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RESEARCH ARTICLE

THE DISCOVERY OF A SECOND POPULATION OF THE MICROENDEMIC *APHELORIA WHITEHEADI* (SHELLEY, 1986), A STATE-LISTED THREATENED SPECIES FOR VIRGINIA

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ABSTRACT

Apheloria whiteheadi (Shelley, 1986), the Laurel Creek millipede, a state-listed threatened species for Virginia, was previously known only from a narrow stretch surrounding the type locality in the Blue Ridge Mountains of southwestern Virginia. Here we present the discovery of a second *A. whiteheadi* population from Virginia's inner Piedmont, approximately 20 km to the east of the type locality, and discuss habitat characteristics of the two sites and the potential implications of this finding.

Keywords: Polydesmida, Xystodesmidae, Laurel Creek millipede, eastern United States, conservation.

INTRODUCTION

First collected by Richard Hoffman in 1983, the Laurel Creek millipede, *Apheloria whiteheadi* (Shelley, 1986), has long stood as the poster child for micro range endemics (MREs; Means & Marek, 2017) in Virginia. Known only from a very restricted area (<1 km^2) in the Blue Ridge Parkway (Fig. 1), *A. whiteheadi* was placed on Virginia's Threatened Species List (at the time as *Sigmoria whiteheadi*; Roble, 2016).

In 2017, Means and Marek conducted a study to determine the evolutionary history of *A. whiteheadi*, and to discover any additional populations in the area near the type locality. Using molecular evidence, Means & Marek (2017) found that the species had been erroneously placed,

based on morphological characters, in the genus *Sigmoria*, and that it belonged in the widespread but poorly understood genus *Apheloria*. While this marked an important discovery for *A. whiteheadi* and underscored the prevalence of morphological convergence in the Diplopoda, the second aim of the study was relatively fruitless; the only additional *A. whiteheadi* individuals found formed a contiguous population, with a distribution tightly confined to the area immediately surrounding the type locality (Means & Marek, 2017).

