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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^2 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_1 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- β 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

Relative Head Width

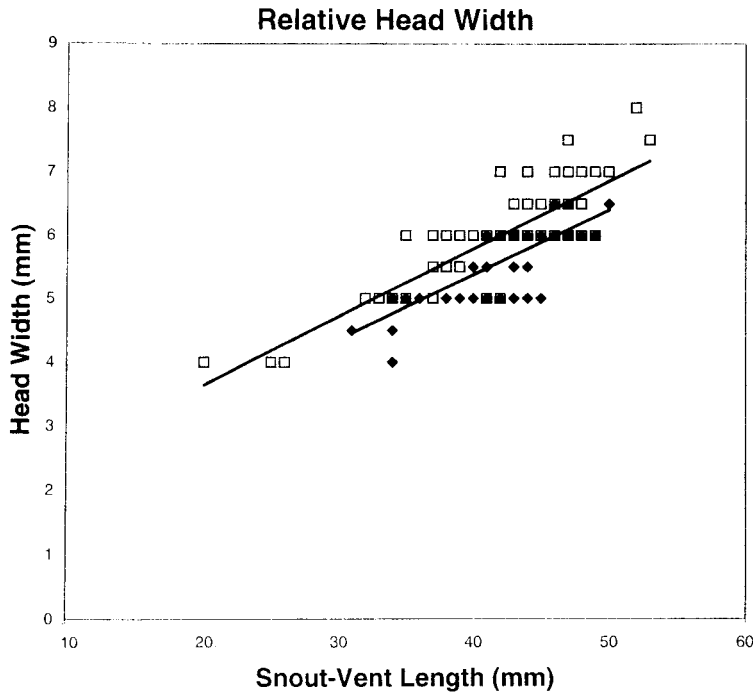*X-axis: Snout-Vent Length (mm); Y-axis: Head Width (mm)*

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*..
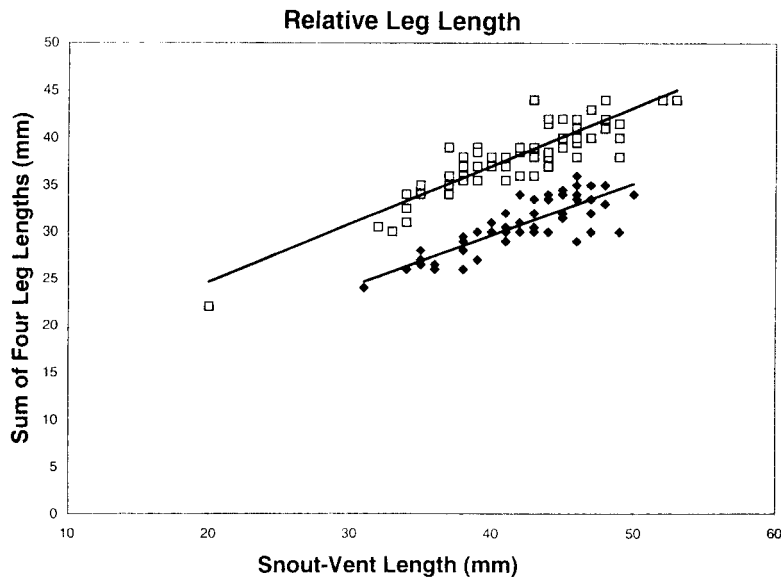
Relative Leg Length

Figure 2. Covariation of the sum of all four leg lengths and body size (snout to anterior angle of the vent) in 110 *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.

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

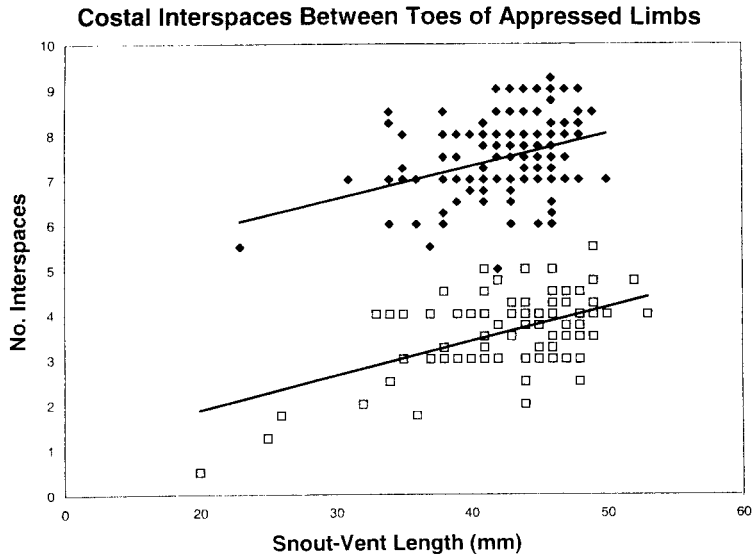**Costal Interspaces Between Toes of Appressed Limbs**

