

Natural history, taxonomy, and phylogenetics of Appalachian flat-backed millipedes (Diplopoda:
Polydesmida)

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ABSTRACT (Academic)

Millipedes (class Diplopoda) are ubiquitous in forests worldwide, with about 12,000 described species and an estimated 30,000 undescribed species. The most species-rich order is the Polydesmida, the flat-backed millipedes, which encompasses about 3,500 species. Appalachia has an abundance of Polydesmida, and is a biodiversity hotspot for millipedes in the family Xystodesmidae. These diplopods are chemically defended with hydrogen cyanide and benzaldehyde, and sometimes form mimicry rings based on shared color. The evolutionary dynamics of model and mimic are incompletely known in these rings, so I investigated a mimicry ring in the central Appalachian Mountains to determine if the species *Apheloria polychroma* functions as a model for the genus *Brachoria*. I measured the size of the chemical gland to assess toxicity in 15 species, and reconstructed the ancestral dimensions of the gland to determine the direction of volume change over evolutionary time. Using a molecular phylogeny, I traced the miniaturization of chemical glands in the mimic genus *Brachoria* and found that in areas without *Apheloria*, *Brachoria* and related xystodesmids have larger chemical glands. Non-aposematic millipedes of the genus *Nannaria* have significantly smaller glands, and ostensibly rely on camouflage to avoid predation. This genus is known as the twisted claw millipedes and occur throughout eastern North America, but have their center of diversity in the Appalachian Mountains. About 22 species are described, but many undescribed species are known. To determine the diversity of this group, field collection and examination of museum specimens took place from 2015-2020. Examination of morphology, combined with molecular phylogenetics, revealed two distinct clades in the genus. One is distributed throughout the eastern United States, while the other is found only in the Appalachian Mountains. This Appalachian clade contains six described species, and I describe an additional 18 species,

quadrupling the diversity of the group. Additionally, the phylogenetics of the polydesmid genus *Pseudopolydesmus* is investigated in an integrative taxonomic framework using five genes. I find that the genus is monophyletic, contains 8 species, and are related to one another in a hierarchical way according to a molecular phylogeny. I provide complete distributional records and live photographs of each species.

ABSTRACT (Public)

Millipedes are common animals in forests. There are about 12,000 known species in the world, with an estimated 30,000 undescribed species still awaiting discovery and description. The largest group of millipedes are the flat-backed millipedes, with about 3,500 known species. They are some of the most common millipedes in North America, and many of these species defend themselves with poisons that are harmful to predators, but smell sweet like cherries to humans. Some of these millipedes have bright red or yellow spots against dark colors to warn predators of their toxins, and look similar to other species that live near them. I wanted to know if some of these species are more or less poisonous than others, and measured how large their poison reserves were. I found that one species, called *Apheloria polychroma*, is more poisonous than similar-looking species called *Brachoria*, which are less poisonous. If *Brachoria* doesn't live near *Apheloria* though, *Brachoria* is more poisonous. Not all of these millipedes are brightly colored, and a group called *Nannaria*, or the twisted claw millipedes, are camouflaged with brown colors on the forest floor. These millipedes only live in the eastern United States, especially the Appalachians, and aren't found anywhere else in the world. We know about 20 species of them, but based on specimens stored in scientific collections in museums and through discovery from fieldwork, we knew that more species existed. Each species of twisted-claw millipede only lives in a small area, sometimes only a few miles wide, and could be threatened by habitat loss and other dangers. So, to learn more about them, we need to find them in the forest and describe what they look like and most importantly, giving them a name. I did this by collecting them, illustrating their anatomy, and sequencing their DNA. I found two groups within *Nannaria*, and focused on the group that only lives in Appalachia. Scientists know 6 of these species, but I found 18 more species and describe them. A related group called

Pseudopolydesmus lives throughout North America, and I studied their anatomy and DNA as well, finding 8 species.

Dedication

To my wife, Marian, for her unwavering love and support.

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Chapter 1 – Introduction

The class Diplopoda (millipedes) is the most species-rich class of the subphylum Myriapoda, with over 12,000 described species (Sierwald & Spelda 2020), with estimates of the true diversity in group ranging from 20,000 - 30,000 species (Brewer et al 2012). Millipedes occur on every continent except Antarctica, and are common inhabitants of forests worldwide, where they inhabit the forest floor, feeding on dead leaves, wood, fungi, and lichen. Some species have also adapted to desert environments or periodically flooded areas, evolving physiological or morphological features to deal with such habitats (Minelli 2015).

About 2,100 species are recorded from North and Middle America (Hoffman 1999), a number that has increased during the past 20 years. The taxonomy of millipedes in North America during the 20th and early 21st centuries has been most influenced by the work of Richard Hoffman (1927-2012), Rowland Shelley (1942-2018), and William Shear (b. 1942), each of whom published extensively, describing new genera and species, and synthesizing knowledge of the millipede families found in North America. One of the most important effects of their research was to bring clarity to the taxonomy of millipedes on the continent, investigating poorly-known species and providing useful illustrations and identification information. Much of Hoffman's work focused on the order Polydesmida, with his early revisions of xystodesmid genera (e. g. Hoffman 1965) clarifying the nomenclature within the group. His 1999 Checklist still stands as an important reference for anyone working with North American millipede taxonomy, and his chapter on Diplopoda in the *Soil Biology Guide* (Dindal 1990) remains the most recent published key to millipede families for the U.S. and Canada. Rowland Shelley's main publishing interest within North American millipedes was also the order Polydesmida, but he published many works on other orders, including the Polyzoniida, Spirobolida, and Julida. His

focus on the Parajulidae during the latter part of his career resulted in a series of 12 papers on the family, with his last published in 2016 (Shelley & Smith 2016). Within the Polydesmida, he focused on the Xystodesmidae, publishing two monographs on the genus *Sigmoria* Chamberlin, 1939 (Shelley 1981; Shelley & Whitehead 1986), a confusing and heterogeneous genus that makes up a large component of xystodesmid diversity in eastern North America. His two publications listing the genus, family and ordinal-level updates to millipede taxonomy since 1958 (Shelley et al. 2000; Shelley & Golovatch 2015) serve as necessary references for millipede taxonomy worldwide. William Shear's main publishing focus has been the order Chordeumatida. He has revised most of the North American groups of the order, including the Cleidogonidae (Shear 1972), Trichopetalidae (Shear 2003; 2010), and most recently, the Urochordeumatidae (Shear & Marek 2019).

The past six decades of North American millipede taxonomy have thus focused on genus and species description, and cleaning up a morass of ill-defined species. Thanks to the diligence of these workers and others, including Nell B. Causey, Harold F. Loomis, Paul E. Marek, millipede taxonomy is reaching a developmental point where species can be confidently identified and placed in a generally stable classification, and sometimes even identified from just a photo. The field still has some distance to go before it reaches the level of knowledge on par with a group such as the insects, but it is no longer such a messy and confusing assemblage of animals. Still, questions remain about the natural history, ecology, and phylogenetics of millipedes.

Most work on millipedes has remained focused on morphology, with genetic investigations only recently becoming more common (see Marek & Bond 2006; Marek 2010; Marek et al. 2018; Rodriguez et al. 2017). Genetic investigations will become more necessary in

the future, as evidence of cryptic species is found and species anomalous in their family placement are questioned. The importance of basic taxonomy remains: many millipede species have restricted distributions (some severely restricted), and any hope for conservation of these species is tied to naming them and communicating their importance, rather than a species name being a forgotten footnote in a scientific paper.

The biogeography of millipedes in North America, especially in Appalachia, are indicative of the geological and ecological history of the continent, with millipede distributions often showing the same patterns as other low-vagility, flightless organisms, such as plethodontid salamanders (Mueller et al. 2004) and harvesters (Hedin & McCormack 2017). Appalachia is a continental hotspot of biodiversity for Polydesmida and particularly Xystodesmidae (Means & Marek 2017), with many still undescribed species. The region also contains interesting ecological and evolutionary interactions between species, which Müllerian mimicry rings known to occur among apheloriines (Marek & Bond 2006).

As part of an effort to document and describe the biodiversity, natural history, and ecology of Appalachian Polydesmida, I conducted three research projects focused on this order. The first was an investigation of the evolution of toxicity in a Müllerian mimicry ring of apheloriine xystodesmids in central Appalachia. The second was a taxonomic revision of the genus *Nannaria*, a group of ostensibly non-aposematic millipedes with its center of diversity in the Appalachian Mountains. The third was an integrated taxonomic revision of the polydesmid millipede genus *Pseudopolydesmus*, a widespread genus in North America, using both morphological and molecular data to delimit species and describe their evolutionary relationships.

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Chapter 2 - Evolution of cyanide defense glands in a Müllerian mimicry ring of millipedes

2.1 Abstract

Cherry millipedes have been documented to form Müllerian mimicry rings in eastern North America, sharing striking aposematic color patterns that warn predators of their potent chemical defenses. However, direct measurements of their toxicity have not been taken, leaving the evolutionary dynamics between the species involved in the mimicry rings unknown. To answer questions of mimicry dynamics and millipede toxicity in a confirmed mimicry ring in the Cumberland Mountains, the area of the chemical gland reservoir chamber was measured in 15 millipede species from the eastern North American clade of the Xystodesmidae. The ancestral state of the chemical gland size was reconstructed on a recently-inferred phylogeny of the family, and the reservoir chamber was found to have increased in size over evolutionary time, particularly in the tribe Apheloriini. The area of the reservoir chamber was compared between the putative model and mimics in the mimicry ring, and was found to have increased relative to the ancestor in the model genus *Apheloria*, and it was found to have decreased in the putative mimicking species in sympatric species of the genus *Brachoria*, supporting previous hypotheses about the mimicry relationship between these species. In other xystodesmid species that do not co-occur with any species of *Apheloria*, their reservoir chamber size was found to be as large or larger than that of *Apheloria*, suggesting that only species sympatric with *Apheloria* have reduced toxicity.