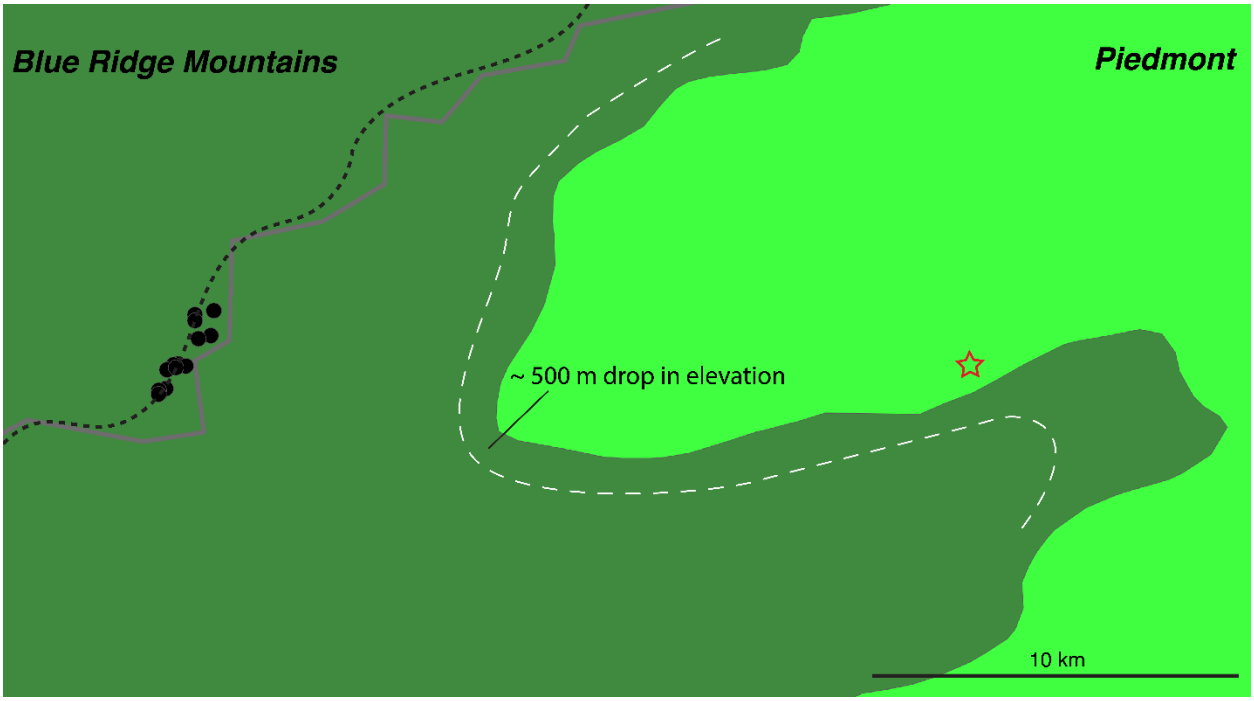

Figure 1. Known distribution of the Laurel Creek millipede, *Apheloria whiteheadi*: type locality and surrounding Blue Ridge Parkway sites, Floyd Co., Virginia (black circles); Patrick Co., Virginia site (red star). The Blue Ridge Parkway is represented by a black dashed line, the Blue Ridge Escarpment by a white dashed line, and the border between Floyd and Patrick counties by a solid grey line.

The majority of Diplopoda are highly dependent on specific habitats, and the discovery of MREs is not uncommon in the group (Means & Marek, 2017; Marek, 2010; Shelley & Whitehead, 1986). However, with a distribution of less than one square kilometer, *A. whiteheadi* represents one of the most extreme examples of MREs in the Xystodesmidae. Therefore, it is not unreasonable to predict that the habitat within which *A. whiteheadi* is found may be unique, and that this species may be particularly vulnerable to climate change and/or anthropogenic disturbances such as clearcutting, which is not uncommon in the area.

Apheloria whiteheadi is a member of the flat-backed millipedes (order Polydesmida, family Xystodesmidae, tribe Apheloriini), which are characterized by lateral elongations (paranota) of the body rings that give the body a "flattened" appearance. In addition, members of this group often display bright warning coloration. The Xystodesmidae produce and secrete hydrogen cyanide from ozopores located on the lateral edges of their paranota, and the reds, yellows, and oranges contrasted against an often dark background color serve as a warning of toxicity to potential predators (Marek & Bond, 2006). Adult *A. whiteheadi* are medium sized xystodesmids, typically around 30 mm in length and 6 mm wide, and display vivid yellow

transverse stripes against a deep black background, with or without a small middorsal spot (Fig. 2). Amongst Appalachian aphelorines, contrasting yellow and black is a fairly common color scheme, and morphology of the male reproductive structures (gonopods), is the best way to discriminate between species. Unlike other members of the genus, *A. whiteheadi* has a relatively robust and tightly curved gonopod. For a comparison of the gonopods of *A. whiteheadi* and *A. virginiensis corrugata*, see Means & Marek (2017; Fig. 7).

Figure 2. Adult *Apheloria whiteheadi* from Patrick Co., Virginia. An immature *A. whiteheadi* can be seen in the bottom left of the image (photograph by B. Williams).

The type locality of *A. whiteheadi* in the Blue Ridge Mountains physiographic province of southwestern Virginia (Floyd Co.) is a narrow riparian corridor stretching along either side of Laurel Creek. The vegetation of the type locality is an acidic cove forest surrounded by stretches of montane mixed oak forest (Fig. 3). The acidic cove forest overstory is composed of variable mixtures of tulip poplar (*Liriodendron tulipifera* L.), red maple (*Acer rubrum* L.), and sweet birch (*Betula lenta* L.), with some eastern white pine (*Pinus strobus* L.) and magnolias (*Magnolia* spp.). The dense understory is dominated by evergreen thickets of great rhododendron (*Rhododendron maximum* L.) interspersed with mountain laurel (*Kalmia latifolia* L.), northern spicebush (*Lindera benzoin* L.) and witch hazel (*Hamamelis virginiana* L.). The surrounding montane mixed oak forest is dominated by mixtures of white oak (*Quercus alba* L.), northern red oak (*Quercus rubra* L.), and chestnut oak (*Quercus montana* Willd.) with admixtures of hickories (*Carya* spp.),

sourwood (*Oxydendrum arboretum* (L.) DC.), tulip poplar, red maple, sweet birch, and white pine depending on site. The herb layer is often patchy and includes New York Fern (*Parathelypteris noveboracensis* (L.) Ching) and a variety of wildflowers, such as American lily-of-the-valley (*Convallaria pseudomajalis* W.Bartram) and Indian cucumber-root (*Medeola virginiana* L.).

