

A Tale of Two Salamanders

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In 1951, Richard Hoffman performed a survey of Virginian amphibians and found what he considered a new subspecies of *Desmognathus monticola* to the east of the Shenandoah Valley, and named the new subspecies *D. monticola jeffersoni* (Hoffman, 1951). This subspecies was considered unique by Hoffman because of its unusual reticulated dorsal pattern caused by less extensive black spotting that merged to form the reticulated coloration and the geographical separation it had from *D. monticola monticola*. However, Petranka (2001), denied Hoffman's subspecies classification of *D. monticola jeffersoni*. Petranka refers to Hoffman's key distinction of *D. m. jeffersoni*: less conspicuous dorsal patterning. Petranka then stated that he has found salamanders that fit Hoffman's description south of where the subspecies should exist. Without geographical separation, any subspecific designation for the Seal Salamander is not valid.

Desmognathus monticola, the seal salamander, occurs in the Appalachian Mountains from Pennsylvania to Georgia and Alabama (Conant, 1998; Petranka, 2010). *Desmognathus monticola* is in the family Plethodontidae, the lungless salamanders which respire entirely through their skin. Usually residing in well oxygenated mountain springs and streams in elevations below 1300 feet, *D. monticola* has a stout, gray body, about three to six inches in length (Conant, 1998; Martof et al., 1980; Petranka, 2010). The dorsal side has irregular black spots scattered randomly, and the tail of the salamander is laterally compressed and knife-shaped at the tip. *D. monticola* has a single line of white spots between the legs, cornified darkened friction pads on the toes, enlarged pre-maxillary teeth, and males are larger than females (Petranka, 2010). The young usually have four pairs of orange dots dorsally (Martof, 1980).

Desmognathus monticola jeffersoni lives towards the east of the Shenandoah Valley. The range of *D. monticola jeffersoni* extends about 15 miles east of the main ridge of the Blue Ridge mountains in Northern Virginia down through the Shenandoah Valley where Hoffman says the

subspecies stops at about Roanoke. He described the habitat for *D. m. jeffersoni* to be steep, cold mountain streams (Hoffman, 1951).

Morphologically there are a few differences between the two subspecies. In the *jeffersoni* form, the dorsal markings are reticulated, sparse, irregular, and sometimes absent on a grayish brown background; the venter is grayish white. The head of the *jeffersoni* form is darker than the back and there are black flecks behind the eyes. The head may also have a yellow diagonal mark from the eye to the lower jaw, edged in black. There is a gradual fading from the lower sides to the venter, with the lower sides faintly mottled. The knifed tail has dark flecks, with the limbs darker at the joints. A juvenile *D. m. jeffersoni* is usually more pigmented in pattern from the adult but lighter in background color.

A well accepted definition of a species is a reproductively isolated group of organisms (de Quieroz, 2005). A species must have mechanisms to prevent interbreeding, whether those are behavioral or morphological. A classification lower than species would have the capability of interbreeding. For an organism to have a subspecies classification, it must be morphologically distinct and be geographically separated. Petranka (2001) observed that *D. m. jeffersoni* mixes with *D. m. monticola* south of the Shenandoah Valley, so it cannot be considered a subspecies since there is not complete geographic separation. However, they may be considered distinct species if there is not interbreeding. The gene pools would be isolated if the two types of salamanders were not interbreeding so reproductive isolation should lead to genetic differences over time.

Salamander morphology is highly conserved across most salamander families. In many cases, the most definitive technique to classify salamanders is through genetic testing. Two

groups of salamanders may be morphologically indistinguishable but do not interbreed. This isolation causes a separation of the gene pool, and genetic differences accumulate over time.

Highton (1989) described protein electrophoresis of several salamanders of the genus *Plethodon*. Three different species, *P. aureolus*, *P. kentucki*, and *P. teyahalee* were found to be genetically separate from *P. glutinosus*, though these salamanders were considered to be one species when only morphological traits were used.

Tilley (1981) used gel electrophoresis to differentiate *D. fuscus* from another parapatric species *D. planiceps*. This is particularly significant because the original idea that these species were genetically distinct was proposed by Richard Hoffman. Tilley used genetic techniques to verify what Hoffman had originally hypothesized from slight differences in morphology.

Salamander morphology is so variable it has not proven to be very useful in differentiating closely related species. A more productive approach is to use molecular techniques, such as mitochondrial DNA sequencing. By performing genetic testing on the two forms of the Seal Salamander, we hope to see if genetic differentiation has occurred. There are no published accounts of genetic analysis on the Seal Salamander.

Like Highton and Tilley, the focus of this research is to use molecular techniques to differentiate salamanders based on DNA sequences. In salamanders, one of the most variable genes is the cytochrome b gene that codes for one of the cytochromes in the mitochondrial electron transport chain. This gene is found in the mitochondria, so after specimens were collected from east, west, and south of the Shenandoah Valley, mitochondria were isolated. Mitochondrial DNA was extracted from the samples and then PCR was used to amplify a 400 bp gene fragment of the cytochrome b gene. The DNA was then sent out to another lab to be

sequenced. The DNA sequence of the two forms of the Seal Salamander will be compared. With these data we hope to see if Hoffman's (1951) idea that they are somehow different, is correct.

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