2.2 Introduction

Aposematic coloration is a ubiquitous anti-predatory mechanism that has evolved multiple times across animals, such as in ladybird beetles (Arenas & Stevens, 2017), danaine butterflies (Prudic et al., 2019), coral snakes (Brodie, 1993), and skunks (Caro et al., 2013). Aposematism often occurs as contrasting colors, such as long wavelength colors with black, but may also be expressed in other ways, such as an olfactory warning via chemical odors (ladybird beetles, Marples et al., 1994) or an auditory warning (rattlesnakes, Rowe & Owings, 1978; Arctiine moths, Dowdy & Conner, 2016). The advantage conferred by a warning signal is predicated on the signal being associated with a defense that deters the predator from attacking (Ruxton et al., 2018). By preventing injury to the individual exhibiting aposematism, the organism defends itself without deadly contact with predators. However, the advantages of being aposematic depends on the space and time in which an organism occurs. If a predator evolves a trait that counters the defense of the aposematic organism, then the advantage of being conspicuous to that predator is diminished (Endler & Mappes, 2004, Mappes et al., 2005). While the advantage of aposematism may vary through evolutionary time (Ruxton et al., 2018), when present, aposematism may result in increased speciation rates, as exhibited by amphibians (Arbuckle & Speed, 2015).

The protection conferred to aposematic organisms has resulted in the emergence of mimicry, in which another organism(s) co-evolve the same aposematic signal. Two main types of mimicry have been identified: Müllerian mimicry, in which two species share an aposematic signal and possess a defense (Müller, 1879); and Batesian mimicry, in which two organisms share an aposematic signal but only one (the model) is defended (Bates, 1862). The traditional view of Müllerian and Batesian mimicry categorizes these types as discrete, but subsequent research has shown that mimicry is a continuum between defended and non-defended statuses;

within this continuum, a variety of mimicry scenarios have been proposed, including quasi-Batesian mimicry, quasi-Müllerian mimicry, and super-Müllerian mimicry (Huheey, 1976, Turner & Speed, 1999, Speed, 1999, Balogh et al., 2008). For example, in quasi-Batesian mimicry, two or more species are defended, but the least-defended species functions as a “quasi-”Batesian mimic that slows down the learning process of the predator—negatively affecting the better defended species in the mimicry complex (Speed, 1993). Quasi-Müllerian mimicry is a category of mimicry in which an undefended mimic reduces predation on the model (Speed & Turner, 1999). Another mimicry category is super-Müllerian mimicry, in which a less defended mimic benefits the model more than an equally defended mimic would (Balogh et al., 2008). Therefore, within the mimicry spectrum, the advantages conferred by aposematism and defenses depend on the local adaptive landscape. Factors such as the predator community, their satiation, and availability of prey species can also affect whether a species involved in a mimicry complex is closer to the classical Müllerian or Batesian end of the spectrum (Balogh et al., 2008). Variation of secondary defenses within a species and a locality may also affect mimicry (Amezquita et al., 2017).

A model case of aposematism and mimicry is found in millipedes of the order Polydesmida, family Xystodesmidae (Marek & Bond, 2009; Zimmer & Emlen, 2016). Known as cherry millipedes due to an aromatic byproduct of their chemical defense, xystodesmids have a center of diversity in the Appalachian Mountains. The Appalachian Region is the family’s worldwide hotspot of biodiversity, where the millipedes are common detritivores in the undergrowth of temperate forests. One western genus, *Motyxia*, exhibits bioluminescence as a warning signal to nocturnal rodents (Marek et al., 2011, Marek and Moore, 2015). Many species of Polydesmida have aposematic coloration, which function as warning signals advertising their

unpalatability to potential vertebrate predators and protect the millipedes from predation (Iniesta et al., 2017). Species in the tribe Apheloriini, in particular, exhibit an array of bold color patterns, and the tribe is the most species rich of the family (Marek et al., 2014). These millipedes are a model system for studying mimicry and evolution of defense, as they lack eyes, so their bold colors do not function in communication with conspecifics and are therefore not a result of sexual selection (Marek & Bond, 2009).

The toxicity of xystodesmid millipedes is due to their paired, bi-compartmental chemical defense glands, which produce a poisonous mix of hydrogen cyanide and benzaldehyde at levels sufficient to kill 18 pigeon-sized birds (Eisner et al., 1967). Millipedes do not sequester their toxins from their diet, rather they synthesize them *in vivo* (Shear, 2015, Duffey & Towers, 1978). The bi-compartmental anatomy of the chemical defense gland in millipedes is only found in the Polydesmida in which a large reservoir chamber stores the stable precursor mandelonitrile and is connected to a thickened reaction chamber by a release valve controlled by a muscle (Eisner & Meinwald, 1966). When the valve is opened, mandelonitrile is released into the reaction chamber, where it is broken down by alpha-hydroxynitrile lyase, producing hydrogen cyanide and benzaldehyde, which is then released through an ozopore outside of the body (Shear, 2015, Duffey & Towers, 1978, Yamaguchi et al., 2018). These paired glands are typically present on the 5, 7, 9, 10, 12, 13, and 15-19 rings of the body of polydesmidan millipedes, opening either laterally or dorso-laterally. The benzaldehyde has a strong odor similar to cherries or almonds (hence the common name of the family) and functions as an olfactory signal, while hydrogen cyanide is a potent toxin conferring deterrence and protection from predators. However, some predators have evolved strategies to avoid these toxins, such as glowworm beetles in the family Phengodidae and ground beetles in the genus *Promecognathus*, which ostensibly quickly

decerebate the millipede and eat through the body, avoiding the poison glands (Labonte, 1983, Sánchez-Echeverría et al., 2016).

Shared color patterns in xystodesmid millipedes were noted by Whitehead & Shelley (1992), and several Müllerian mimicry rings among seven species in three genera (*Apheloria*, *Appalachioria*, and *Brachoria*) were recently studied in the Appalachian Mountains (Marek & Bond, 2009, Marek et al., 2018). These mimicry rings were inferred to be a consequence of unilateral ‘advergence’ (Mallet, 1999) by the mimic to the color pattern of the model, rather than convergence of each species to a shared color pattern (Marek & Bond, 2009). The genus *Apheloria* was hypothesized to be the more toxic model, and *Appalachioria* and *Brachoria* were hypothesized to be the less defended mimics. This was suggested because *Apheloria* is the more abundant species and aposematic coloration of *Brachoria* varies geographically as a function of *Apheloria* (Marek & Bond, 2009). While these qualities indicate that *Apheloria* is a widespread model species, which is mimicked by several narrowly endemic *Brachoria* species, the hypothesis that *Apheloria* is more toxic in this mimicry ring has not been directly evaluated.

With our hypothesis that *Brachoria* species are mimics, we expect them to have smaller chemical glands, and thus be less toxic than the *Apheloria* models. Their divergence from the model, with lower toxicity levels and less distinct color patterns, is an example of imperfect mimicry (Kikuchi & Pfennig, 2013). The color patterns in the mimicry ring were identified as impressionistic aposematic signals (Mallet, 1999), due to the mimicry being imperfect between presumed models and mimics, with the mimicking *Brachoria* and *Appalachioria* having “fuzzier” patterns that were not as distinct and clear as the patterns in *Apheloria* (Marek & Bond, 2009). According to mimetic theory, when purifying selection is relaxed, there is higher survivorship, which may allow for more variability in color pattern. At the study’s localities with

more abundant millipede populations and more species, more color morphs were observed, supporting this conclusion (Marek & Bond, 2009). Such a state where purifying selection is relaxed could result from predator learning, as inexperienced predators may not exert strong selection pressure for accurate Müllerian mimicry, allowing unequally defended co-mimics to survive (Ihalainen et al., 2007).

Within the mimicry continuum, Batesian and quasi-Batesian mimics exhibit dishonest signals, which indicate greater risk to a predator than actually exists. A prediction of dishonest signaling is that in species mimicking a more toxic model, the dishonest signal can be stable if there is a frequency-dependent advantage (Müller 1879; Dawkins & Guilford, 1991). In the case of *Apheloria* and *Brachoria*, *Apheloria*'s greater abundance at a site may confer such an advantage to the co-mimicking and rarer *Brachoria* (Marek & Bond, 2009). Since both genera are toxic, *Brachoria* may gain an additional advantage from evolving smaller chemical glands and relying on the protection granted by the sympatric *Apheloria* millipedes' comparatively greater toxicity. Additionally, cyanide-based defenses are inherently costly due to their toxicity and minimizing the volume of cyanide may be advantageous to the dishonest signaler. Congruent warning patterns in areas of sympatry between the two genera have been documented (see Fig. 3 in Marek & Bond, 2009), supporting this hypothesis of reliance on a more-toxic model.

Since a direct measurement of toxicity of the millipedes involved in the Appalachian mimicry ring has not been carried out—Eisner and colleagues only quantified overall cyanide concentration in *Apheloria* species—questions about the chemical ecology of these millipedes remain. How toxic are the millipedes in this shared mimicry ring? How does toxicity vary among species in the model-mimic ring? Is it affected by shared color patterns? Has the poison gland changed size earlier in time, for example throughout the family? Is *Apheloria polychroma* more

toxic, thereby providing more evidence that it's the model for *Brachoria* in the Appalachian mimicry ring? Here, we address these questions by comparing the toxicity of the poison glands by measuring their area and tracking their evolution in millipedes.

Our main objectives were to (1) test if the model *Apheloria polychroma* possesses larger chemical glands based on size of the reservoir chamber, and (2) test a prediction of dishonest signaling that the model species will have larger cyanide glands, and over evolutionary time the toxicity of the mimic declined. As an evolutionary context to determine direction of cyanide gland volume change, we used a recent phylogeny of Xystodesmidae to infer the ancestral volume of the gland of species in mimicry rings.

2.3 Materials and Methods

Xystodesmid millipedes were collected from broadleaved forests in the eastern United States with a millipede rake, according to methods described in Means et al. (2015). Millipedes were found under leaf litter during the day or crawling on top of leaves at night, sometimes with the use of an ultraviolet flashlight. Millipede species were subsampled from across the phylogeny of Xystodesmidae and were selected from each recognized tribe to investigate the evolution of the size of the defense glands across clades. Representative species and genera were selected from each tribe in the eastern North American xystodesmid clade as exemplars for the size of the defense gland in their respective tribes. Species from the large radiation Apheloriini were selected from throughout the tribe, including from the basal genus *Deltotaria*, the genus *Rudiloria*, which is most closely related to *Apheloria*, and from the genera *Sigmoria*, *Dixioria*, and *Appalachioria*, to assess the defense gland's evolution in this tribe. To test predictions of

how toxicity is affected by mimicry rings (Marek & Bond, 2009), specimens were selected from within areas of a mimicry ring, and in areas without a mimicry ring. The area (squared millimeters) of their chemical glands were measured and compared. The mimicry ring between the three species—*Apheloria polychroma* (listed as *Apheloria* clade A in Marek & Bond, 2009), *Brachoria cedra*, and *B. mendota*—in the central Appalachian Mountains of Virginia, Kentucky, and Tennessee (see Fig. 1 in Marek & Bond, 2009) was chosen for analysis due to the availability of material and preexisting information on population size and color patterns for this mimicry ring.

