RANGE DELIMITATION OF A NORTH CAROLINA ENDEMIC SALAMANDER, THE BLUE RIDGE GRAY-CHEEKED SALAMANDER, *PLETHODON AMPLUS.*

A thesis presented to the faculty of the Graduate School of Western Carolina University in partial fulfillment of the requirements for the degree of Master of Science.

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RANGE DELIMITATION OF A NORTH CAROLINA ENDEMIC SALAMANDER, THE BLUE RIDGE GRAY-CHEEKED SALAMANDER, *PLETHODON AMPLUS.*

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The Southern Appalachian Mountains are a global hotspot for salamander biodiversity including many endemic species. These endemic montane salamanders have limited ranges and are vulnerable to anthropogenically induced habitat shifts. *Plethodon amplus* (the Blue Ridge Gray-Cheeked Salamander) is a North Carolina endemic salamander whose current published range is likely inaccurate due to data deficiencies. *Plethodon amplus* is visually indistinguishable from other Gray-Cheeked Salamanders which occupy adjacent mountain ranges, making it difficult to locate exact boundary lines between species. To re-delineate the range of *P. amplus*, I collected tissue samples from Gray-Cheeked Salamanders from sites surrounding and within the known range of *P. amplus*. I extracted DNA from each tissue sample then amplified and sequenced mtDNA using primers for three protein-coding regions. This study found evidence that current published boundary lines for two of the Gray-Cheeked Salamander species (*P. amplus* and *P. meridianus*) are larger than currently accepted. The results did not indicate clear species boundaries and suggested that there was likely genetic exchange between species in their past evolutionary history which led to mitochondrial capture. Future studies using next-generation

sequencing techniques will be necessary to draw accurate boundary lines between species of Gray-Cheeked Salamander.

INTRODUCTION

Documenting biodiversity is increasingly important during the current global wave of defaunation. Loss of biodiversity negatively impacts ecosystem function and services. For example, it has decreased carbon cycling, increased soil erosion, and decreased water quality and stream respiration (Dirzo et. al. 2014). Biodiversity loss can also have widespread and unexpected results on the impacted communities. For example, in ecosystems that experienced amphibian defaunation, snake communities declined in biodiversity as well as body condition, particularly in species for which frogs are a main food source (Zipkin et al. 2020). Amphibians are disproportionately impacted by defaunation. Nearly half of all amphibian populations are currently in decline (Barnosky 2011, Dirzo et al. 2014). Amphibian species with small ranges are particularly vulnerable to anthropogenically driven habitat loss (Sodhi et al. 2008). Understanding the limits of a species' range, and factors that contribute to these limits, are key to developing management plans aimed at addressing the specific threats to a species.

Cryptic species present a challenge to determining species' ranges and documenting biodiversity. Although morphologically indistinguishable, cryptic species represent distinct lineages. The recent development of molecular methods for examining genetic relatedness has contributed to a surge in the identification of cryptic species (Tilley & Mahoney 1996, Highton & Peabody 2000, Pages et al. 2009). Plethodontidae, a family of lungless salamanders, includes a large number of cryptic species that have been recently described with the aid of molecular methods (Camp & Wooten 2016). The *Plethodon jordani* complex a group of seven cryptic species, once considered a single species, that researchers described with the help of allozyme analyses (Highton & Peabody 2000). Although analyses using allozyme data were influential in

the recognition of cryptic species within Plethodontidae, current molecular methods use DNA sequencing to detect interspecific differences in DNA that were previously undetectable with allozyme data, including those in non-coding genes and silent mutations in coding genes (Camp & Wooten 2016, Patton et al. 2019).

Salamanders of the family Plethodontidae represent the majority of salamander biodiversity within the Southern Appalachian Mountains. The Appalachian Mountains are a global diversity hotspot for many plant and animal taxa, harbor relatively high concentrations of endemic species, and are a world center of salamander diversity (Parmesan 2006, Lyons et al. 2016, Stein et al. 2000, Petranka 1998). In montane regions, changes in elevation are often accompanied by changes in abiotic conditions such as temperature and precipitation which help shape community compositions (Acharya et al. 2011, Lyons et al. 2016). These gradients can produce multiple diverse habitats on a single mountain which contributes to the phenomenon of most plant and animal taxa reaching their highest species richness in these regions (Parmesan 2006, Lyons et al. 2016). This diversification of habitat types across elevations can restrict montane species to particular elevations. For example, in the "Sky Islands" of Arizona, mesic forest habitat is surrounded by desert conditions at lower elevations which limit dispersal of forest species to suitable habitat on adjacent mountains (Heald 1951). The ranges of Plethodontidae in the Southern Appalachians are also limited by differing habitat suitability based on elevation (Kozak & Wiens 2006). Plethodontidae are lungless salamanders that rely on having moist skin for respiration. This trait largely restricts them to cool and moist habitats within their ranges (Wake 1967). These restrictions, along with climatic-niche conservatism of plethodontid salamanders over evolutionary timescales, prevent the dispersal of some species through warmer, drier lowlands, resulting in isolated populations confined to mid to high