Figure 3. Habitat at the *A. whiteheadi* type locality in Floyd Co., Virginia: **left**) Acidic cove forest bordering Laurel Creek; **right**) Montane mixed oak forest (photographs by J. Means).

Shelley and Whitehead (1986) categorized *A. whiteheadi* as a cove species, a term Shelley described in his 1981 *Sigmoria* revision as "a species found in riparian corridors, typically beneath hardwood leaf litter adjacent to rhododendron" (Shelley, 1981). Although we agree that *A. whiteheadi* falls into the cove species category, the utility of such a category is questionable as species in a wide variety of xystodesmid genera have been found in these cove habitats by Means and others. Whether there exist unique environmental factors on which *A. whiteheadi* relies for survival is currently unclear. We present here the discovery of a second population of *A. whiteheadi*, which suggests that the type locality may represent one of a few remaining localities of a formerly more widespread species.

MATERIALS AND METHODS

We observed and collected juvenile and adult *A. whiteheadi* on private property in Patrick Co., Virginia. The site (36.7662°N, -80.1827°W), situated in the southern reaches of Virginia's inner Piedmont, lies approximately 20 km east of the species' type locality in Floyd Co., Virginia (Fig. 1). Notably, elevation at the Patrick Co. site is 436 m, less than half that of the type locality (~930 m). Logging took place on the property intermittently between the 1920s and early 1940s but it has been left relatively untouched since.

The habitat where the *A. whiteheadi* specimens were collected lies along the northern side of the property and is bisected by an old abandoned logging road. The apparent age and composition of the surrounding forest suggests that the area immediately to the west of the road was logged in the past, while the steeper west-facing slope to the east was left undisturbed. The habitat of the west-facing slope is a mature montane mixed-oak forest similar to that surrounding the type locality. The canopy is dominated by white, northern red, and chestnut oak, with a few interspersed red maples and white pines (Fig. 4). The dense understory is composed of mountain laurel and scattered great rhododendron, with an extensive rhododendron thicket located approximately 300 m southeast of where the *A. whiteheadi* individuals were observed. The habitat

west of the logging road is a relatively young forest almost exclusively (~90%) dominated by tulip poplar with a few scattered red maples and a number of small patches of Virginia pine (*Pinus virginiana* Mill.). The understory is open with no visible shrub layer and only a few scattered small American holly (*Ilex opaca* Aiton), American beech (*Fagus grandifolia* Ehrh.), and sourwood. The domination of this area by young tulip poplar and red maple may be indicative of a recent trend in oak-hickory forest succession towards more shade-tolerant species due to a lack of fire disturbance (Rose, 2008). Conversely, the apparent young age and composition of the forest west of the logging road may be due to the presence of xeric, nutrient-poor soils, which favor tulip poplar and red maple over northern red oak (Rose, 2008).

Figure 4. Habitat at the site in Patrick Co., Virginia. *Apheloria whiteheadi* individuals were found under leaf litter accumulated along the inside edge of this old logging road. The habitat to the right (east) of the road is a montane mixed oak forest, while the habitat to the left (west) is a successional forest dominated by tulip poplar and red maple (photograph by B. Williams).

Searching for millipedes involved the removal of leaf litter and turning over cover objects, such as large logs and rocks, to expose specimens on the surface of the soil. Initial observations took place on 4 April 2020, when we discovered adult male and female *A. whiteheadi* along the northern side of the property during a routine outing for leaf litter invertebrates. The male specimen was collected, placed in a sealed plastic container containing moss, leaves, and other moist detritus, and brought to the lab for identification. The identity of the specimen was confirmed by the senior

author through a combination of color pattern and gonopod morphology (Fig. 5). The specimen was preserved in 100% ethanol and is deposited in the invertebrate collection at the Virginia Museum of Natural History (VMNH110620).

Figure 5. *Apheloria whiteheadi* gonopod illustrated based on a male specimen from Patrick County, Virginia (VMNH110620): **left**) medial view; **right**) anterior view; dashed line indicates prostatic groove.