The 12th body ring (segment) of 7 – 30 specimens of each species was separated from the trunk of the millipede, and its width and paranotal length were measured with a Leica M125 stereomicroscope with an eyepiece reticule at between 8x and 100x magnification. The 12th body ring was selected because it is in the middle of the body, easy to access, and is largest gland-containing ring. The 12th body ring was incubated overnight in a 2% trypsin solution (Amresco Inc., Solon, OH) at 37°C to digest muscle, proteins, and soft tissue. Following trypsin digestion, the cyanide glands were carefully removed from the body ring. The cyanide glands were then placed in a watch glass submerged in water under a glass cover slip to stabilize them for photography, and a pure silver scale bar was placed beside them. The cyanide glands (Fig. 2.1) were then photographed with a Canon 6D camera and a MPE 65-mm lens, and the resulting images were focus stacked with the program Helicon Focus Pro v.6.7.1 into one composite image. The precursor reservoir chambers of each specimen's cyanide glands were outlined in Adobe Illustrator CS6, and their areas calculated in ImageJ 1.51k (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <https://imagej.nih.gov/ij/>, 1997-2018, Schneider et al., 2012) with an automated threshold routine via the Fill Holes command in the

Process and Binary menu. Area was calculated with the Analyze Particles command after setting the individual scale for each image. For each specimen, the largest and most intact reservoir chamber's area was measured with ImageJ and used in calculations. The measurements were subsequently averaged together by species to obtain a gland size measurement for each species. Males were preferentially measured, but if male specimens were lacking, females were also used and a weighted average was calculated. In instances where only male specimens were measured, the average was calculated instead of a weighted average.

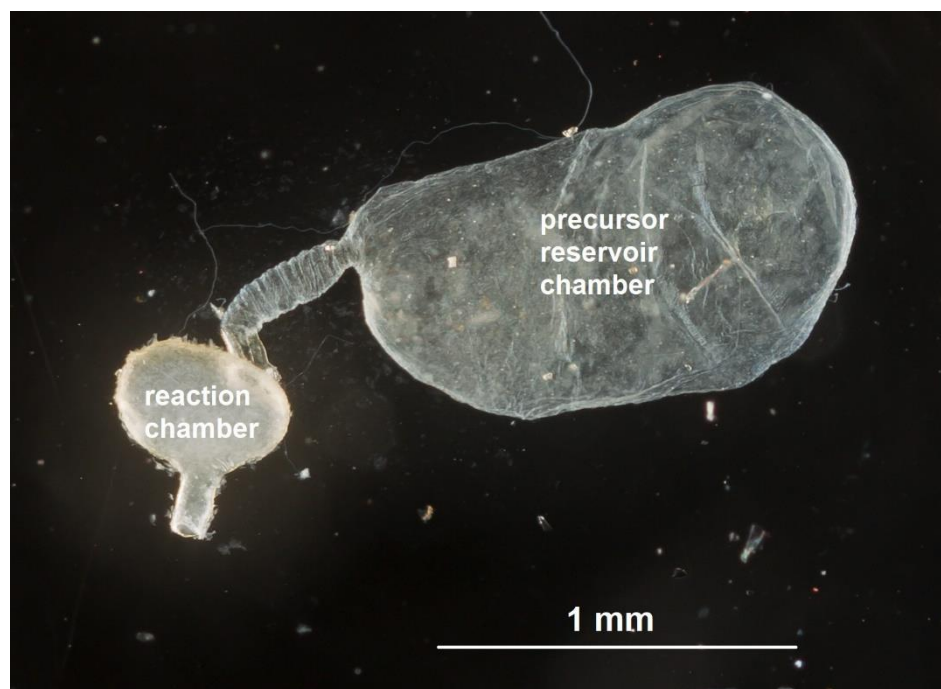


Figure 2.1. Bicompartmental chemical gland of xystodesmid millipedes, showing the large precursor reservoir chamber where stable mandelonitrile is stored and the reaction chamber, where an enzyme catalyzes the chemical reaction, resulting in the production of benzaldehyde and hydrogen cyanide. The gland has been digested in trypsin, removing the associated muscle that controls the valve connecting the two chambers.

An exemplar chemical gland from *Apheloria polychroma* (MMC0276) was imaged with a scanning electron microscope (SEM). The gland was attached to a 12.7 mm diameter

aluminum SEM stub with double-sided carbon tape and subsequently plasma coated under stable argon pressure with 20 nm of platinum and palladium metals with a Leica EM ACE 600 high vacuum coater, and imaged on a FEI Quanta 600 FEG environmental SEM (5 kV, 3.5 spot size).

To test the hypothesis that in the mimicry ring between *Apheloria polychroma* and *Brachoria mendota* and *B. cedra* (Marek & Bond, 2009), *A. polychroma* is the more toxic model and the two *Brachoria* species are less toxic mimics, the area of the reservoir chamber of the glands between the two genera were compared with a two-sample t-test. Natural log-transformed data were used in the test to account for non-linear variation resulting from the differing body-sizes of the millipedes.

Phylogeny Estimation: To investigate the evolution of the area of the reservoir chamber of the cyanide gland, a phylogenetic tree of the family Xystodesmidae was estimated from six mitochondrial (16S including tRNA-Val, 12S, CO1) and nuclear (28S, EF1-a) genes. The published phylogeny from Means & Marek (2017) was used for our analysis, and phylogenetic estimation methods can be found in that study.

Ancestral Trait Reconstruction: The evolution of the area of the reservoir chamber of the cyanide gland was inferred on the tree with a least-squares parsimony method of estimating ancestral states, in which the cost of a state change is equivalent to the squared difference in state values, $(x - y)^2$ (Maddison, 1991). This method of inference was chosen because we can assume that the chosen trait evolves by Brownian motion in an independent manner across the species. Species for the ancestral state reconstruction analysis were selected from several genera and clades across the phylogeny of the eastern North American Xystodesmidae to ensure independence of species. This is to make certain that the data are drawn independently from the phylogeny and to avoid a situation where our chosen species are from the same distribution

(Felsenstein, 1985), which would result in a statistically unsound inference. Since the phylogeny of the xystodesmids is known (Means & Marek, 2017), we can be reasonably sure that we have accurate knowledge of relationships within the family.

A directional evolutionary trend of increased toxicity was tested for by calculating phylogenetic distance (Marek & Moore, 2015). Phylogenetic distance was calculated by adding the branch lengths from the root to the reconstructed species node. If millipedes became more toxic over time, a strong positive correlation would be found between a node's distance from the root and its level of toxicity.

2.4 Results

A total of 15 millipede species from 4 tribes and 11 genera were collected from 8 U.S. states. Depending on availability of material, 7 – 30 specimens of each species were dissected and measured, resulting in 222 individual measurements. Three species (*Apheloria polychroma*, *Brachoria cedra*, *B. mendota*) were collected from areas of known mimicry rings, while the other 12 species were from areas without the presence of mimicry rings.

The area (in mm²) of the chemical gland reservoir chamber in the three species known to form a mimicry ring are in Figure 2.2. Sizes are given as the weighted average (by sex) for each species. The model species, *Apheloria polychroma*, was found to have the largest chemical gland size, with a weighted average of 1.947 mm², while *Brachoria cedra* has the second largest gland size of 0.951 mm², and *Brachoria mendota* had the smallest gland size at 0.810 mm². A total of 30 specimens of *A. polychroma* were measured, seven specimens of *B. cedra*, and 25 specimens of *B. mendota*. To test if the area of the chemical glands differed significantly between the model

A. polychroma and the mimic *Brachoria* species, a two-sample t-test assuming unequal variances was calculated. We failed to reject the null hypothesis that gland size did not differ significantly between both *Apheloria polychroma* and *Brachoria cedra* ($df = 11$, $P < 0.001$) and *Apheloria polychroma* and *Brachoria mendota* ($df = 50$, $P < 0.001$).

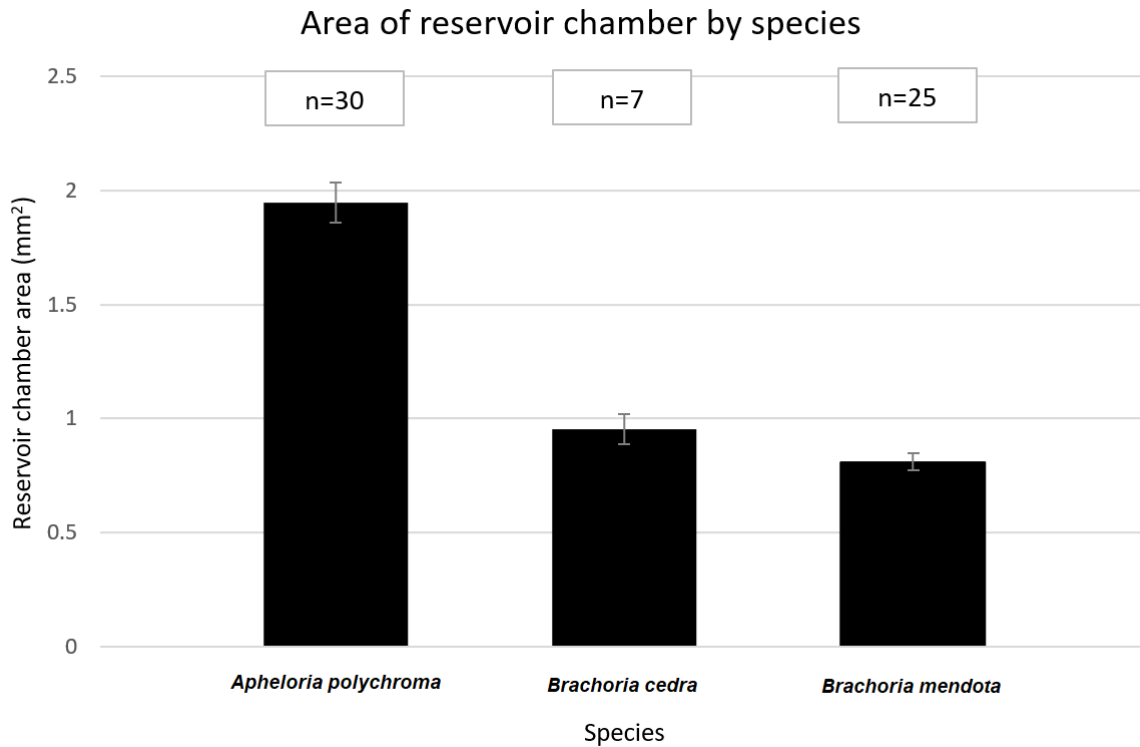


Figure 2.2. Chemical gland reservoir chamber area (weighted average by sex, in mm²) of each species in the *Apheloria polychroma* mimicry ring, with standard error bars.