elevation habitats (Kozak & Wiens 2006, Wake 1967, Wake & Lynch 1976). Biotic factors such as competitive interactions have also contributed to elevational range limits of species including *P. jordani* and *P. teyahalee*, which exhibit interspecies aggression (Hairston 1980). Unfortunately, these factors that limit elevational mobility, along with low vagility, have made members of the family Plethodontidae particularly vulnerable to climate change and land development (Lyons & Kozak 2019). Currently, it is believed that over half of all Plethodontid salamanders are imperiled (IUCN 2017).

The Blue Ridge Gray-Cheeked Salamander (*Plethodon amplus*) is a species within Plethodontidae that is found only in a small area of North Carolina, specifically in the mountains around Hickory Nut Gorge in Buncombe, Henderson, and Rutherford counties (Fig. 1; Highton & Peabody 2000). This species is immensely data deficient, is labeled as a "Knowledge Gap Research Priority" by the North Carolina Wildlife Resources Commission (2015), and it been little studied apart from the species' original description 20 years ago. The species' current published range is not well supported by data and may be largely inaccurate (J. Apodaca pers. comm., D. Beamer, pers. comm..). *Plethodon amplus* is a cryptic species within the *P. jordani* complex, a group of closely related salamanders restricted to the Southern Appalachian Mountains (Hairston 1951). Other species in this complex with ranges directly adjacent to *P. amplus* include: *P. metcalfi* (Southern Gray-Cheeked Salamanders), *P. meridianus* (South Mountain Gray-Cheeked Salamanders), and *P. montanus* (Northern Gray-Cheeked Salamanders). *Plethodon metcalfi* occupies the Blue Ridge Mountains in Haywood, Buncombe, Henderson and Macon counties, NC, and Oconee County, SC. *Plethodon meridianus* (South Mountain Gray-Cheeked Salamanders) occupies the South Mountains in Burke, Cleveland, and Rutherford counties in NC. The range of *Plethodon montanus* (Northern Gray-Cheeked

Salamanders) extends from the Blue Ridge Province of North Carolina, Virginia, and Tennessee including Avery, Wilkes, Buncombe, McDowell, and Yancey counties, NC, to the Valley and Ridge Province of Virginia (Highton & Peabody 2000). These three Gray-Cheeked Salamanders are morphologically indistinguishable from *P. amplus*, which makes it difficult to locate the exact boundary lines between each species.

As an amphibian with a limited range, *P. amplus* is particularly vulnerable to habitat loss and has been labeled as a species at "High Threat" from residential and commercial development (North Carolina Wildlife Resources Commission 2015). *Plethodon amplus* is currently listed as "Vulnerable" by the IUCN (International Union for Conservation of Nature; Hammerson & Beamer 2004), a "Significantly Rare" species by the NC Natural Heritage Program, and a "Species of Greatest Conservation Need" in the *North Carolina Wildlife Action Plan* (NC Wildlife Resources Commission 2015). *Plethodon meridianus* also occupies a very limited range and is listed as "Vulnerable" by the IUCN (Highton & Peabody 2000, Hammerson & Beamer 2004). Recently, the habitat of *P. meridianus* has been reduced due to residential development. As development in the area continues, its range will likely be restricted to South Mountains State Park and South Mountains Game Land, the only state protected lands within their range (Lannoo 2005). In 2015, the U.S. Fish and Wildlife Service denied a petition to list *P. amplus* and *P. meridianus* under the Endangered Species Act citing insufficient information (U.S Fish and Wildlife Service et al. 2015, U.S Fish and Wildlife Service et al. 2016). Accurate range de-lineations are crucial to developing species management plans aimed at conserving species of Gray-Cheeked Salamanders and their contributions to this region's biodiversity.

