

The Distribution and Identification of Two-lined Salamanders in Virginia

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The two-lined salamander, *Eurycea bislineata* (Green) has a broad distribution in eastern North America, occurring from southeastern Canada to Florida, and from the Mississippi River to the Atlantic ocean (Conant, 1975). The subspecies *bislineata*, *cirrigera*, *rivicola*, and *wilderiae* have traditionally been recognized according to the following morphological diagnostic characters: (1) cirri are present in *cirrigera* and some *wilderiae* (only detectable in sexually mature and active males). (2) Costal groove counts are 15-16 for *bislineata*, 13-14 for *cirrigera*, and 14-16 for *rivicola*. (3) The stripe rarely extends beyond the midpoint of the tail in *bislineata* and *wilderiae*, extends to the distal fourth or apex of the tail in *cirrigera*, and extends beyond the midpoint of the tail in *rivicola* (Mittleman, 1966). These morphological criteria have clearly been of value in distinguishing these subspecies. However, the existence of many and different range maps (Conant, 1958, 1975; Mittleman, 1966; Tobey, 1985; Jacobs, 1987) suggests that morphological data alone may not be sufficient.

A recent study by Jacobs (1987), which analyzed genetic variation in the group by protein electrophoresis, suggested that the complex comprises at least three separate species. He proposed elevating the previously recognized subspecies of *E. b. bislineata*, *E. b. cirrigera*, and *E. b. wilderae* to specific status. Although *E. b. bislineata* and *E. b. cirrigera* appear to behave as genetically distinct species, Jacobs called for a thorough investigation of the contact zone to determine if there really is reproductive isolation as one would expect to occur between species. The common occurrence of these two subspecies in Virginia makes the Commonwealth an ideal study site for such an investigation.

This is a preliminary report on our attempt to determine the ranges of *E. b. bislineata* and *E. b. cirrigera* in Virginia, using a combination of morphology and protein electrophoresis.

Materials and Methods

The morphological traits used to distinguish between species were the number of costal grooves, length of the tail stripe, and presence of cirri (in breeding males only). Protein electrophoresis used standard starch gel techniques (Selander et al., 1971) and the same proteins used by Guttman and Karlin (1986) and Jacobs (1987) to differentiate these salamanders. Samples were prepared using whole-body homogenates.

Both larvae and adults were captured by hand or hand net from 46 different populations. The collection sites are presented in Appendix A. Although whole-body homogenates were used for electrophoresis, specimens were usually photographed. These slides, and for some populations voucher specimens, are currently deposited in the Liberty University Museum of Natural Science collection.

Results and Discussion

Our first observation was that the electrophoretic protein markers were able to consistently differentiate the two forms. A clearly defined contact zone was found near the Greene/Albemarle County line and extended Westwards (Figure 1). Samples to the north were determined consistently to be *E. b. bislineata* and samples to the south were *E. b. cirrigera*.

A second observation was that none of the morphological characters was able to consistently discriminate the two forms, at least near the contact zone. The number of costal grooves was 14-16 for *E. b. bislineata* and 13-15 for *E. b. cirrigera*. There was an overlap with 14-15 costal grooves being found in both forms.

There was likewise no consistent pattern for the length of the tail stripe. *E. b. bislineata* had tail stripes from <50% the length of the tail to >75%. While most *E. b. cirrigera* had tail stripes >75% the length of the tail, many were observed to be <50%.

The presence of cirri in *E. b. cirrigera* and their absence in *E. b. bislineata* can only be compared in sexually active males. Not all the collections in this study were made late enough in the fall breeding season for a complete analysis to be performed. However, it was noted that some male *E. b. bislineata* from central Greene County did possess cirri. This observation eliminates the morphological trait for which *E. b. cirrigera* is named as a rigorous taxonomic indicator, at least in populations near the contact zone.

A possible reason for the breakdown of morphological characters in Virginia could be introgression. Most morphological traits are controlled by more than a single genetic locus. Gene flow between these two forms at one or more of these loci could account for the small number of individuals in many Virginia populations which do not appear to correspond with the subspecies expected at that locality. The finding of at least limited introgression beyond the contact zone of the genes coding for the protein markers tends to support this view.