The full tree for the eastern North American xystodesmid clade is shown in Figure 2.3, and the taxa used for the ancestral state reconstruction analysis have their branches highlighted. The results of the ancestral state reconstruction are shown in a pruned tree (Figure 2.4), with a total of 15 species used for the analysis. The ancestral root node state of the chemical gland size was found to be -1.73 mm² (negative value due to the natural log transformation applied for the

analysis). The sister species of the remaining taxa in the phylogeny, *Gyalostethus monticolens*, (part of the polyphyletic tribe Rhysodesmini) has the smallest chemical glands, at -4.29 mm^2 , and has the shortest phylogenetic distance to the ancestor. The chemical gland size increases to -0.885 mm^2 in *Nannaria* n. sp. “aenigma”, and to -0.266 mm^2 in *Dicellarius atlanta*—members of the tribes Nannariini and Pachydesmini. The chemical gland size continues to increase in the clade that includes *Cherokia georgiana latassa* (tribe Rhysodesmini) and the rest of the crown-group species, all in the tribe Apheloriini. The gland is largest in the species *Apheloria polychroma*, *Dixioria pela*, *Sigmoria nantahalae*, *Brachoria initialis*, and *Dixioria coronata*, with the species *Brachoria initialis* having the largest measured chemical glands.

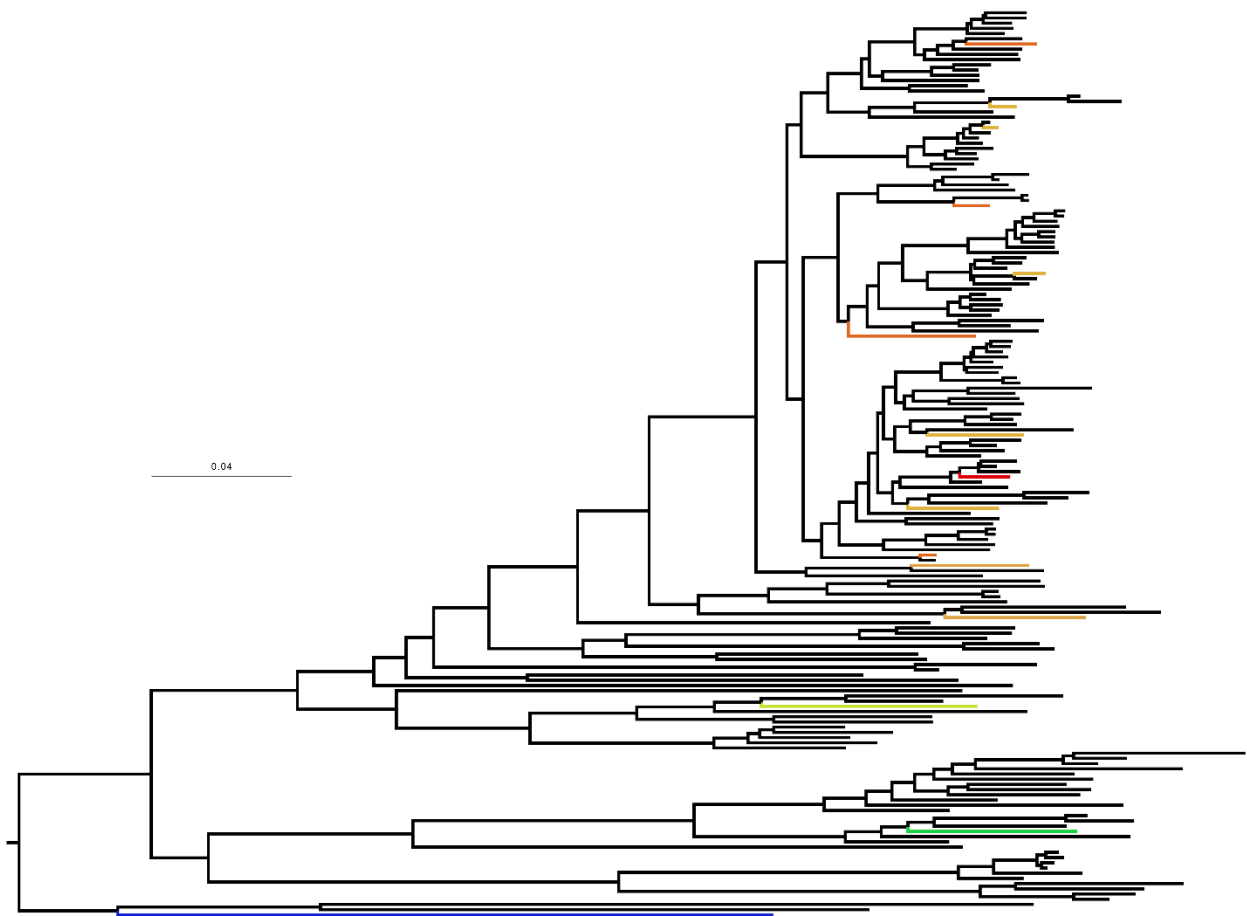


Figure 2.3 Phylogeny of the eastern North American Xystodesmidae. Highlighted branches indicate species used for ancestral state reconstruction analysis, colors correspond to colors in Fig. 2.4. Scale is in site substitutions.

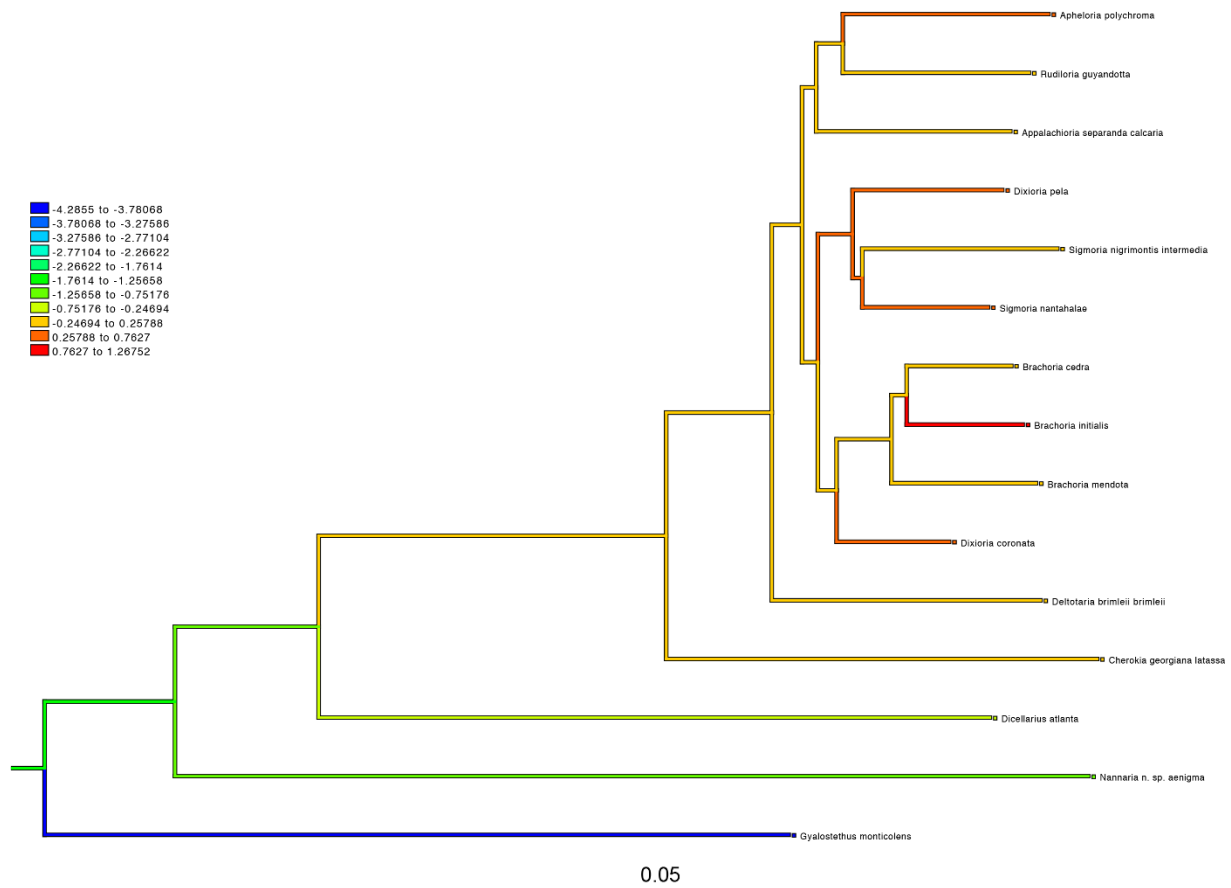


Figure 2.4. Least-squares parsimony ancestral state reconstruction of cyanide gland size in the family Xystodesmidae. Blue color indicates a smaller gland size, increasing in size through green to yellow to red coloration. Scale is in site substitutions.