In this study, I sought to reduce the knowledge gap for *P. amplus* by providing a revised range delimitation which included boundaries between Gray-Cheeked Salamander species. To

achieve this objective, I collected tissue samples from Gray-Cheeked Salamanders from areas surrounding and throughout the known range of *P. amplus*. I used mitochondrial DNA (mtDNA) primers for two tRNA genes to amplify DNA extracted from the collected tissues, then sequenced the DNA and identified each sample to species. Data from my project will help inform future studies of Gray-Cheeked Salamanders and contribute to the management of the species.

METHODS

To delineate the range of *P. amplus*, I collected tissue samples from 60 Gray-Cheeked Salamanders from 15 field sites in the areas within and surrounding the species' current published range. I surveyed potential sites then selected field sites based on the presence of Gray-Cheeked Salamanders, with an emphasis on those located between the known range of *P. amplus* and the known ranges of either *P. montanus*, *P. meridianus*, or *P. metcalfi*. I collected salamanders used in the study by hand at night when they were outside of their burrows or rock crevices. I collected tissue samples from each individual via tail clip (5-10mm) and recorded snout-to-vent length (mm) and mass (g) before returning them to where each was initially found. I recorded location data, elevation, ground temperature, and general forest composition for each field site.

To prepare samples for DNA sequencing, I extracted DNA using the QIAGEN DNeasy Blood and Tissue Kit according to the manufacturer's protocol (Qiagen,Valencia, CA). I amplified the extracted samples according to the PCR methods outlined in Schuelke (2000) and Kozak et al. (2006). I used the mtDNA primers L4437, which amplifies a $tRNA^{Met}$ gene (5'-AAGCTTTCGGGCCCATACC-3'), and H5692, which amplifies a $tRNA^{Asn}$ gene (5'-

GCGTTTAGCTGTTAACTAAA-3') (Macey et al. 1997, Weisrock et al. 2001). The amplified PCR product was then sent to an external lab, GeneWiz (South Plainfield, NJ, USA), for sequencing using Sanger Sequencing methodology. I identified each sample based on highest percent match using the Genbank database (Benson et al. 2015, Appendix 3). I mapped the results of mtDNA analyses for each site using arcGIS (ESRI 2018; Fig. 1).

RESULTS

I located six sites that did not have historic records of Gray-Cheeked-Salamanders (3 - 5, 7, 12, & 13; Fig. 1). Four of my sites were chosen due to their location within the published ranges of either *P. amplus* (8, 9 & 10) or *P. metcalfi* (1 & 2) (Fig 1). Samples collected from these four sites were used as references for my species identifications. Sites 2, 6, 11, 14, and 15 were known to have Gray-Cheeked Salamanders present, but DNA analyses were needed to confirm species identities. Of nine field sites in which the samples were identified as *P. amplus*, five of the sites (3-5, 7, & 11) were located outside of the current published range of *P. amplus* and were not in the range of any other Gray-Cheeked Salamanders. Four sites (6, 8-10) were consistent with the published range of *P. amplus*. Of five field sites which the samples were identified as *P. metcalfi*, three (12-14) were located east of the published range of *P. metcalfi* and outside of any other Gray-Cheeked Salamander's range. Sites 1 and 2 are within the published range of *P. metcalfi*. At one field site located west of the known range of *P. meridianus* (15), the tissue samples were identified as having *P. meridianus* mtDNA. This site is also not located within any known Gray-Cheeked Salamander range.

At each site, except for site 2, the forest composition was a mixture of young and mature hardwoods with leaf litter over soil as ground cover. Site 2 was predominantly pine trees with

minimal leaf litter and mainly pine needles and sand as ground cover. Minimum elevations for captures of the three species of Gray-Cheeked Salamanders ranged from 300-520 m. The ground temperatures for where each Gray-Cheeked Salamander was found ranged from 9.1-22 (°C). Snout-to-vent lengths (SVL) for the collected Gray-Cheeked Salamanders ranged from 39.9 to 80 mm and mass ranged from 4.1 to 12.75 g (Table 1, see Appendix 1 for the raw data).