Because Jacobs' (1987) study examined a large geographic area with widely separated samples, one can conclude only that the contact zone in central Virginia lies somewhere between Staunton and Bedford. In our study, the contact zone was identified at one specific point, namely the Roach River at County road 603, just north of the Greene/Albemarle County line. This is considerably north of the contact zone suggested previously and based on morphological characters (Conant, 1958; Mittleman, 1966; and Tobey, 1985), but consistent with data of Jacobs (1987).

In delineating the contact zone west of Charlottesville, we attempted to collect along north-south drainage systems. Along the Little Calfpasture and Calfpasture Rivers, the Augusta/Rockbridge County line marks the approximate location of the contact zone. *Eurycea b. bislineata* occurs along the Little Calfpasture north of Craigsville, and in the Calfpasture River drainage, in Augusta County. *Eurycea b. cirrigera* occurs along the Little Calfpasture south of Craigsville and in Stuart Run, a tributary of the Cowpasture River, in Rockbridge and Bath Counties.

In the very western part of Virginia, the contact zone was found to be just north of Lake Moomaw. *Eurycea b. bislineata* was found in a small tributary of Mill Creek, which like Back Creek, also flows into Lake Moomaw. The town of Mountain Grove, just west of Warm Springs, marks the approximate contact zone.

It should be noted that the contact zone does not always follow obvious geographic barriers such as drainage systems. As the contact zone was being narrowed, it was expected that the Buffalo Branch of Middle River with a northern drainage would be occupied by *E. b. bislineata* while the adjacent Little Calfpasture River with a southern drainage might be occupied by *E. b. cirrigera*. Instead, *E. b. bislineata* extends about ten km south into the headwaters of the Little Calfpasture River at Craigsville. It is possible that either the contact zone is not static but is moving either north or south or that geographic boundaries, such as river drainage systems play only minor roles in determining the position of the contact zone.

A more extensive analysis of the contact zone at the Greene and Albemarle County line and the distribution of the northern and southern forms is continuing. Such analysis of the Greene-Albemarle County contact zone is necessary to determine the taxonomic status of these salamanders, although preliminary results support Jacobs (1987) elevation to specific status. Analysis of the distribution is necessary to more fully delineate the contact zone. We have only examined a few drainage systems in widely separated areas to obtain the approximate range in the western portion of the State where the salamanders are most abundant. The precise contact zone is currently known only at the Roach River in Greene County. There remains considerable work to do not only in western Virginia to establish the contact zone in additional river systems, but in eastern Virginia where we currently have no data at all.

As work on this species complex continues, it must be emphasized that the ability of protein markers to consistently differentiate between *E. b. bislineata* and *E. b. cirrigera* with a higher degree of confidence than morphological markers alone necessitates their use in future studies of this complex.

Acknowledgements

Robert Littlejohn and Joseph Mitchell made significant contributions to this manuscript. A large number of individuals aided in the collection of specimens, including: Terry Spohn, George Damoff, Mike Hayslett, Steve Rhodes, Jeff Schmidt, Jeff Hampell, and Aubrey Neas.

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Appendix A. Collecting sites designated by stream and road intersections

Species N Locality

- E. b. cirrigera* (3) Tributary to Vaughns Creek, 1.5 km S. of US 460 Prince Edward Co., VA
- E. b. cirrigera* (1) Bell Creek at CO 626, 0.6 km N. CO 658, Prince Edward Co., VA
- E. b. cirrigera* (6) Brown's Branch of Buffalo Creek at CO 664, 1.4 km S. CO 669, Prince Edward Co., VA
- E. b. cirrigera* (4) South Fork of Spring Creek at CO 757, 0.6 km S. of CO 663, Prince Edward Co., VA
- E. b. cirrigera* (4) Rock Castle Creek at VA 8, 4.6 km NW. of VA 40, Patrick Co., VA
- E. b. cirrigera* (7) Austin Creek at US 60, Buckingham Co., VA
- E. b. cirrigera* (15) Stony Creek 0.8 km from end of CO 751, Nelson Co., VA
- E. b. cirrigera* (4) Tributary to Buffalo Creek at CO 722, 0.8 km S. of VA 56, Nelson Co., VA
- E. b. cirrigera* (3) Opossum Creek at CO 667, Campbell Co., VA
- E. b. cirrigera* (3) Right Hand Fork of Cub Creek at CO 644, Appomattox Co., VA
- E. b. cirrigera* (5) Bowler Creek at CO 628, Appomattox Co., VA
- E. b. cirrigera* (7) Suanee Creek at CO 621, Appomattox Co., VA
- E. b. cirrigera* (1) Rough Creek at CO 705, Appomattox Co., VA
- E. b. cirrigera* (1) Blackfoot Creek at CO 665, Appomattox Co., VA
- E. b. cirrigera* (15) North Fork of David Creek at CO 654, 1.9 km N. of VA 24, Appomattox Co., VA
- E. b. cirrigera* (1) Tributary to David Creek at CO 654, 0.6 km S. of CO 615, Appomattox Co., VA
- E. b. cirrigera* (1) Buck Mountain Creek at Co., 601, 0.5 km N. of CO 671, Albemarle Co., VA
- E. b. cirrigera* (14) North Fork of Rocky Creek at CO 671, 0.8 km E. of CO 609, Albemarle, Co., VA