Over evolutionary time (as measured by the path length from the root to each species node), the area of the chemical gland reservoir chamber increased (Figure 2.5). A strong positive correlation was found between cyanide gland size and phylogenetic distance ($r=0.701$). With regards to the *Apheloria polychroma* mimicry ring, *A. polychroma* was found to have a larger chemical gland, compared to the other species that were measured. The two *Brachoria* species in

the mimicry ring (*B. cedra* and *B. mendota*) were determined to have smaller chemical glands than *Apheloria polychroma*. Relative to the most recent common ancestor of the Apheloriini clade, *Apheloria polychroma*'s chemical glands were found to have increased in area ($r = 0.994$), while the two *Brachoria* species' chemical glands decreased in area (*B. cedra*: $r = -0.821$; *B. mendota*: $r = -0.927$) (Figure 2.6).

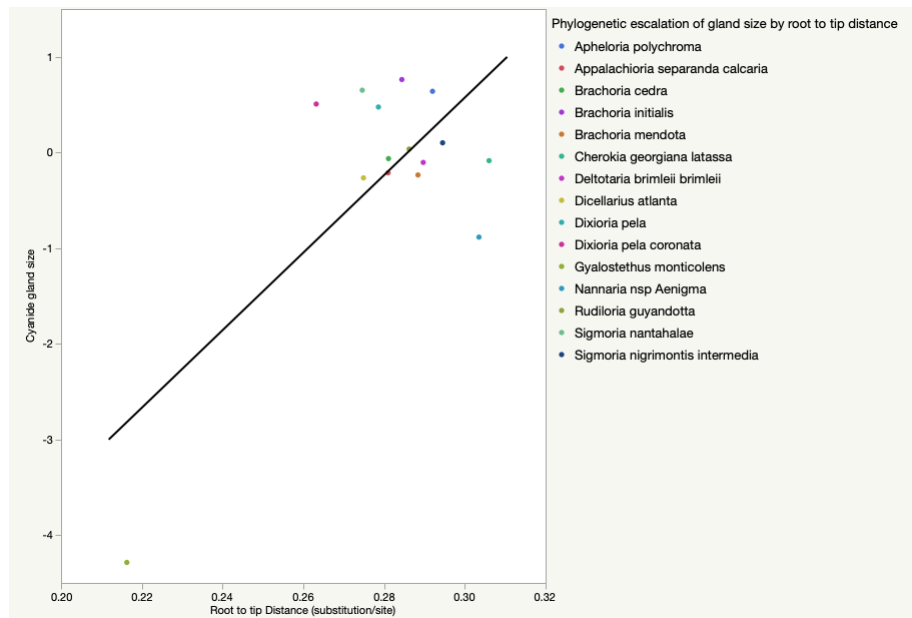
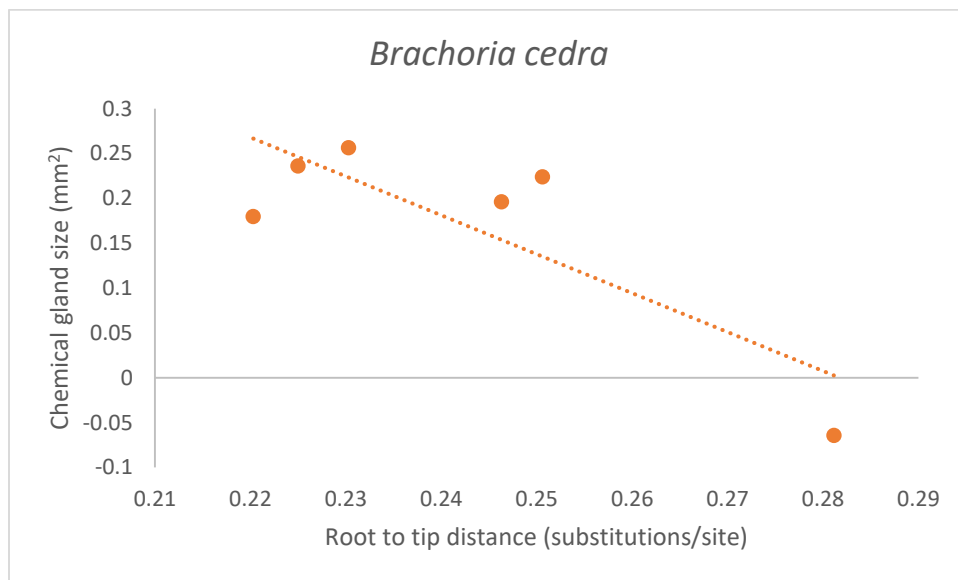
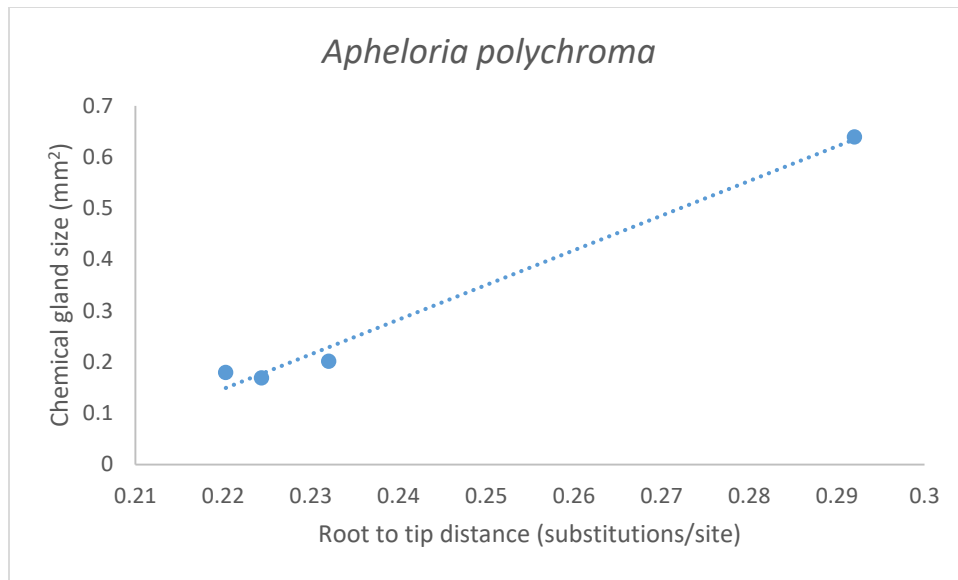


Figure 2.5. Phylogenetic escalation of gland size by phylogenetic distance, measured by root to tip distance.



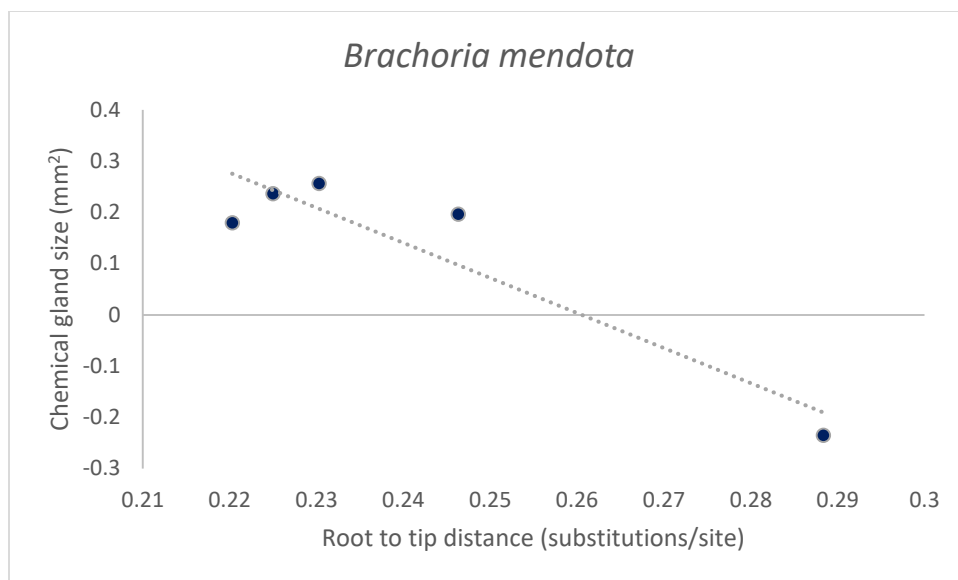


Figure 2.6. Phylogenetic change in gland size of three species in a known mimicry ring. From top to bottom, *Apheloria polychroma*, *Brachoria cedra*, and *Brachoria mendota*.

The reaction chamber of the cyanide gland of *Apheloria polychroma* has many pores (Fig. 2.7), and was opened to allow examination of the interior and exterior surfaces of the chamber. The largest pores (the hole-type pores) are about 3 μm wide and are flush with the surface of the chamber. Another observed type of pore (the salt-shaker pores) is much smaller, about 0.5 μm wide, and occur in clusters of 2 – 8 similarly-sized pores set in a slight depression at the top of a small mound about 10 μm wide. These clusters have the appearance of salt shakers and are numerous within the interior of the chamber, but do not have corresponding structures on the outer surface of the chamber, as the hole-type pores do. These pores are likely the avenues through which the catalytic enzyme is injected into the chamber, causing the disassociation of mandelonitrile into the toxic byproducts hydrogen cyanide and benzaldehyde.

