



# JEFFERSONIANA

*Contributions from the  
Virginia Museum of Natural History*

---

Number 14

October 15, 2004

A New Species of Woodland Salamander  
of the *Plethodon cinereus* Group from  
the Blue Ridge Mountains of Virginia

Richard Highton

ISSN 1061-1873

A New Species of Woodland Salamander of the  
*Plethodon cinereus* Group from the Blue Ridge  
Mountains of Virginia

Richard Highton

Department of Biology, University of Maryland,  
College Park, MD 20742 USA

ABSTRACT

A tenth species of Woodland Salamander of the *Plethodon cinereus* group from the central Blue Ridge Mountains of Virginia is described as *Plethodon sherando*. It differs morphologically from all other species of *Plethodon*. A preliminary analysis of allozyme variation indicates it also differs genetically from the other species of the *P. cinereus* group. It is known to occur at elevations between 579-1091 m in a small area of <100 km<sup>2</sup> in the vicinity of Big Levels, Augusta County, Virginia. Within most of its known range, *P. sherando* is the only eastern small *Plethodon* present, but at lower elevations it has been taken sympatrically with *P. cinereus* at five sites. A transect through the overlap zone between the two species near Stony Creek indicates that the altitudinal overlap is at least 46 m. The geographic distance between the highest and lowest sympatric populations in the overlap zone is at least 0.7 km. Since no F<sub>1</sub> hybrids were found, apparently the two species do not hybridize frequently, although rare introgression may occur.

Key words: Allozymes; Morphological variation; Plethodontid salamanders;  
*Plethodon cinereus*; *Plethodon sherando*, new species

## INTRODUCTION

The number of species of salamanders was greatly underestimated by classical morphological studies. Highton (1962) recognized only 16 species of woodland salamanders in the genus *Plethodon*. As a result of new discoveries and molecular systematic studies, 53 species are now recognized (Highton, 1995, 1997, 1999; Highton & Peabody, 2000; Lazell, 1998). This paper reports the discovery of an undescribed species of the *P. cinereus* group from an area known as Big Levels, in the Blue Ridge Physiographic Province of Augusta County, Virginia. A survey of small *Plethodon* populations at 30 localities in that area found the new species at 15 sites and *P. cinereus* at 20 sites. The two species were taken sympatrically at five sites with no evidence of current hybridization.

Nine species of the eastern North American *P. cinereus* group are currently recognized (Highton & Larson, 1979; Highton, 1999). Morphologically, there appear to be three clades in the group: (1) two geographically widespread species (*P. cinereus* and *P. serratus*), characterized by the frequent presence of a polymorphism in dorsal coloration (striped and unstriped morphs), an approximately equal amount of gray and white ventral mottling, and geographic variation in the modal number of trunk vertebrae (19-21); (2) four elongated species with moderate-sized ranges (*P. electromorphus*, *P. hoffmani*, *P. richmondi*, and *P. virginia*), usually occurring only in the unstriped color morph, characterized by darker venters resulting from reduced white mottling, and geographic variation in the modal number of trunk vertebrae (21-23); and (3) three dark-bellied montane species (*P. hubrichti*, *P. nettingi*, and *P. shenandoah*), isolated in small ranges at high elevations in the Blue Ridge mountains of central Virginia (*hubrichti* and *shenandoah*) and the Cheat Mountains in the Appalachian Plateau of West Virginia (*nettingi*), with a modal number of 19 (*nettingi* and *shenandoah*) or 20 (*hubrichti*) trunk vertebrae. Of the last seven species, the striped morph occurs only in *P. shenandoah* and *P. virginia*, but in the latter species it is rare. The first and second groups usually have relatively smaller heads and relatively shorter limbs than the third group. The undescribed species resembles the first group (*P. cinereus*

and *P. serratus*) in its dorsal color pattern polymorphism and its extensive ventral white pigmentation, and resembles the third group in its low modal number of trunk vertebrae (19), larger head, longer limbs, and its restricted montane geographic distribution. It is known only from a small area of the central Blue Ridge Physiographic Province of Virginia where it occurs at both high and low elevations.