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

the striped morph resembles that commonly occurring in striped *P. cinereus* and *P. serratus*. The striped morph of *P. sherando* often has less red pigment in the dorsal stripe than most striped individuals of *P. cinereus* and *P. serratus*. The borders of the stripe in *P. sherando* are not serrated, as in the Arkansas-Oklahoma isolate and some Louisiana P. *serratus*, but are straight-edge as in most striped *P. cinereus* and most *P. serratus* from the Missouri and Alabama-Georgia-North Carolina-Tennessee isolates. A color pattern, rarely present in the other striped species of the *P. cinereus* group, often occurs in *P. sherando*: some of the dorsal red pigment may be

Figure 4. Photograph of a male striped morph (holotype, USNM 556159) and a male unstriped morph (paratype, USNM 556161) of *P. sherando* in life.

concentrated into a series of small irregular-shaped dorsolateral spots (as in the striped holotype, Figure 4). In the dorsum of the unstriped morph of *P. sherando,* the gray coloration in the area of the dorsum occupied by the stripe in the striped morph is often slightly lighter (as in the unstriped paratype, Figure 4). Thus a faint outline of a stripe (but without red pigment) may be present in the unstriped morph of *P. sherando,* resembling that often seen in unstriped individuals of *P. dorsalis* and *P. ventralis* of the *P. welleri* group. The modal number of trunk vertebrae in *P. sherando* is 19 (18 costal grooves when counted by the method of Highton, 1957). There is geographic variation in the modal number of trunk vertebrae in *P. cinereus* and *P. serratus,* but in the Blue Ridge Province of Virginia, the modal number in *P. cinereus* is usually 20 (Highton, 1962; Table 5). The head of *P. sherando* averages about 0.5 mm wider than the head of *P. cinereus* of the same size (Figure 1). In *P. sherando,* leg length is longer than that of *P. cinereus* with very little overlap in variation between the two species when the sum of the lengths of all four limbs is compared (Figure 2). Because of the longer limbs and shorter trunk of *P. sherando,* the number of costal interspaces between the toes of appressed limbs (Bishop, 1943) is a good diagnostic character to separate the two species (Figure 3). *Plethodon sherando* also differs genetically from the other species of the *P. cinereus* group as discussed above.

Holotype: USNM 556159, an adult male of the striped morph collected on the northwest slope of Bald Mountain (37° 55' 09" N, 79° 04' 00" W) at an elevation of 1055 m, Augusta County, Virginia, by Glenn A. Marvin and Richard Highton on 17 May 2003 (Figure 4).

Allotype: USNM 556160, an adult female of the unstriped morph with the same collecting data as the holotype.

Description of holotype: Before preservation, length from tip of snout to anterior angle of the vent 42 mm, to posterior angle of vent 44 mm, and total length 95 mm; 18 costal grooves; vomerine teeth 7 on the right side and 6 on the left side. Head width at widest point 5 mm, head length (snout to gular fold) 9 mm; front limbs 9.5 mm (right) and 9 mm (left); both hind limbs 10 mm in length; 3 costal interspaces between the toes of appressed limbs on both sides, with typical *P. cinereus* group semicircular mental

gland and premaxillary teeth with enlarged anterior cusps. In life, the holotype had a straight-edged red dorsal stripe of which about 80% is of red pigment and 20% dark gray pigment, the latter is the same color as that on the remainder of the dorsum and sides. Much of the dorsal red pigment is concentrated in 18 dorsolateral pairs of irregular-shaped red spots where the gray pigment is largely absent (this color pattern often occurs in striped individuals of *P. sherando* in which the central region of the stripe consists primarily of gray pigment). The red stripe continues on the tail with abundant red pigment on the anteriormost 8 mm, but red pigment is reduced posteriorly on the tail. There is a red "V" on the top of the head behind the snout with the apex pointing anteriorly. There are no small white spots on the dorsum, but there are a few brassy flecks on the dorsum both inside and outside of the stripe. Both small white spots and brassy flecks are present on the top of the head and on the eyelids. There is abundant brassy pigmentation in the upper part of the iris of the eyes. Laterally there is about an equal amount of white and gray pigmentation. About 75% of the venter is covered with yellow pigmentation and 25% with gray pigment. There is little gray melanophore pigmentation on the chin. About half the chin has yellow pigment and the remaining half is pigment-free. The legs have small white spots and brassy flecking dorsally.