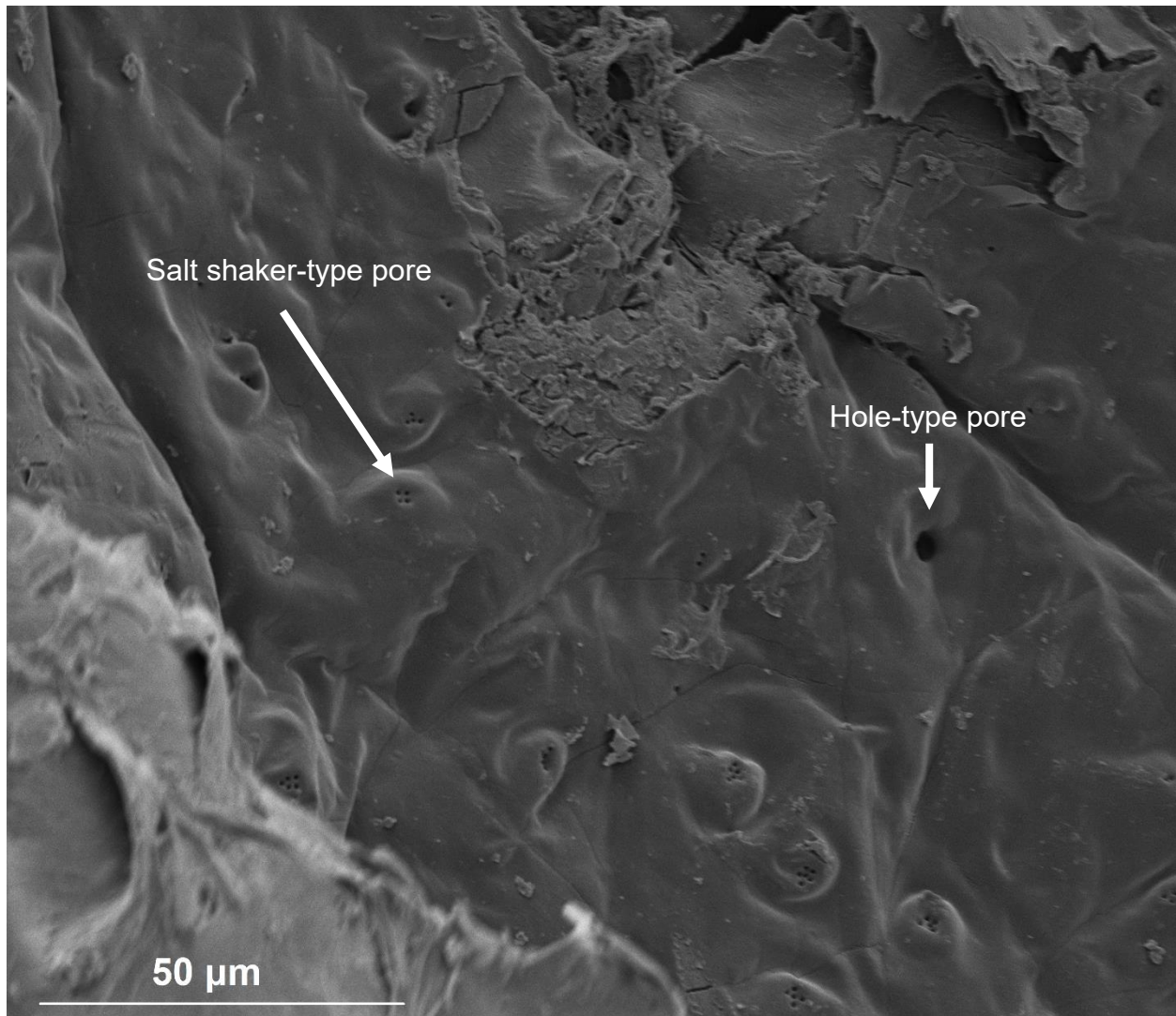


Figure 2.7. Interior surface of the reaction chamber of a chemical gland of *Apheloria polychroma* (MMC0276), showing hole-type and salt shaker-type pores.

2.5 Discussion

In the Appalachian Mountains, mimic millipedes of the family Xystodesmidae evolved greater toxicity over evolutionary time. However, this escalation in toxicity of defenses was not equal among species. In our analyses, we show that over evolutionary time, *Apheloria polychroma* has become more toxic than two sympatric *Brachoria* species, which have evolved

lower toxicity in tandem with their advergent color patterns. Therefore toxicity, initially starting at 0.1797 mm² in the most recent common ancestor of *Apheloria* and *Brachoria*, diverged in separate directions. This asymmetric divergence is consistent with a prediction of dishonest signaling in the mimic species of the genus *Brachoria*.

While the aforementioned *Brachoria* species were found to have comparatively small glands, *B. initialis* was found to have a greater cyanide gland area (indicating a greater toxicity) than *A. polychroma*. *Brachoria initialis* is not a known mimic and observations indicate that co-occurring species are dissimilar in appearance (Marek, 2010). Similarly, large glands were also observed in *Sigmoria nanatahalae*, *Dixioria pela*, and *Dixioria coronata*. The explanation for these large glands may be due to a lack of protection from an established mimicry ring. The distribution of these species are allopatric to any *Apheloria* species. If *Apheloria* typically functions as a Müllerian model throughout its range, in its absence, taxa that might otherwise evolve lower toxicity may be vulnerable to the greater predation that *Apheloria*'s presence would otherwise deter. Perhaps the additive effect of toxicity among mimetic species equates to the solitary effect of a non-mimicking species.

Brachoria may not be the only Apheloriine genus with a relationship with the genus *Apheloria*. The species *Rudiloria guyandotta* and *Appalachioria separanda calcaria* have chemical gland reservoir chamber areas similar to the mimicry ring's *Brachoria* species. They are also sympatric with an *Apheloria* species, *Apheloria virginiensis corrugata*. Their color patterns and possible mimicry have not yet been investigated, but they also have aposematic coloration and perhaps may also form a mimicry ring within their distribution.

The evolutionary trend of increased toxicity over time within the Xystodesmidae may coincide with the evolution of aposematism. The clades with the smallest chemical gland sizes—

Rhysodesmini, Nannariini, and Pachydesmini—all have color patterns that are not very vivid or striking. Mostly, they consist of drab hues of brown and orange, or in the Nannariini, black or brown with white or pink lateral spots. These taxa tend to be cryptic in their native microhabitats of dark soil and dead leaves, and are often not encountered atop leaf litter as in *Apheloria*. These characteristics, combined with their small chemical gland size, suggests a primary defense of background matching, crypsis, and secondary chemical defenses for use if ultimately uncovered by a potential predator. In contrast, the clade containing the Rhysodesmini and Apheloriini is almost exclusively aposematic, with vivid contrasting colors of yellow, orange, and red against a black base color. This adaptive radiation encompasses about 140 species (Marek et al., 2014). The striking coloration and large chemical glands thus suggest a different survival strategy from the cryptic species, with a reliance on being apparent to predators and posing a danger to attack as their primary defense.

Based on our analyses of chemical gland size and phylogenetics of the Xystodesmidae, we show that the model species *Apheloria polychroma* is a more toxic species than *Brachoria cedra* and *B. mendota*. *Brachoria* species have evolved lower toxicity over evolutionary time in sympatry with *A. polychroma*. We conclude that dishonest signaling evolved unilaterally in the mimic *Brachoria* species over evolutionary time as a result of the additive effects of Müllerian mimicry.

2.6 Acknowledgements

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Chapter 3 - Phylogenetic systematics of the *Nannaria wilsoni* species group (Polydesmida: Xystodesmidae)

3.1 Abstract

Dozens of new species of the xystodesmid genus *Nannaria* Chamberlin, 1918 have been known from museum collections for decades, but a systematic revision has not been undertaken, until now. Two species groups are known within the genus, the *minor* species group and the *wilsoni* species group. In this study, the *wilsoni* species group is investigated throughout its range in the Appalachian Mountains of the eastern United States. 374 specimens were collected from seven states and used for molecular phylogenetic analysis of six genes: COI, 16S, 28S, EF1a, RNAPolII, and fbox. A maximum likelihood phylogenetic tree using RAxML was inferred, and relationships of the species are discussed. A total of 18 new species are described, bringing the composition of the *wilsoni* group to 24 species, quadrupling its known diversity. Museum holdings of *Nannaria* are catalogued, and a total of 1,835 records are used to produce distribution maps for each species. Live photographs, illustrations of species identification characters, and ecological notes for each species are given. The *Nannaria wilsoni* species group is found to be restricted to the Appalachian region, unlike the widely-distributed *minor* species group, and has a gap in its distribution in northeast Tennessee and adjacent northwest North Carolina. The *wilsoni* species group appears to be adapted to moist microhabitats in middle to high elevation forests in eastern North America, and additional new species are expected to be found in the southern Appalachian Mountains.

3.2 Introduction

Millipedes in the family Xystodesmidae encompass a large component of the diplopod fauna in the deciduous forests of North America, with around 400 currently known species (Marek et al., 2014). Many of the species in the family are strikingly aposematic, with bold coloration of black paired with hues of yellow, orange, and red, advertising their toxic chemical defenses of hydrogen cyanide and benzaldehyde (Marek & Bond, 2009; Marek et al., 2018). While all members of the family are chemically-defended, a few groups within the family do not appear to rely heavily on chemical defense and aposematism as a main form of defense. This is exemplified by the genus *Nannaria* Chamberlin 1918, an assemblage of small-bodied (15-38 mm long) millipedes distributed in eastern North America. They are typically chestnut brown to black with a bimaculate pattern of orange to red, or white spots.

The small size and comparatively subdued colors are atypical for xystodesmids, and previous authors have classified *Nannaria* as non-aposematic due to these traits, but suggested *Nannaria* may be mimicking colorless juveniles of aposematic xystodesmids (Whitehead & Shelley, 1992). Additionally, *Nannaria* do not exhibit as much diurnal surface level activity, traits typically associated with aposematic organisms, as other xystodesmids, such as the apheloriine species *Apheloria polychroma* (Marek et al., 2018). More commonly, *Nannaria* are collected under forest detritus such as dead leaves and stones, or with passive trapping methods such as pitfall traps. They are difficult to deliberately collect through active methods (Hennen & Shelley, 2015). Part of this difficulty stems from their behavior: individuals tend to remain buried in the soil, even as adults. They either stay completely beneath the surface, or with only a portion of their body exposed above the soil.

The range of *Nannaria* extends from Arkansas and Missouri east through northern Mississippi to central North Carolina, and north to New York. The genus has the highest number

of species described from the Appalachian Mountains. The genus is classified in the tribe Nannariini, which also includes the monotypic genus *Oenomaea* Hoffman, 1964, known from eastern Tennessee, northern Alabama, and northern Georgia. A recent taxonomic article reduced the Nannariini to a subtribe of the tribe Eurymerodesmini within the Xystodesmidae, but this hypothesis has yet to be tested with phylogenetic data (Shelley & Smith, 2018).