#### MATERIALS AND METHODS

Based on sample sizes of 25 or more individuals, studies of geographic variation in the common Eastern Red-backed Salamander (*P. cinereus*) in the middle Atlantic states were reported by Highton (1962, 1972, 1977). A large proportion (90 of 94) of samples from the Blue Ridge Physiographic Province of Virginia have a modal number of 20 trunk vertebrae (mean=19.9; range 19.3-20.4). Only four samples have a modal number of 19 vertebrae (mean, 19.3-19.5). A sample with an even lower mean number of vertebrae (19.0) was taken from near the top of Bald Mountain, Augusta County, Virginia. It has some distinct structural features, while the other four samples with a modal number of 19 trunk vertebrae resemble *P. cinereus* in structure. In order to investigate further both anatomical and molecular variation in populations of small *Plethodon* in the Big Levels area, a survey of small *Plethodon* from 30 sites was made during 2003. A total of 509 salamanders from these populations are now available (including museum specimens from the earlier studies) of which 185 represent an undescribed species and 324 are *P. cinereus* (Table 1).

Estimates of the number of trunk vertebrae were made using the method of Highton (1957). The following characteristics were recorded from anesthetized animals in life: (1) number of costal interspaces (mean of both sides) between the toes of appressed limbs (Bishop, 1943); (2) head width, measured at its widest point; (3) body length, measured from the snout to the anterior angle of the vent; (4) the sum of the lengths of the four limbs (excluding specimens with regenerating limbs); and (5) in striped individuals the proportion of the area of the stripe containing red pigment was estimated by examination under a dissecting microscope.

Analysis of protein variation by the same methods used in the allozyme study of the *P. cinereus* group (Highton, 1999) was accomplished on 78 individuals of the new species from 14 sites and 127 *P. cinereus* from 18 sites. Common allelomorphs (hereafter referred to as alleles) of the new samples were compared with those of the 71 samples of the nine previously known species of the *P. cinereus* group in Highton (1999). One protein (LAP) from the latter study was not evaluated because one of the chemicals used in the assay may be carcinogenic to humans. Common alleles at each locus in the new samples were compared in side by side comparisons with the alleles of the nine species of the *P. cinereus* group from the earlier study. Some rare alleles could not be compared because all of the samples containing those alleles had been depleted in previous comparisons. Therefore identifications of a few rare alleles in the *P. cinereus* group are not known and so there may be minor errors in the estimation of genetic distances between the new species and the other nine species. Nei (1972) genetic distances ( $D$ ) were calculated by the PHYLIP program (Felsenstein, 1989) and these were used to estimate the phylogeny of the species of the group by the UPGMA method (Sokal & Rohlf, 1962). Cavalli-Sforza & Edwards (1967) chord distances were also calculated by the PHYLIP program and these were used to generate a NJ tree using the method of Saitou & Nei (1987). Genetic distances were calculated from mean frequencies of alleles at each locus within each of the nine previously recognized species from the data in Highton (1999), as well as the grouped samples of the new species and the grouped samples of *P. cinereus* from the Big Levels area. The samples within each of the two species were grouped because several samples are of small size and there is little geographic genetic variation in the Big Levels area within either species..

Whole animal homogenates were used in electrophoresis according to the methods given in Highton (1999). The remaining salamanders were preserved and are catalogued in the collection of the National Museum of Natural History (USNM).

## RESULTS

A total of 160 of 185 (86%) individuals of the new species possess 18 costal grooves, equivalent to 19 trunk vertebrae (range 18-20, mean 19.1), while 225 of 324 (69%) of *P. cinereus* possess 19 costal grooves, equivalent to 20 trunk vertebrae (range 19-22, mean 20.1). Salamanders of the new species are also different from local *P. cinereus* in several other morphological features.

Estimates of the proportion of the stripe area of the dorsum that was occupied by red pigment in 88 striped *P. cinereus* from 15 populations in the Big Levels area ranged from 30-98% (mean=80%). In 71 striped individuals of the new species from 11 sites, the range is 1-95% (mean=42%). In *P. cinereus*, 64 (73%) of striped individuals have >79% of the dorsal stripe region occupied by red pigment, but in the new species 55 (77%) striped individuals have <80% of the stripe occupied by red pigment.

The new species has a slightly larger head than *P. cinereus*. Figure 1 shows that the width of the head of the new species at all body sizes averages about 0.5 mm larger than that of *P. cinereus*, although there is considerable overlap in the range of variation between the two species.

