performed using 12.5 µL Apex Red Primer Mix, 8 µL water, 2 µL sample, and 2.5 µL of primer.
- We sequenced gene fragments from 16S rRNA.
- We used standard PCR protocol to amplify each gene fragment.
- 5 µL of the PCR product was analyzed by agarose gel electrophoresis to ensure that the amplified DNA was present.
- For 16S rRNA PCR products were cleaned and concentrated using the Genesee DNA Clean & Concentrator-5 Kit protocol.
- Samples were then sent to Eurofins for sequencing (Figure 3).
- Sequences were analyzed using Geneious software (Figure 2), a phylogenetic tree-building algorithm, to indicate the degree of genetic differentiation between the populations.

Figure 1. *Desmognathus monticola jeffersoni* (Photo by P. Sattler).

Figure 3. Desmognathus monticola monticola (Photo by P. Sattler)

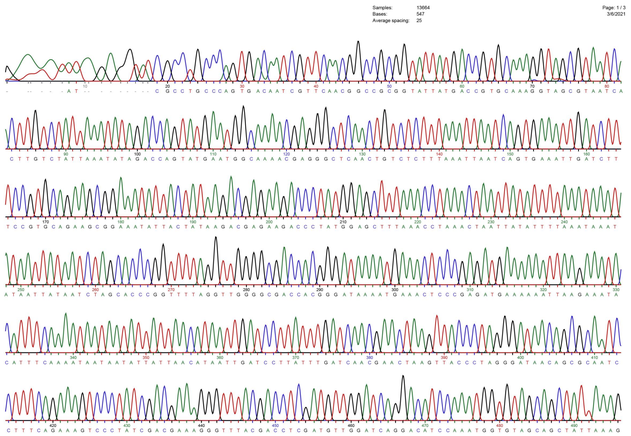

Figure 5. DNA sequence for 16S rRNA (sequence supplied by Eurofins Scientific).

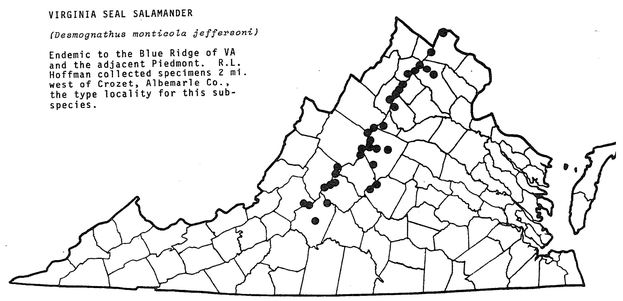

Figure 2. Range map for *Desmognathus monticola* (used with permission of VHS).

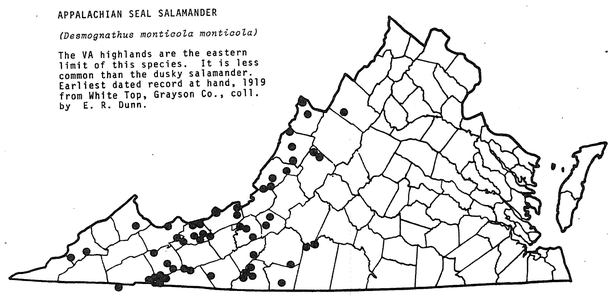

Figure 4. Range Map for *Desmognathus monticola monticola* (used with permission of VHS).

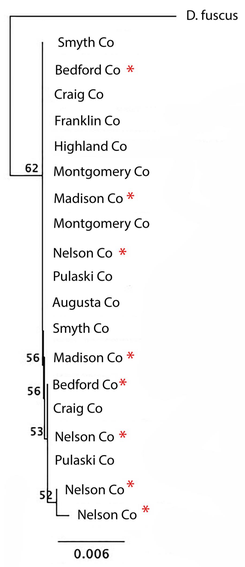

Figure 6. Cladogram of D. monticola sites by county (M. Becker)

* D. m. jeffersoni

Results and/or Conclusion

The purpose of this study was to determine the validity of the subspecific status of *Desmognathus monticola jeffersoni*, a subspecies of the Seal Salamander first described by Richard Hoffman in 1951. The subspecific designation was based on the difference in morphology between populations east and west of the Shenandoah Valley in northcentral Virginia. Those in the western portion of the state had the more typical spotted pattern, with small black spots on a gray background. Those in the more eastern portions of the state had a "reticulated" pattern with a more extensive black spotting which merged into wavy lines on the gray background. Because salamander morphology is very conserved, to the point where morphologically similar species are still being described on the basis of molecular rather than morphological differences, any morphological differences within what is presumed to be one species, is worthy of investigation. The subspecific status of *Desmognathus monticola jeffersoni* has been rejected because the two morphologies are not geographically separated. However, the different morphology may still have a genetic component. We used DNA sequence analysis to investigate the genetic status of populations of *Desmognathus monticola* from throughout Virginia.

From Figure 6 it can be seen that populations previously recognized as *Desmognathus monticola monticola* and *D. m. jeffersoni* group together when 16S rRNA sequences are compared. There was no separation of populations from a genetic standpoint. Those from east and west (*) of the Shenandoah Valley are interspaced among each other, showing no genetic differentiation on a geographic basis. From this, we ould conclude there is genetic basis for the recognition of *Desmognathus monticola jeffersoni* at any taxonomic level. This conclusion supports those reached by Beamer and Lamb (2020).

Future Work

The results presented here represent the work of more than two years of collecting tissue samples, extracting DNA, performing PCR reactions, and analyzing these data. While additional genetic work could be performed by utilizing additional gene fragments, it is doubtful additional analysis will produce differences not revealed by this study. We consider the question "is there a genetic basis for the recognition of *Desmognathus monticola jeffersoni* at any taxonomic level" to be settled, with the answer of no, there is not.

References and/or Acknowledgments

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