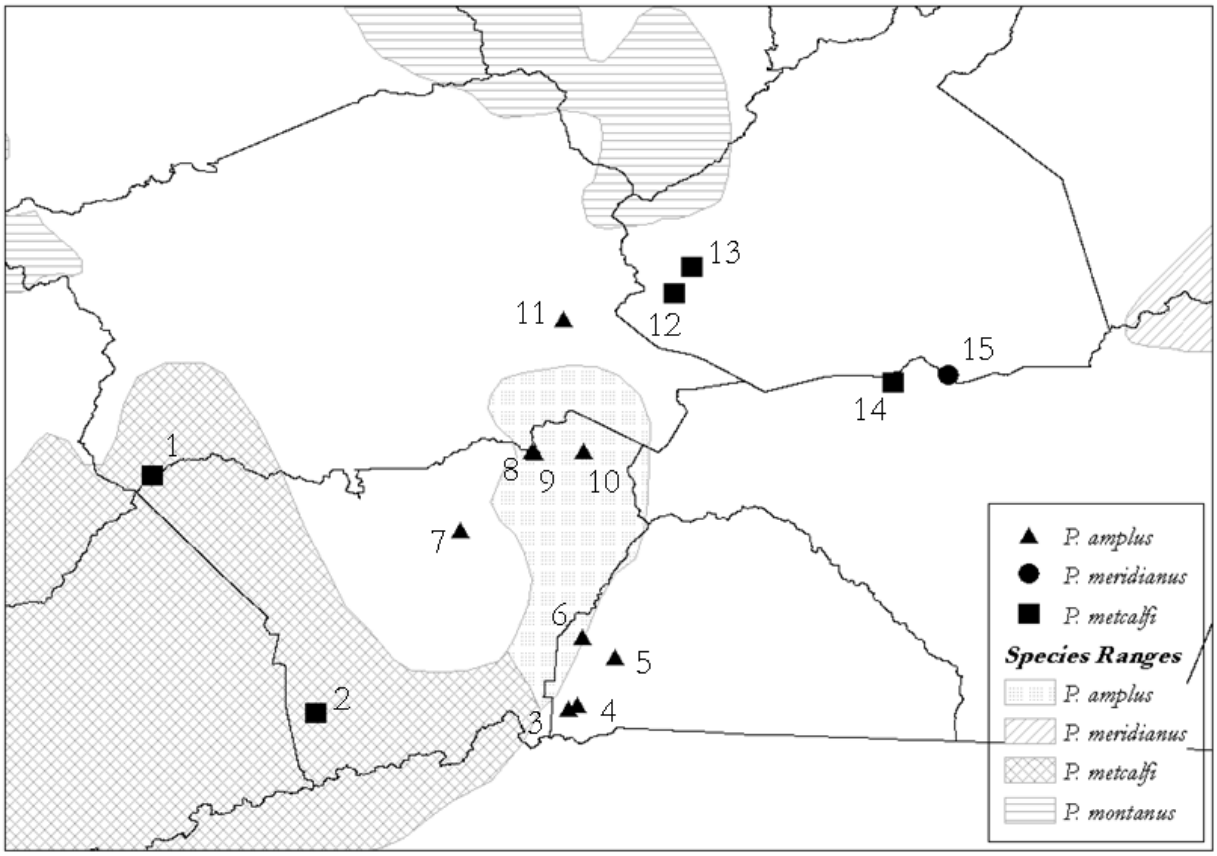

Fig 1. Species identifications determined by mtDNA sequences mapped for each site. The map displays a portion of Western North Carolina with county boundaries and the published ranges for each species of Gray-Cheeked Salamander (Weisrock & Larson 2006). Samples were collected from Henderson, Polk, Rutherford, Buncombe, and McDowell counties.

Table 1. Summary of mass, snout-to-vent length (SVL), ground temperature, and elevation data (± SD) by mtDNA species identification. Raw values provided in Appendix 1.