The new species has longer limbs than *P. cinereus*. Figure 2 indicates little overlap between the two species in the Big Levels area when the sum of the lengths of the four limbs is compared to body length. Since the undescribed species has fewer vertebrae and longer legs than *P. cinereus*, the number of costal interspaces between the toes of the appressed limbs is also diagnostic (Figure 3) with little overlap between the species.

A comparison of allozymes also provides convincing evidence that the two morphotypes in the Big Levels area represent different species. Hass (1985), in an allozyme study of geographic variation within *P. cinereus*, found that the species includes four differentiated geographic groups. The genetic divergence among these groups is high (range of Nei  $D=0.11-0.16$ ), but because there is extensive hybridization between groups at their geographic contacts (Hass, unpublished data), she retained all four groups within a single species. Three of her groups (II, III, IV) are represented in

Highton (1999). The local sympatric *P. cinereus* closely resemble Hass' Group II with a Nei  $D=0.02$ . At 16 loci evaluated electrophoretically there is little or no difference in allele frequencies between *P. cinereus* and the undescribed species. There are differences in the remaining seven genetic loci.

At the EST- $\beta$  locus, the frequency of allele  $n$  in *P. cinereus* is 0.97, but this allele is absent from all samples of the new species. It possesses nine alleles:  $f, h, i, j, k, m, q, r,$  and  $s$ . Alleles  $j$  (0.36),  $m$  (0.38), and  $q$  (0.16) are

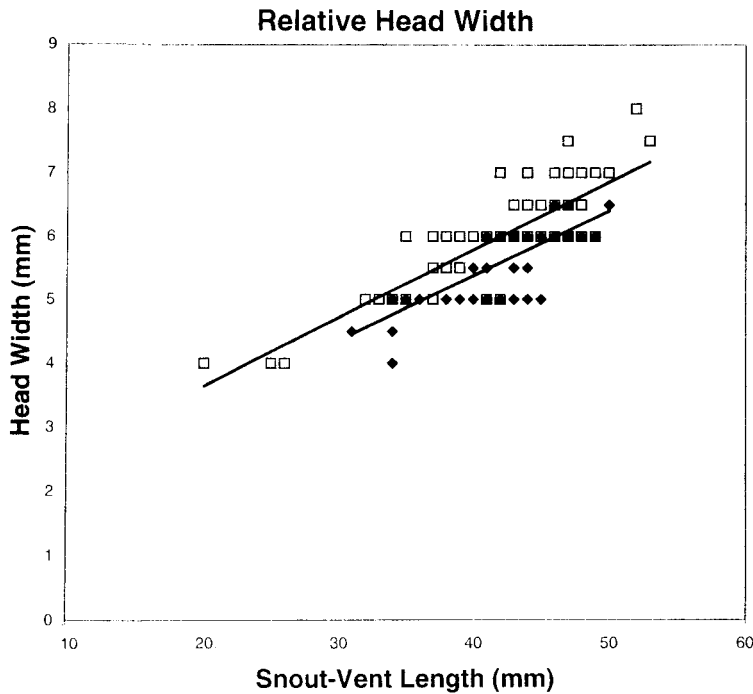


Figure 1. Covariation of head width (at the widest point) and body size (snout to anterior angle of the vent) in 112 *P. sherando* (hollow squares) and 148 *P. cinereus* (solid diamonds). Some symbols represent more than one individual. Linear regression lines calculated by the method of least squares are shown for both species.

the most frequent. All but three (*h*, *i*, *k*) of these alleles occur in *P. shenandoah*, and some also occur in other species of the *P. cinereus* group.

At the AAT-1 locus, all local *P. cinereus* surveyed are monomorphic for allele *e*, but this allele was not found in the new species. The most common of three alleles in the new species is *d* with a frequency of 0.98. Allele *d* is also common in *P. electromorphus*, *P. hubrichti*, *P. richmondi* and *P. shenandoah*.