We revisited the Patrick Co. site on the 26 of September and the 16 of November 2020. These visits yielded 11 additional *A. whiteheadi* observations, including 2 males, 2 females, and 7 immatures on September 26 (Fig. 2), and a single deceased female on November 16. Interestingly, all *A. whiteheadi* were found under a thick leaf litter layer accumulated along the inside edge of a single 25 m stretch of an abandoned logging road (Fig. 4). Extensive searches of the surrounding area, including a large *R. maximum* thicket situated ~ 300 m to the southeast, yielded no additional *A. whiteheadi*, although multiple specimens of *Apheloria virginiensis virginiensis* (Drury, 1770) were observed.

Field photography of specimens was performed with a Panasonic Lumix DMC-G7 equipped with an Olympus 60 mm macro lens. Gonopods were photographed using an Omax trinocular microscope with an Omax 18MP mounted camera (Omax Microscope, South Korea). Illustrations were made in Adobe Illustrator CC 2021 (Adobe, San Jose, CA). For a more detailed description of Xystodesmidae collection and curation methods see Means et al. (2015).

DISCUSSION

The discovery of a second population of *A. whiteheadi* approximately 20 km east of the type locality is notable for a number of reasons. First, this discovery demonstrates that the existence of additional undiscovered populations of *A. whiteheadi* is both possible and likely, especially in the poorly sampled intervening area. Second, there is now a possibility that *A. whiteheadi* may exist on other protected lands in the nearby area, namely Fairy Stone State Park, Spring Cove Park Recreation Area, and Mines Branch Recreation Area. Third, currently available data suggests that *A. whiteheadi* is associated with montane mixed oak forests as well as the rhododendron thickets characteristic of the co-occurring acidic cove forests. However, the

existence of a population of *A. whiteheadi* in a physiographic province differing from that at the type locality, and the substantial difference in elevation between the two sites suggests that this species may be more general in its habitat requirements, and may therefore display some resiliency in the face of various natural and anthropogenic disturbances.

Somewhat surprisingly, all *A. whiteheadi* specimens encountered over three separate visits to the Patrick Co. location were found within the confines of an abandoned logging road. The lower profile of the logging road allows it to accumulate large amounts of leaf litter and small woody debris resulting in higher moisture levels as compared to the surrounding, more exposed, areas. The combination of these factors may offer some protection against predators and/or the elements and therefore serve as an ideal site for xystodesmid millipedes. In the authors' experience, however, millipedes in the family Xystodesmidae are often found in aggregates scattered throughout a generally favorable habitat, and the apparent lack of any observed *A. whiteheadi* outside of the logging road may be due to sampling bias.

The *A. whiteheadi* type locality is one of many locations around Virginia experiencing *Rhododendron maximum* dieback (Brooks et al., 2019; Fig. 6), the driving factors for which are still unclear. The disappearance of rhododendron will likely have a lasting impact on the microclimate of the type locality, and may have a negative effect on multiple associated taxa, including *A. whiteheadi*. The senior author has found immature *A. whiteheadi* under accumulated leaf litter within *R. maximum* thickets, and it is possible that the dense canopy provides some degree of protection from predators and the elements during molting. It is fortunate, therefore, that an additional population of *A. whiteheadi* exists in a habitat which, thus far, has not shown evidence of rhododendron dieback. Additionally, the Patrick Co. site has been left relatively undisturbed for nearly eight decades, which likely contributes to the continued presence of *A. whiteheadi*, and underscores the importance of habitat conservation for the preservation of endemic, and other, taxa.

Figure 6. Dead and dying great rhododendron (*Rhododendron maximum*) at the type locality of *A. whiteheadi* (photographs by J. Means).

Maxent (software for modeling species niches and distributions) and other species distribution modeling programs offer a potentially powerful method by which habitats suitable to species in need of conservation may be revealed, and collection efforts made more efficient (Phillips et al., 2004; Phillips at al., 2017). With the discovery of a second population of *A.*

whiteheadi the number of positive inputs for these modeling programs has doubled, thereby increasing model output precision.

This discovery also highlights the need for natural history collections on private property, within which often lay parcels of land untouched for generations, and the importance of engaging private landowners in citizen science initiatives.

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