Despite the small size and behavior of *Nannaria*, millipede workers have described 19 species of Nannariini in three genera: *Nannaria*, *Mimuloria*, and *Oenomaea* (Marek et al., 2014; Hennen & Shelley, 2015). The first species of *Nannaria* was described in 1847 by C. L. Koch, as *Fontaria oblonga*, with its type locality simply stated as Pennsylvania (Koch, 1847). Two more species were described in the late 1800s (McNeill, 1887; Bollman, 1888), but the genus *Nannaria* was not erected until the early 20th century, with *N. minor* designated as the type species (Chamberlin, 1918). Later papers by Chamberlin (1928, 1940, 1947, 1949), Williams & Hefner (1928), Causey (1942, 1950), Hoffman (1948, 1949, 1950), Loomis & Hoffman (1948), Shelley (1975), and Hennen & Shelley (2015) added more species and characters to define the Nannariini. Previous species included in *Nannaria* have been transferred to other genera such as *Boraria* Chamberlin, 1943; *Stenodesmus* De Saussure, 1859; and the genus *Castanaria* Causey, 1950, which was later synonymized with *Nannaria*. The monophyly of genera in the tribe have not been tested using molecular phylogenetics. However, the monophyly of the tribe Nannariini has been tested using a limited taxon sample, and found to be monophyletic and closely related to the tribes Apheloriini and Rhysodesmini (Marek & Bond, 2006). The tribe is diagnosed by the following main characters: sterna with lateral triangular spines; males with twisted, spatulate pregonopodal claws; gonopods with straight or curving acropodites; and a stout, long prefemoral process (Hoffman, 1964; Hennen & Shelley, 2015).

Previous authors have indicated that many undescribed species and possibly even genera remain to be found in the tribe (Hoffman, 1999; Hennen & Shelley, 2015; Marek et al., 2018), and this is generally supported by a cursory examination of material in museum collections. Upon closer study of material from the Virginia Museum of Natural History amassed by Richard Hoffman and museum staff during decades of field collecting and preliminary molecular phylogenetics conducted over the past decade, two main morphological groups within the genus were apparent. One group, whose gonopod morphology was relatively simple, was referred to as the *Nannaria minor* species group, based on *N. minor*, the oldest name in the group. The second group, whose gonopod morphology was more “complex” due to the twists and additional processes on the acropodite branch, was labeled the *Nannaria wilsoni* species group, based on the species *N. wilsoni*. The *Nannaria minor* species group is more widespread in eastern North America, found across the entire generic range, while the *Nannaria wilsoni* species group is restricted to the Appalachian Mountains, where it occupies two separated distributions, a northern and a southern portion.

With the discovery of about 50 hypothesized undescribed species between the two groups, the ambitious goal of describing them all was split between two projects. The species of the *Nannaria minor* species group are treated in a forthcoming manuscript lead by the second author (JCM), while the species of the *Nannaria wilsoni* species group are treated here. The aims of this study were: (1) to test the monophyly of the genus *Nannaria* using molecular phylogenetics, (2) describe new species within the *Nannaria wilsoni* species group, and (3) investigate the ecology of the genus.

3.3 Materials and Methods

Nannaria specimens were collected by raking leaf litter and digging at and beneath the soil-litter interface in deciduous forest habitats throughout the eastern United States, according to the methods described in Means et al. (2015). Millipedes were collected with the goal of preserving material for both genetic and morphological analysis, and efforts were made to collect samples of all described species and any undescribed species represented in museum collections. *Nannaria* holdings from the Virginia Museum of Natural History (VMNH, Martinsville, Virginia), Virginia Tech Insect Collection (VTEC, Blacksburg, Virginia) and the North Carolina Museum of Natural Sciences (NCSM, Raleigh, North Carolina) were examined and georeferenced if geographical coordinates were not originally provided with the program GEOLocate (Rios, 2018), and databased with catalogue numbers beginning with the prefix NAN (e.g. NAN0001). This museum material represented the bulk of *Nannaria* holdings worldwide, and provided locality data for putative new species within the genus. Along with this museum material, *Nannaria* specimen records from the Symbiota Collections of Arthropods Network (SCAN) database (<https://scan-bugs.org/>) were acquired, along with records from the National Parks Service's Great Smoky Mountains National Park natural history collection.

Sampling sites were selected based on the historical museum material, along with a focus on unsampled areas within the generic distribution. Field collecting took place in seven states: Georgia, Kentucky, North Carolina, South Carolina, Tennessee, West Virginia, and Virginia. Scientific collecting permits were acquired for collecting and are listed in the acknowledgements section.

After field collection of specimens, millipedes were photographed on a moss background in a laboratory setting with a Canon 6D camera and a 50-mm lens. Afterwards, they were given a catalog number with the prefix MPE (e.g. MPE02123), and 24 legs were removed from the right

side of the body and preserved in RNAlater or 100% ethanol for molecular analysis. The rest of the body was preserved in 70% isopropanol for morphological analysis and deposited in the Virginia Tech Insect Collection (VTEC, <https://www.collection.ento.vt.edu>). Measurements for the following six characters were taken from a male exemplar of each species using a Leica M125 stereomicroscope, after Marek (2010), and all following measurements are given in millimeters: body length (BL), collum width (CW), intergenal width (IW), interantennal socket width (ISW), body ring 10 width (B10W), and body ring 10 height (B10H). Morphological coding and scoring of characters was accomplished using the program Mesquite (Maddison and Maddison, 2010) to produce a Nexus file of qualitative male and female exoskeletal and genitalic characters for species diagnosis and comparison (Appendix A). The left gonopod of each species was photographed with a Canon 6D dSLR camera with a 65 mm Canon MP-E macro lens mounted on a Passport II Portable Digital Imaging System (Canon, Tokyo, Japan; Visionary Digital, Charlottesville, USA). Photographs were focus stacked with the program Helicon Focus Pro v.6.7.1 (HeliconSoft, Kharkiv, Ukraine), and the composite image was outlined in Adobe Illustrator CS6. Distribution maps were made using the program SimpleMappr (Shorthouse, 2010).

A phylogenetic tree of the genus *Nannaria* (including both the *Nannaria minor* species group and *Nannaria wilsoni* species group) was estimated from six mitochondrial (16S, CO1) and nuclear (28S, EF1-a, fbox, and RNAPolII) gene regions. Genomic DNA was extracted from millipede legs and purified with a Qiagen DNeasy kit according to the manufacturer's instructions. The DNA was then amplified via polymerase chain reaction and sequenced with a Sanger protocol, as in Means & Marek, 2017. Chromatograms were trimmed, bases called, and overlapping fragments assembled into contiguous sequences with the program Mesquite using

phred and phrap in the Chromaseq module (Maddison and Maddison, 2010; Ewing et al., 1998). The program Prank (Probabilistic Alignment Kit, Löytynoja and Goldman 2005) was used for sequence alignment using the default gap opening and extension probabilities. MrBayes (Ronquist et al., 2012) was used for Bayesian phylogenetic inference, with one-quarter of the trees from the MCMC analysis being discarded as the burn-in phase, and the subsequent topologies averaged with the commands “sumt” and “contype=half-compat” to generate a consensus phylogeny.

3.4 Results

Field Collection. In total, 1,835 *Nannaria* specimens and specimen records in the wilsoni group were examined for this study. Of these, 374 specimens were recently-collected (i.e. between 2003 and 2019, with 97% collected between 2013 and 2019) and were deposited in the VTEC. During field collection, it became clear that *Nannaria* are not as abundant as other xystodesmid millipedes in eastern North America. At collecting sites, individuals often exhibited a patchy distribution, with only a few individuals in each aggregation. However, it is unclear if this was a true reflection of their abundance, or an artifact of their comparatively cryptic habits (compared to other large xystodesmid millipedes). Their cryptic body coloration and burrowing behavior resulted in them being difficult to collect with regularity, and when found, were most often partly buried in the soil, with only a small portion of their dorsum exposed above the soil. Due to this difficulty, sampling strategies were adjusted, with field collectors’ focus shifting towards habitats with abundant hemlock (*Tsuga* spp.) and rhododendron (*Rhododendron* spp.), as these plants provide shady, moist microhabitats in their understory (Rohr et al., 2009). Preference was also given to riparian habitats, which also tended to have cool, moist soil that *Nannaria* seem to

prefer. In addition to hemlock and rhododendron, the most commonly noted plant species include: oaks (*Quercus* spp.), maples (*Acer* spp.), tulip tree (*Liriodendron tulipifera*), pawpaw (*Asimina triloba*), witch hazel (*Hamamelis* spp.), birches (*Betula* spp.), American beech (*Fagus grandifolia*), dogwoods (*Cornus* spp.), black cherry (*Prunus serotina*), hickories (*Carya* spp.), and sassafras (*Sassafras albidum*). An example of typical habitat for the *Nannaria wilsoni* group is given in Figure 3.1.



Figure 3.1. Typical habitat of species in the *Nannaria wilsoni* group. Rhododendron (*Rhododendron* sp.) and American beech (*Fagus grandifolia*) can be seen in the foreground. Bare dirt can be seen, where specimens were collected after scraping away the surface litter. Lumpkin County, Georgia.

The *wilsoni* species group is restricted to the Appalachian region of the eastern United States, within the following US Level III ecoregions: Blue Ridge, Ridge and Valley, Southwestern Appalachians, Central Appalachians, and Western Allegheny Plateau (Fig. 3.2). The distribution of the species group is split into two separate sections: the central Appalachians portion (West Virginia, Virginia, and Kentucky) and the southern Appalachians portion (North Carolina, South Carolina, Tennessee, and Georgia). Interestingly, there is a 108 km gap in the distribution in northeastern Tennessee and adjacent northwestern North Carolina. This gap was initially thought to be a result of collecting bias, but despite concerted collecting trips to this area, only *minor* group species were found in this area, indicating this is a true gap in the distribution.