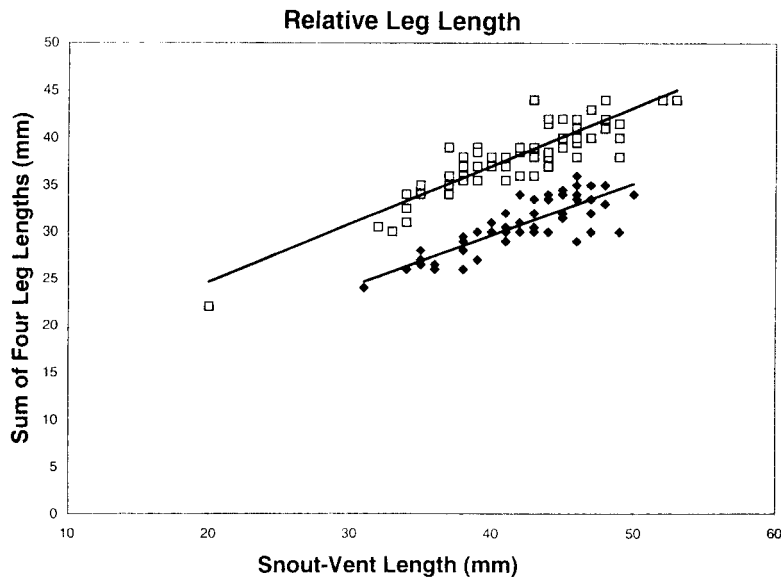


Figure 2. Covariation of the sum of all four leg lengths and body size (snout to anterior angle of the vent) in 110 *P. shenandoah* (hollow squares) and 148 *P. cinereus* (solid diamonds). Some symbols represent more than one individual. Linear regression lines calculated by the method of least squares are shown for both species.

At the ICD-2 locus, all local *P. cinereus* are homozygous for allele *d*, while in the new species all but one individual is homozygous for allele *b*. That individual is a *bd* heterozygote. Allele *b* is also common in *P. electromorphus*, *P. richmondi*, and *P. serratus*.



At the PEP locus, *P. cinereus* is monomorphic for allele *d*. All individuals of the new species are homozygous for allele *b* (also common in *P. serratus*), except for one individual heterozygous for a slower allele (probably *d*).

At the PT-5 locus, *P. cinereus* is variable with at least five alleles, all slower than the single allele found in the new species (either allele *a* or an allele with a mobility close to *a*).

At the PGDH locus, both species in the Big Levels area are variable for the same three alleles (*a*, *b*, and *d*), but they occur at different frequencies in the two species: (0.04 *a*, 0.73 *b*, 0.23 *d*, in *P. cinereus*) and (0.49 *a*, 0.43 *b*, 0.08 *d*, in the new species). An unidentified slower allele occurred in one *P. cinereus* and an unidentified faster allele in one individual of the new species, both as heterozygotes.

At the TRF locus, the new species has two alleles not found in any other species of the *P. cinereus* group, both with a mobility between alleles *b* and *c* of the Highton (1999) study. The fastest of these two alleles is the most common one with a frequency of 0.90. In *P. cinereus* of the Big Levels area there are five TRF alleles. The two most common alleles in *P. cinereus* are *g* with a frequency of 0.61, and a slower allele (*j* or *k*) with a frequency of 0.27.

There is no evidence of current hybridization between the two species. In the loci with significant genetic divergence between the two species, there are no individuals that are heterozygous for alleles that distinguish the two species at most or all of the seven diagnostic loci, as would be expected in  $F_1$  heterozygotes. Rare hybridization might account for the genotypes of the few individuals that are heterozygous at a single locus for diagnostic alleles of both two species, but this does not necessarily indicate recent hybridization because it is possible that the rare alleles could be descended from a common ancestor.

Although a number of rare alleles were not identified, tentative genetic distances were calculated between the new species, local *P. cinereus*, and the other nine species of the *P. cinereus* group. The UPGMA tree obtained by using Nei (1972) *D*-values has the same topology for the other nine