Species	Values	Mass (g)	SVL (mm)	Ground Temperature	Elevation (m)

				(°C)	
P. amplus	mean ± SD	8.5 ± 2.1	61.7 ± 9.0	15.2 ± 3.6	808.7 ± 216.6
	min	4.1	39.9	9.1	474.6
	max	12.75	76.6	22	1200
	n	33	34	34	34
P. metcalfi	mean ± SD	8.2 ± 2.4	61 ± 9.5	16.75 ± 2.2	784.2 ± 283.7
	min	4.8	47.2	13	520.5
	max	14.5	80	19.4	1204
	n	20	20	20	20
P. meridianus	mean ± SD	10 ± 0.5	65.9 ± 4.9	15.9 ± 0	300 ± 0
	min	9.25	57.6	15.9	300
	max	10.5	72.1	15.9	300
	n	3	5	5	5

DISCUSSION

In this study, I conducted extensive surveys to locate and collect tissues from genetically unidentified populations of Gray-Cheeked Salamanders. I also collected tissue samples from previously identified populations of Gray-Cheeked Salamanders at sites within their respective ranges (sites 8-10 for P. *amplus* and sites 1 and 2 for *P. metcalfi)* (Highton & Peabody 2000). Salamanders with *P. amplus* mtDNA were identified at sites 3, 4, 5, 7 and 11 which are located outside of any published Gray-Cheeked Salamanders' range. Due to the absence of any obvious boundaries to dispersal between *P. amplus* populations and these sites, I am confident in these species identities. Therefore, the range of *P. amplus* likely spans farther than its current published range suggests. The range of *P. amplus* extends north into the Swannanoa Mountains, south of the Green River, southeast further into Polk County, and further west into Henderson County. This study did not find evidence to support any range reductions for *P. amplus.*

Three sites where I identified the salamanders as *P. metcalfi* are located well outside of the species' published range (sites 12-14; Fig. 1). For these sites, I suspect the mtDNA identities

are misleading and do not represent an isolated occurrence of *P. metcalfi.* The Asheville basin, which runs north and south along the center of Buncombe County, serves as a geographic boundary in Western North Carolina for several cryptic salamander species such as *Desmognathus wrighti* and *D. organi*, because it is lower in elevation, drier, and generally warmer than the surrounding mountains. (Kozak & Wiens 2006, Crespi et al. 2010). The Asheville basin also likely serves as a boundary for Gray-Cheeked Salamanders, separating *P. metcalfi* from *P. amplus* and *P. montanu*s populations. The discrepancy between the published range of *P. metcalfi* and our results at sites 12-14 are most likely due to past gene flow between species of Gray-Cheeked Salamanders which led to mtDNA exchange. This mtDNA exchange was also observed by Weisrock and Larson (2006) when they performed mtDNA analyses paired with historic allozymic data to build a phylogeny for the *P. jordani* complex. However, their phylogeny did not form monophyletic groups and placed *P. metcalfi* and *P. amplus* within a clade (D2), *P. meridianus* and *P. metcalfi* within a clade (D1) and *P. montanus* and *P. metcalfi* within a clade (E). Although a large portion of variation in mtDNA can be explained by the four species groups, incomplete lineage sorting or mitochondrial capture in the species' evolutionary history likely resulted in discordance between the species tree and gene trees (Weisrock & Larson 2006, Degnan & Rosenburg 2009). During the Pleistocene, environmental conditions within Southern Appalachian Mountains altered with cyclic glaciation events. The conditions associated with glacial expansions allowed boreal forests to expand into low elevations, which connected populations of Gray-Cheeked Salamanders and allowed for genetic flow between populations. Glacial expansion was followed by glacial recession, which resulted in lowland environmental conditions that separated populations of *Plethodon* (Kozak et al. 2006). These cycles continued over tens of thousands of years and isolated and remerged many species'

habitats (Acharya et al. 2011). These contact events would have allowed for hybridization and for mitochondrial introgression in the Gray-Cheeked Salamanders' genomes which has also been documented in *P. jordani* and *P. shermani* (Weisrock et al. 2005, Degnan & Rosenburg 2009). Mitochondrial introgression is most likely the cause of the discrepancy between the species identities and mtDNA identities at sites 12-14. The actual species identities of the samples from sites 12-14 are more likely one of the three other Gray-Cheeked Salamander species *(P. amplus, P. meridianus,* or *P. montanus*) which have range boundaries closest to the sites from which the samples were collected. Sites 12 and 13 are geographically continuous with *P. montanus* habitat and the salamanders at these sites are most likely *P. montanus* rather than *P. metcalfi.*

Additionally, the range of *P. meridianus* likely extends farther west than the published range acknowledges. I am confident in these results because site 15 is closest to the published range of *P. meridianus* with no obvious geographical boundaries preventing dispersal. Sites 14 and 15 are located on the same mountain with site 14 near the peak, at an elevation of approximately 625 m, and site 15 is at the base, at an elevation of 300 m. Given that all five tissue samples from individuals at site 15 matched with *P. meridianus* mtDNA and the tissue sample from site 14 matched with *P. metcalfi*, it is probable that the salamanders at sites 14 and 15 represent two separate species of Gray-Cheeked Salamanders that occupy separate niches restricted by elevation and which do not currently exchange genetic information. The most likely identity of the salamander at site 14 is *P. amplus* or *P. montanus*. These results demonstrate the need for additional genetic analyses to redraw the range maps of both *P. amplus* and *P. meridianus*.