- E. b. cirrigera* (7) Muddy Run on CO 687, 1.0 km E. of CO 810, Albemarle Co., VA
E. b. cirrigera (3) Doyles River at the intersection of CO 629 and CO 810, Albemarle Co., VA
E. b. cirrigera (3) Doyles River at the intersection of CO 687 and CO 810, Albemarle Co., VA
E. b. cirrigera (15) Lynch River at CO 603, Albemarle Co., VA
E. b. cirrigera (9) Lynch River at CO 810, 0.4 km N. of CO 663,
E. b. bislineata (2) Albemarle Co., VA
E. b. bislineata X *cirrigera* hybrids (2)
E. b. cirrigera (2) Welsh Run at CO 617, Green Co., VA
E. b. cirrigera (4) Roach River at CO 603, Greene Co., VA
E. b. bislineata (22)
E. b. bislineata X *cirrigera* hybrids (2)
E. b. cirrigera (2) Parker Branch of Roach River at CO 633, Greene Co., VA
E. b. bislineata (20) Haneytown Creek, N. of CO 631, 0.8 km W. of CO 630, Greene Co., VA
E. b. bislineata (13) Roach River at CO 810, Greene Co., VA
E. b. bislineata (29) Swift Run along US 33, 0.6 km N. of CO 625, Greene Co., VA
E. b. bislineata (22) Lynch River W. of CO 614 and E. of CO 628, Greene Co., VA
E. b. bislineata (5) Hazel River at CO 600, 3.4 km S. of CO 608, Rappahannock Co., VA
E. b. cirrigera (16) Stuart Run at CO 629, 0.2 km N. of CO 633, Bath Co., VA
E. b. cirrigera (16) Tributary of Mill Creek at CO 600, 10.6 km S. of VA 39, Bath Co., VA
E. b. bislineata (3) Tributary of Back Creek at CO 600, 2.9 km N. of Forest Route 6003, at High Voltage lines, Bath Co., VA
E. b. bislineata (2) Townsend Draft at VA 84, 33.6 km W. of CO 600, at Roadside Table, Highland Co., VA
E. b. cirrigera (3) Goshen Branch of Calfpasture River on VA 42, 8.3 km N. of CO 780 and 2.4 km S. of CO 687, Rockbridge Co., VA
E. b. cirrigera (5) Bratton Run on CO 780, 1.1 km S. of VA 42, Rockbridge Co., VA
E. b. cirrigera (6) Bratton Run at intersection of CO 780 and CO 850, Rockbridge Co., VA
- E. b. bislineata* (13) Jerkemtight Creek of Hamilton Branch of Calfpasture River on CO 399, 1.1 km W. of CO 629, Augusta Co., VA
E. b. bislineata (10) West Dry Branch of Calfpasture River on CO 688, 7.4 km E. of CO 629, Augusta Co., VA
E. b. bislineata (13) Smith Creek of Little Calfpasture River on CO 601, 0.8 km S. of VA 42 at Augusta Springs, Augusta Co., VA
E. b. bislineata (14) Little Calfpasture River at VA 42, 10.6 km S. of Buffalo Gap, Augusta Co., VA
E. b. bislineata (6) Headwaters of Little Calfpasture River at VA 42, 8.0 km S. of Buffalo Gap, Augusta Co., VA
E. b. bislineata (16) Buffalo Branch of Middle River, 4.3 km S. of Buffalo Gap on Va 42, Augusta Co., VA
E. b. bislineata (2) Buffalo Branch of Middle River, 2.2 km S. of Buffalo Gap on VA 42, Augusta Co., VA

***Catesbeiana* 1990, 10(1):11-18**