Description of allotype: Before preservation, length from tip of snout to anterior angle of the vent 46 mm, to posterior angle of vent 49 mm, and total length 105 mm; 18 costal grooves; vomerine teeth are 6 on the right side and 7 on the left side; head width at widest point 6 mm, head length 10.5 mm; front limbs 10 mm in length; hind limbs 11 mm in length; 4 costal interspaces between the toes of appressed limbs on both sides. The pigmentation of the unstriped allotype is similar to that of the holotype, except for the complete absence of red pigment and reduced lateral yellow pigmentation.

Paratypes: USNM 419385-406, 419408-11, 556161-62, all from the same locality as the holotype.

Distribution: *Plethodon sherando* was found at 15 sites in the vicinity of Big Levels, Augusta County, Virginia (Figure 5), at elevations ranging from 579 m (Lake Sherando) to 1091 m (top of Bald Mountain) (Table I).

At all higher elevations sites in the Big Levels area (Bald Mt., Flint Mt., Green Pond, Kennedy Ridge, Cellar Mt.), as well as those at intermediate elevations along the Stony Creek road, it was the only species of small *Plethodon* encountered. A transect was made along the Stony Creek road in the area of overlap between the two species. Both species were taken sympatrically at four sites (samples 3-6 of *P. sherando* and 20-23 of *P. cinereus*). The ratio of *P. sherando*: *P. cinereus* declines through the overlap zone from 26:3 at the highest site to 1:17 at the lowest site. The elevation of the lowest site in the overlap zone on this transect is 646 m, and that of the highest site is 692 m, indicating an altitudinal overlap zone of at least 46 m. The geographic distance between the highest and lowest sites is 0.7 km. (0.9 road km). At another low elevation site (628 m) at the Cellar Mt. trail head on Coal Road, the two species were also taken sympatrically (samples 1, 16). At the lowest site (579 m) for *P. sherando* (sample 13), at Lake Sherando, three individuals were taken in a rocky talus slope just west of the dam, but at the campground only 0.7 km to the southwest, 26 *P. cinereus* (sample 27) were taken at about the same elevation. Based on the available information, the distribution of the two species appears to be largely parapatric with little overlap in their ranges.

Most of the rocks at the surface in the range of *P. sherando* are members of the Antietam Formation, a resistant quartzite interbedded with less resistant sandstone of late Precambrian and/or Lower Cambrian age (Schwab, 1970). The Big Levels area of Virginia is one of five widely scattered high elevation areas of Virginia that receives an average of <50 inches of rainfall per year. The only other area in the north-central part of the state that receives this much rainfall also is in the Blue Ridge Physiographic Province and includes the range of *P. shenandoah* (van der Leeden, 1993).

Name: *Plethodon sherando* is named for Sherando Lake, a George Washington National Forest Recreation area. The lake in turn is named for an Iroquois Indian chief who lived in the Shenandoah Valley of Virginia. I suggest the common name: Big Levels Salamander. The known range of *P. sherando* encompasses a section of the Blue Ridge known as "Big Levels" which includes a fairly wide flat ridge top 10 km long at about the