Gray-Cheeked Salamanders are considered high elevational species (>500 m) (Weisrock & Larson 2006). However, several sites for Gray-Cheeked Salamanders in this study were

located below 500 m indicating that the species' are not restricted to high elevations. The lowest elevation I found *P. amplus* was 474.6 m and the lowest site for *P. meridianus* was 300 m (Table 1). Research on another member of the complex, *P. jordani*, showed physiology restricted lower range limits to about 750 m (Gifford & Kozak 2012). One explanation for the difference in elevation between Gray-Cheeked Salamander sites and *P. jordani* habitat could be the difference in body sizes. The SVL Gifford and Kozak (2012) used for calculating the lower limit of *P. jordani* was 55 mm, an average taken from 63 *P. jordani* in a previous study, while my SVL means for *P. amplus, P. metcalfi*, and *P. meridianus* were 61.7, 61, and 65.9 mm respectively (Table 1). The larger body sizes of Gray-Cheeked Salamanders could reduce water loss due to lower surface area to body mass ratios compared to other *Plethodon* which would allow them to survive at lower elevations.

Based on personal observations made over the course of this study, it appears *P. amplus* is more evenly disbursed throughout its range compared to other salamander species of conservation concern in the Hickory Nut Gorge area (*P. longicrus* and *Aneides caryaensis*). Site 14 had the lowest catch-per-unit-effort (CPUE) of my sites at 0.14 and site 10 had the highest CPUE at 15 (CPUE for each site is shown in Appendix 2). I observed a few habitat characteristics that were present at most of the sites that I found *P. amplus*. Mature hardwood trees and leaf litter ground coverage were present at each site, aside from site 11 which had pine trees as the dominant tree type and minimal to no leaf liter. At high elevation sites (>1000 m) within the Hickory Nut Gorge, *P. amplus* was tightly associated with rock outcrops. At lower elevation sites (<500 m), *P. amplus* was often found less than 6 m from streams. Site 15, which had *P. meridianus* and was at 300 m, was also less than 6 m from a stream. While generally considered a mid-elevational species (500-1000 m), these associations exhibited by high and low

elevation Gray-Cheeked Salamander populations could indicate microhabitat requirements (Highton & Peabody 2000). The Green River Game Land, the location of sites 5 and 6, is heavily managed for game animals which includes clear cutting forests to encourage early successional growth. Clear cutting can be detrimental to terrestrial salamanders and, based on my observations, may eliminate *P. amplus* habitat (Homyack & Haas 2009).

The majority of land within the range of *Plethodon amplus* is privately owned with isolated patches of state-owned land. Habitat loss and fragmentation from land development is likely the largest threat to *P. amplus* populations (NC Wildlife Resources Commission 2015). The private properties on which I found *P. amplus* had tracts of preserved forest. Three of my sites, 7, 8, and 9, are on private land with conservation easements secured by Conserving Carolina, a non-profit organization. At these sites, I was able to find high abundances of *P. amplus* as well as *P. longicrus*, a state listed species. Based on my observations during this study, the land management actions I would recommend are to preserve old growth trees, protect corridors in heavily fragmented habitat areas, and to expand conservation easements where possible among private lands throughout the range of *P. amplus.* Additional actions to conserve *P. amplus* that I recommend are to establish baseline population information (e.g., densities, site occupancy, and detection probabilities), monitor populations to detect declines and examine trends over time, collect tissues for use in population genetics studies, and collect physiological data on *P. amplus*.

A future study using next-generation sequencing to analyze a larger portion of the genomes of each Gray-Cheeked Salamander species may provide further insight into the genetic relationships and differences between the species which will likely result in new boundary lines for *P. amplus* and *P. meridianus*, if not for all four Gray-Cheeked Salamander species.