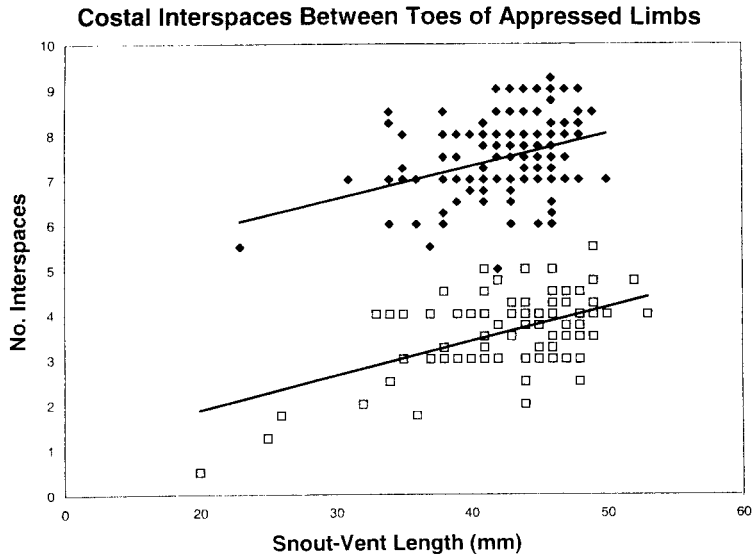


Figure 3. Covariation of the number of costal interspaces between the toes of appressed limbs and body size (snout to anterior angle of the vent) in 106 *P. sherando* (hollow squares) and 148 *P. cinereus* (solid diamonds). Some symbols represent more than one individual. Linear regression lines calculated by the method of least squares are shown for both species.

species of the *P. cinereus* group as that in Highton (1999) even though it is based on a slightly different set of 23 loci. The new species is a sister species to *P. serratus* ( $D=0.34$ ) on the new UPGMA tree, although its  $D$ -values to two other species are slightly lower; to *P. shenandoah* (0.31), and to *P. cinereus* (0.33). The  $D$ -values of the new species to other species of the *P. cinereus* group are: *P. electromorphus* (0.36), *P. richmondi* (0.37), *P. virginia* (0.43), *P. nettingi* (0.45), *P. hoffmani* (0.49); and *P. hubrichti* (0.56). The local samples of *P. cinereus* Group II have a mean  $D=0.02$  to the two Group II *P. cinereus* samples in Highton (1999), and a  $D=0.27$  to the new species. In the new NJ tree, the new species is also a sister species to *P. serratus*, but the remainder of the tree is partially different from the NJ tree in Highton (1999). The results of previous molecular studies on the *P.*

*cinereus* group using allozymes (Highton, 1999; Highton & Larson, 1979), albumin immunological distances (Maxson et al., 1979), and mtDNA sequencing (Sites, et al., 2004) all indicate that the species are closely related and thus may all have diverged from each other at about the same time, probably during arid conditions in the late Pliocene Epoch (Highton, 1995). If this is the case, it is not surprising that the topology of trees attempting to reconstruct the phylogeny of the species of this group have low statistical support (Highton, 1991, 1993, 1999). On the other hand, the monophyly of the group has strong support, based on morphology (Highton, 1962), allozymes (Highton, 1991, 1993, 1999, Highton & Larson, 1979), immunology (Maxson, et al. 1979); and mitochondrial DNA sequences, Sites et al., 2004).

Both morphological and genetic data strongly support the recognition of the new form as a distinct, reproductively isolated, species.

***Plethodon sherando*, new species**

*Diagnosis:* An eastern small *Plethodon* of the *P. cinereus* group (Highton & Larson, 1979). Males possess the diagnostic characters of the species group (Highton, 1962): small size, elongated body, enlarged anterior cusps on the male premaxillary teeth, and a large semicircular mental gland on the anterior chin of adult males. Of the nine previously known species of the *P. cinereus* group, *P. sherando* most closely resembles *P. cinereus* and *P. serratus* morphologically. All three have abundant gray and white (or yellow) ventral pigmentation whereas the other seven species usually have reduced white ventral pigmentation. In *P. cinereus* and *P. serratus* there is often approximately an equal amount of both pigments, but in *P. sherando*, there usually is more white pigment than gray, so that the mean amount of gray ventral pigmentation is less than in all the other species of the group. Both striped and unstriped morphs occur in *P. sherando*, as in many populations of *P. cinereus* and *P. serratus*, two of the three isolates of *P. shenandoah*, and several other species of *Plethodon* in other species groups (*P. angusticlavius*, *P. dorsalis*, *P. ventralis*, *P. websteri*, *P. vehiculum*). The red coloration on the dorsum of