same elevation (900-1040 m). From it side ridges extend north, northeast, and northwest from the main crest of the Blue Ridge at Bald Mountain (1091 m). This is not the only "Big Levels" in the southern Appalachians; there is another one in Nelson County, Virginia, only 12 km to the east.
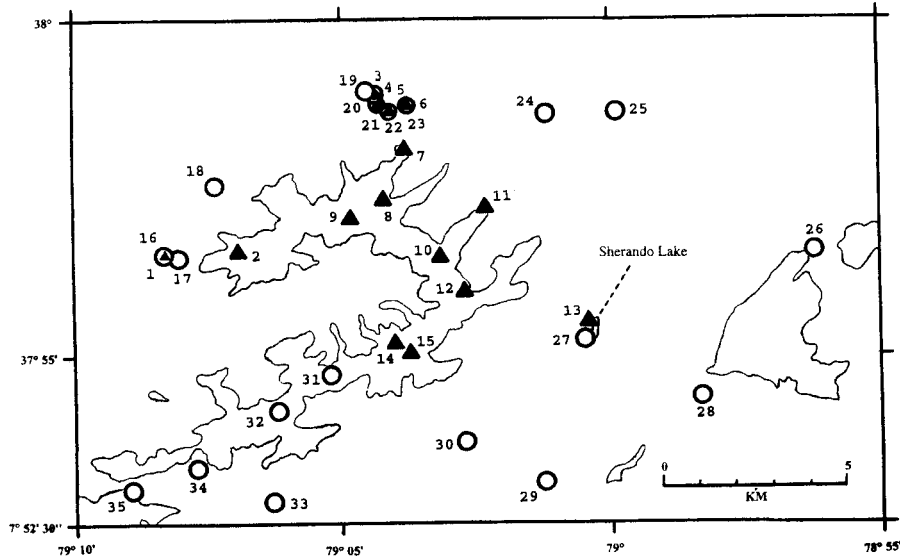

Figure 5. Map of the Big Levels area of the Blue Ridge Physiographic Province of Augusta and Nelson counties, Virginia, indicating sites (numbered as in Table 1) for 15 populations of *P. sherando* (solid triangles) and 20 populations of *P. cinereus* (hollow circles). The 3000 ft (=914 m) contour line is shown.

DISCUSSION

The known locality records of *P. sherando* indicate that it may be restricted to a limited area of the central Blue Ridge Province of Augusta County, Virginia. After the discovery of *P. shenandoah* on three of the highest mountains in Shenandoah National Park in the northern part of the Blue Ridge Province of Virginia in the mid-1960s (Highton &

Worthington, 1967), I searched at many sites in the central Blue Ridge Mountains of Virginia in the hope of finding *P. shenandoah, P. hubrichti*, or a new form allied to the dark-bellied trio of montane species, without success. Later, Thurow (1999) searched the same area and apparently misidentified Group II *P. cinereus* from three sites as *P. shenandoah* (Carpenter et al., 2001; Sites et al., 2004). One of the sites I visited three times from 1965-1968 was on Bald Mountain, the type locality of *P. sherando* (locality 14), obtaining 26 specimens (paratypes, USNM 419385-406, 419408-11). At that time I noted the unusual morphological features of the population. However, the morphological differences between *P. sherando* and *P. cinereus* are among several morphological characters that are known to be geographically and locally variable in *P. cinereus* (Highton, 1960, 1972, 1977). At that time, when the diagnostic characters that taxonomists used to classify animals were usually morphological, one had to be careful not to recognize populations as distinct species by using characters that were known to be so locally variable within species. This was before it was known that the genus *Plethodon* (as well as numerous other animal groups) had numerous cryptic species hidden within many of its recognized taxa. Moreover, the Bald Mountain salamanders had heavily mottled bellies, unlike the three montane endemics (*P. hubrichti, P. nettingi*, and *P. shenandoah*) of the *P. cinereus* group. Thus I failed to recognize that the sample from Bald Mountain represented an undescribed species until 2003 when proteins of two new specimens of *P. sherando* were first analyzed electrophoretically.

Between the 1960s and 2003, I did little fieldwork in the central Blue Ridge Mountains of Virginia and have not investigated all of the highest mountains of the area. My collections of >25 salamanders from seven high elevation (>1000 m) sites (Cow Camp Gap, Pompey Mt., The Priest, Rocky Mt., Salt Log Gap, Wiggins Spring, and Yankee Horse Ridge), as well as one series of 10 specimens from Cole Mt., include only *P. cinereus*, as do others reported in Sites et al. (2004). Similarly, lower elevation collections also include only *P. cinereus*. Now that it is easy to distinguish *P. sherando* and *P. cinereus* both morphologically and genetically, another search of the area may be in order to see if other populations of *P. sherando* exist.