By identifying additional locations of Gray-Cheeked Salamanders and providing evidence for the need for range extensions for *P. amplus* and *P. meridianus,* this study contributes to the limited understanding of these species. Additional studies on the range boundaries, population densities, and genetic relationships of Gray-Cheeked Salamanders are necessary to help evaluate the need for special listing status for these endemic species facing habitat loss across their limited range. Understanding and documenting the biodiversity of the Appalachian Mountains is critical to protecting the region's species' and their contributions to the health of its ecosystems.

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APPENDICES

Appendix 1. Raw data from each sample including mass, SVL, ground temperature, and elevation. Some samples have a mass >10 g due to the limitations of the scale used. NR indicates value not recorded.

Site Number	Species	Mass (g)	SVL (mm)	Ground Temperature (°C)	Elevation (m)
3	*P. amplus*	7.9	59.9	18.9	474.6
3	*P. amplus*	8.5	64.2	17.6	474.6
3	*P. amplus*	7.3	58.6	16.8	474.6
4	*P. amplus*	<10	71.7	22	497.1
4	*P. amplus*	9.5	66.5	19	497.1
6	*P. amplus*	9.2	65.8	18.3	618.8
6	*P. amplus*	<10	68.1	18.9	618.8
6	*P. amplus*	<10	69.5	18	618.8
7	*P. amplus*	12	75.8	11.3	760
7	*P. amplus*	6	51	9.2	760
7	*P. amplus*	4.75	39.9	9.5	760
7	*P. amplus*	8.5	63.1	9.3	760
7	*P. amplus*	10	66.1	9.1	760
8	*P. amplus*	7.2	58.8	15.5	1068
8	*P. amplus*	8.15	59.2	15.4	1068
8	*P. amplus*	7.05	58.9	16.4	1052
8	*P. amplus*	5.25	47	15.9	1052
8	*P. amplus*	11	67.7	16	1052
9	*P. amplus*	7	53.1	17.5	1105.2
9	*P. amplus*	5.5	46	17.5	1105.2
9	*P. amplus*	4.1	44.1	17.5	1105.2
9	*P. amplus*	8.3	56.8	17.5	1200
9	*P. amplus*	10.8	64.8	18.5	1200
10	*P. amplus*	10.75	65.6	17.2	696
10	*P. amplus*	12.75	65.6	17.2	696
10	*P. amplus*	8.75	66	16.9	696
10	*P. amplus*	9.25	66.1	17.4	696
10	*P. amplus*	7.25	57.4	16.8	696

11	*P. amplus*	9	63.4	9.9	822
11	*P. amplus*	12	74.2	10.1	822
11	*P. amplus*	6	49.9	9.9	822
11	*P. amplus*	7	62.4	11.6	822
11	*P. amplus*	9.5	72.4	11.1	822
14	*P. amplus*	NR	76.6	14.1	825.3
15	*P. meridianus*	9.25	72.1	15.9	300
15	*P. meridianus*	10.5	68.4	15.9	300
15	*P. meridianus*	10.25	57.6	15.9	300
15	*P. meridianus*	NR	67.4	15.9	300
15	*P. meridianus*	NR	63.9	15.9	300
1	*P. metcalfi*	5.75	53.8	13.1	1204
1	*P. metcalfi*	7	57.8	13.1	1204
1	*P. metcalfi*	5.25	47.2	13.4	1204
1	*P. metcalfi*	6	54.3	13	1204
1	*P. metcalfi*	6.25	54.1	13.3	1204
2	*P. metcalfi*	7.5	59.4	17.3	885
2	*P. metcalfi*	8.75	67.7	17.3	885
2	*P. metcalfi*	9.25	62.4	16.7	885
2	*P. metcalfi*	5	48.5	16.7	885
2	*P. metcalfi*	6.5	51.7	16.5	885
12	*P. metcalfi*	4.8	51.4	18.7	529.7
12	*P. metcalfi*	>10	72.3	18.1	524
12	*P. metcalfi*	>10	80	17.9	524
12	*P. metcalfi*	>10	73	18.4	524
12	*P. metcalfi*	>10	73.1	18.5	534.3
13	*P. metcalfi*	>10	68.5	19.4	520.5
13	*P. metcalfi*	8	49.9	19.1	520.5
13	*P. metcalfi*	14.5	70.2	19.2	520.5
13	*P. metcalfi*	8.5	59.5	18	520.5
13	*P. metcalfi*	10.5	64.4	17.4	520.5

Appendix 2. Catch-per-unit-effort (CPUE) for each site.

Site number	CPUE
1	1.25
2	1.25
3	0.75
4	4
5	0.16
6	0.75

7	0.56
8	1.25
9	0.83
10	5
11	3.3
12	1
13	2.5
14	0.14
15	2

Appendix 3. Genbank Database BLAST search results. Reference sequences are sourced from Weisrock et al. (2005) and clades are sourced from Weisrock & Larson (2006).

Site	Sample number	Species mtDNA ID	Percent Identity	Accession	Clade
1	56	*P. metcalfi*	100	AY874995.1	E
1	57	*P. metcalfi*	100	AY874995.1	E
1	58	*P. metcalfi*	100	AY874995.1	E
1	59	*P. metcalfi*	100	AY874995.1	E
1	60	*P. metcalfi*	100	AY874995.1	E
2	51	*P. metcalfi*	100	AY874995.1	E
2	52	*P. metcalfi*	99.83	AY874950.1	E
2	53	*P. metcalfi*	99.75	AY874954.1	E
2	54	*P. metcalfi*	99.83	AY874954.1	E
2	55	*P. metcalfi*	100	AY874950.1	E
3	8	*P. amplus*	100	AY874886.1	D
3	9	*P. amplus*	100	AY874886.1	D
3	10	*P. amplus*	100	AY874886.1	D
4	6	*P. amplus*	100	AY874886.1	D
4	7	*P. amplus*	100	AY874886.1	D
5	42	*P. amplus*	99.75	AY874886.1	D
6	43	*P. amplus*	99.92	AY874883.1	D2
6	44	*P. amplus*	99.9	AY874884.1	D2
6	45	*P. amplus*	99.92	AY874883.1	D2
7	21	*P. amplus*	99.92	AY874880.1	D2
7	22	*P. amplus*	99.57	AY874880.1	D2
7	23	*P. amplus*	99.86	AY874882.1	D2
7	24	*P. amplus*	100	AY874880.1	D2
7	25	*P. amplus*	100	AY874880.1	D2

8	37	*P. amplus*	100	AY874880.1	D2
8	38	*P. amplus*	100	AY874880.1	D2
8	39	*P. amplus*	100	AY874880.1	D2
8	40	*P. amplus*	100	AY874880.1	D2
8	41	*P. amplus*	100	AY874880.1	D2
9	11	*P. amplus*	99.92	AY874880.1	D2
9	12	*P. amplus*	100	AY874880.1	D2
9	13	*P. amplus*	100	AY874880.1	D2
9	14	*P. amplus*	99.83	AY874880.1	D2
9	15	*P. amplus*	100	AY874880.1	D2
10	46	*P. amplus*	100	AY874880.1	D2
10	47	*P. amplus*	100	AY874880.1	D2
10	48	*P. amplus*	100	AY874880.1	D2
10	49	*P. amplus*	100	AY874880.1	D2
10	50	*P. amplus*	100	AY874880.1	D2
11	32	*P. amplus*	100	AY874880.1	D2
11	33	*P. amplus*	99.91	AY874880.1	D2
11	34	*P. amplus*	100	AY874880.1	D2
11	35	*P. amplus*	99.33	AY874880.1	D2
11	36	*P. amplus*	99.58	AY874880.1	D2
12	1	*P. metcalfi*	99.49	AY874854.1	D2
12	2	*P. metcalfi*	99.58	AY874854.1	D2
12	3	*P. metcalfi*	99.41	AY874854.1	D2
12	4	*P. metcalfi*	99.49	AY874854.1	D2
12	5	*P. metcalfi*	99.32	AY874854.1	D2
13	16	*P. metcalfi*	99.41	AY874954.1	D2
13	17	*P. metcalfi*	99.58	AY874954.1	D2
13	18	*P. metcalfi*	99.58	AY874954.1	D2
13	19	*P. metcalfi*	99.58	AY874954.1	D2
13	20	*P. metcalfi*	98.62	AY874954.1	D2
14	26	*P. metcalfi*	99.58	AY874954.1	D2
15	27	*P. meridianus*	99.75	AY874898.1	D1
15	28	*P. meridianus*	99.83	AY874898.1	D1
15	29	*P. meridianus*	99.92	AY874898.1	D1
15	30	*P. meridianus*	99.92	AY874898.1	D1
15	31	*P. meridianus*	99.75	AY874898.1	D1