The four montane endemics of the *P. cinereus* group occur in somewhat different habitats. As far as is known, *P. hubrichti* and *P. sherando* occur at both high and low elevations throughout their respective small ranges and both are found sympatrically with *P. cinereus* only at the periphery of their ranges. *Plethodon nettingi* is largely restricted to high elevation spruce forests, where it is sometimes the only small *Plethodon* present, but it often also occurs in sympatry with *P. cinereus*. The interaction of *P. shenandoah* and *P. cinereus* is unusual: the former species is confined to high elevation northwest facing rocky talus slopes that *P. cinereus* rarely enters in two isolates of *P. shenandoah*, but in the third isolate, *P. cinereus* occurs in sympatry with *P. shenandoah* throughout the isolate (Highton & Worthington, 1967; Highton, 1972). Thus it is difficult to predict the habitats where new species of *Plethodon* might occur, and considering the number of morphologically cryptic species already discovered in the genus, whether or not undescribed species will be morphologically distinguishable.

ACKNOWLEDGEMENTS

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Table 1. Locality information and data on two morphological characters for samples of 15 populations of *Plethodon sherando* and 20 populations of *P. cinereus* from the Blue Ridge Physiographic Province of Augusta and Nelson Counties, Virginia.

Pop No.	Species Plethodon	County	Latitude ° ′ ″	Longitude ° ′ ″	Elevation m	Sample Size	Mean No. Trunk Vertebrae	No. Striped	No. Unstriped
1	sherando	Augusta	37 56 34	79 08 14	628	1	19	1	0
2	sherando	Augusta	37 56 31	79 07 14	1024	4	19.0	3	1
3	sherando	Augusta	37 58 52	79 04 15	646	1	19	1	0
4	sherando	Augusta	37 58 47	79 04 15	661	7	19.3	6	1
5	sherando	Augusta	37 58 43	79 01 06	671	4	19.5	4	0
6	sherando	Augusta	37 58 43	79 03 49	692	38	19.2	24	14
7	sherando	Augusta	37 58 05	79 03 48	924	6	19.2	4	2
8	sherando	Augusta	37 57 21	79 04 17	975	7	19.0	5	2
9	sherando	Augusta	37 57 08	79 04 52	1039	2	19.0	2	0
10	sherando	Augusta	37 56 32	79 03 02	975	29	19.0	24	5
11	sherando	Augusta	37 57 09	79 02 20	945	3	19.3	2	1
12	sherando	Augusta	37 55 01	79 02 49	985	6	19.0	5	1
13	sherando	Augusta	37 55 33	79 00 16	579	3	19.0	3	0
14*	sherando	Augusta	37 55 09	79 04 00	1055	72	19.0	54	18
15	sherando	Augusta	37 55 02	79 03 49	1091	2	18.5	2	0
					Total		19.1	140	45

* Type locality of *Plethodon sherando*

Table 1. Continued.

16	*cinereus*	Augusta	37 56 34	79 08 14	628	29	20.1	25	4
17	*cinereus*	Augusta	37 56 27	79 07 58	747	4	20.0	4	0
18	*cinereus*	Augusta	37 57 55	79 07 00	628	4	20.0	3	1
19	*cinereus*	Augusta	37 58 54	79 04 27	585	27	20.4	19	8
20	*cinereus*	Augusta	37 58 52	79 04 15	646	17	20.6	17	0
21	*cinereus*	Augusta	37 58 47	79 04 15	661	27	20.3	22	5
22	*cinereus*	Augusta	37 58 43	79 01 06	671	2	20.5	0	2
23	*cinereus*	Augusta	37 58 43	79 03 49	692	3	20.7	3	0
24	*cinereus*	Augusta	37 58 35	79 01 17	549	4	20.0	3	1
25	*cinereus*	Augusta	37 58 34	78 59 49	488	27	20.4	25	2
26	*cinereus*	Augusta	37 56 29	78 56 12	884	37	20.2	22	15
27	*cinereus*	Augusta	37 55 15	79 00 30	573	26	20.1	24	2
28	*cinereus*	Nelson	37 54 22	78 58 22	716	1	20	1	0
29	*cinereus*	Nelson	37 53 04	79 01 17	747	12	20.0	12	0
30	*cinereus*	Augusta-Nelson	37 53 41	79 02 38	792	41	20.1	29	12
31	*cinereus*	Augusta	37 54 43	79 05 10	1006	1	19	1	0
32	*cinereus*	Nelson	37 54 10	79 06 20	1024	13	20.1	10	3
33	*cinereus*	Nelson	37 52 52	79 06 20	683	7	19.9	6	1
34	*cinereus*	Nelson	37 53 23	79 07 42	853	9	20.1	8	1
35	*cinereus*	Nelson	37 53 03	79 08 55	945	33	19.7	26	7
						Total	20.1	260	64