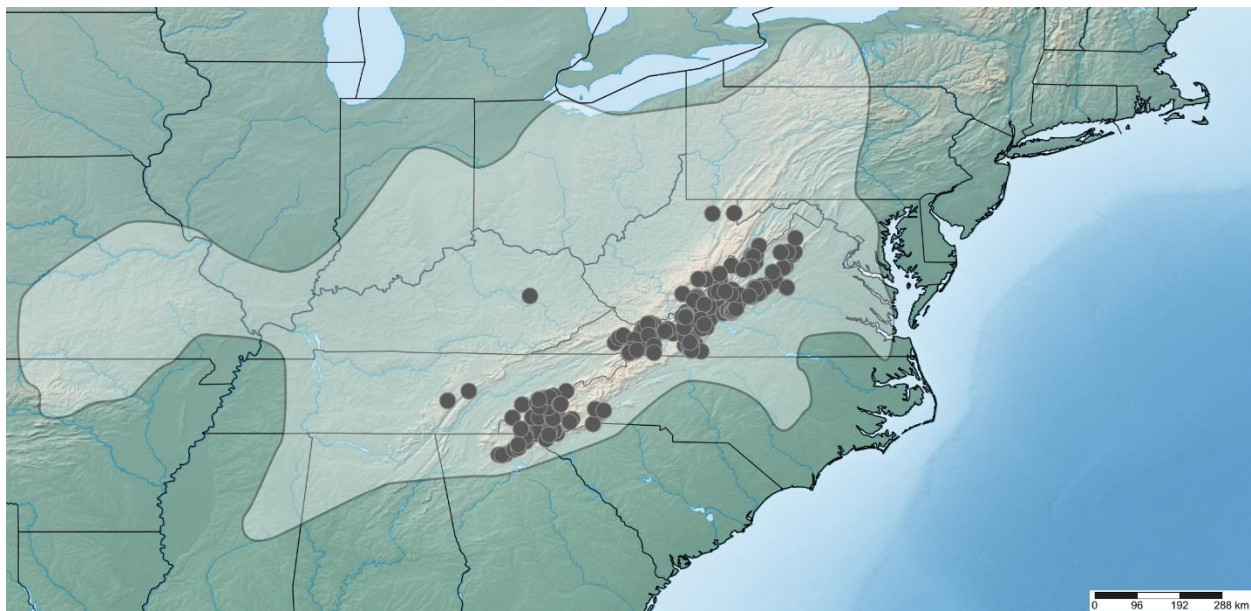


Figure 3.2. Distribution of the *Nannaria wilsoni* species group (black dots) within the *Nannaria* generic distribution (white shaded region).

Species of the *wilsoni* group are sympatric with the *minor* group throughout most of the *wilsoni* group's distribution, except for much of northern Georgia and adjacent southwestern

North Carolina. Outlying records of the *wilsoni* group in east-central Tennessee, eastern Kentucky, and northeastern West Virginia indicate the *wilsoni* group likely has a larger distribution than is currently known, however these areas are poorly-collected for *Nannaria*.

Molecular Phylogenetics. An estimation of the phylogenetic history of the *Nannaria wilsoni* group found the *wilsoni* group clade sister to the *minor* group clade (Fig. 3.3). Outgroups for the tree were *Oenomaea pulchella* (Bollman, 1889), and the genera *Eurymerodesmus* Brölemann, 1900, *Euryurus* C. L. Koch, 1847, and *Auturus* Chamberlin, 1942, which comprise the taxa with the closest hypothesized relationships to *Nannaria* (Shelley & Smith, 2018).

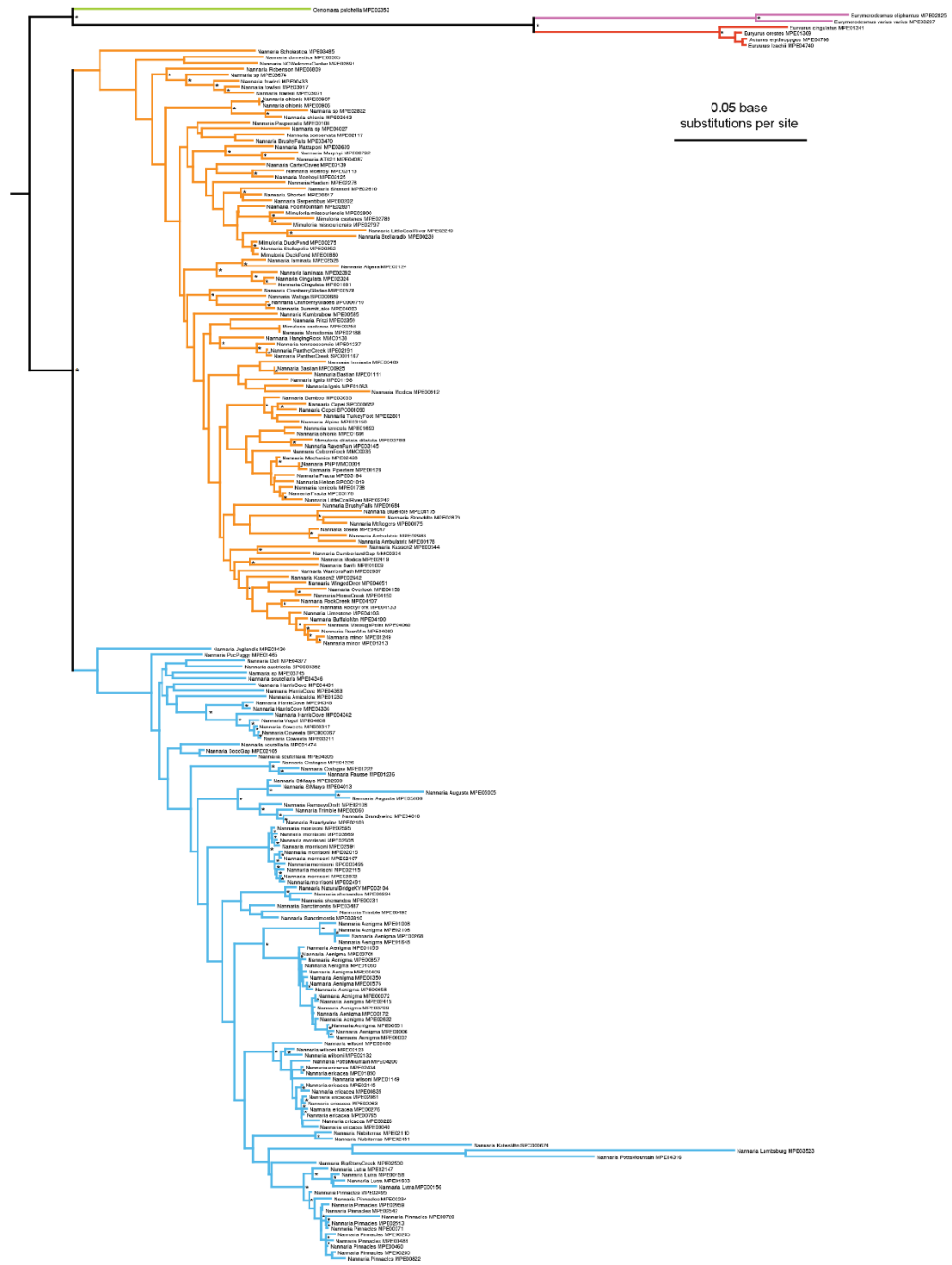


Figure 3.3. Phylogenetic tree of the Nannariini. Branches colored according to clades:

Oenomaea pulchella (green), *Eurymerodesmus* (purple), the “Euryurina” *Euryurus* and

Auturus (red), *Nannaria minor* group (orange), and *Nannaria wilsoni* group (blue).

Bootstrap support values > 70 indicated by asterisks (*).

The nodes separating *Oenomaea pulchella* from the outgroup and the *Nannaria* species group clades were well-supported, with bootstrap values > 70. Within the *Nannaria wilsoni* group clade (Fig. 3.4), species level clades were generally well-supported with bootstrap values > 70. Higher level relationships within the clade, however, were not well-supported with average bootstrap values of 5.8.

supported by both morphology and molecular phylogenetics. When species clades were not well-supported by the molecular results, priority was given to the morphological results. The identification of 18 new species brings the total number of species in the group to 24, from the previously known diversity of six species. These species are treated in the following pages, with the described species treated first, followed by the new species, listed alphabetically.

Taxonomic Part.

Class Diplopoda de Blainville in Gervais, 1844

Order Polydesmida Pocock, 1887

Family Xystodesmidae Cook, 1895

Subfamily Xystodesminae Cook, 1895

Tribe Nannariini Hoffman, 1964

Genus *Nannaria* Chamberlin, 1918

Type species: *Nannaria minor* Chamberlin, 1918.

Taxa included in the *wilsoni* species group:

Nannaria austriicola Hoffman, 1950

Nannaria ericacea Hoffman, 1949

Nannaria morrisoni Hoffman, 1948

Nannaria scutellaria Causey, 1942

Nannaria shenandoa Hoffman, 1949

Nannaria wilsoni Hoffman, 1949

Genus diagnosis: The genus *Nannaria* can be separated by the following combination of characters. The tribe Nannariini have triangular sternal projections laterally on the sterna, while

non-Nannariini lack lateral sternal projections, but may have rounded sternal lobes (as in *Pleuroloma* Rafinesque, 1820), “scooped out” sterna (as in *Gyalostethus* Hoffman, 1965), or unmodified sterna; males with pregonopodal claws twisted and spatulate, non-Nannariini males with pregonopodal claws simple, bisinuate; and gonopods with straight or gently curving acropodites and stout to long prefemoral processes, gonopods never with circular or sigmoid acropodites as in *Apheloria* Chamberlin, 1921 or *Sigmoria* Chamberlin, 1939, and gonopods never with small, hooked prefemoral processes as in *Apheloria*; size small to moderate (length 15-38 mm), dorsum chestnut brown to black with orange to red, sometimes white, paranotal spots. *Nannaria* is distinguished from the genus *Oenomaea* by having caudal paranotal corners only slightly projecting or rounded, not distinctly projecting caudally. The *Nannaria minor* species group and *Nannaria wilsoni* species group can be separated by the following characters: acropodital setae extending to at most halfway up length of acropodite, not reaching distal zone, as in *Nannaria minor* species group; anterior bend of acropodite typically with a gently curving or abrupt helical twist, not absent as in *Nannaria minor* species group; prefemoral process arising from the base of the acropodite, or rarely, from the side of the acropodite, but never from an acropodital shelf as in *Nannaria minor* species group. Females often with cyphopod receptacle modified or enlarged, the cyphopod receptacle is unmodified in the *Nannaria minor* species group; female 2nd leg coxae laterally expanded, partly or entirely covering cyphopod aperture, female 2nd leg coxae not expanded in *Nannaria minor* species group.

***Nannaria wilsoni* species group:**

***Nannaria austriicola* Hoffman, 1950**

Vernacular name: “The Highlands twisted claw”

Fig. 3.5.

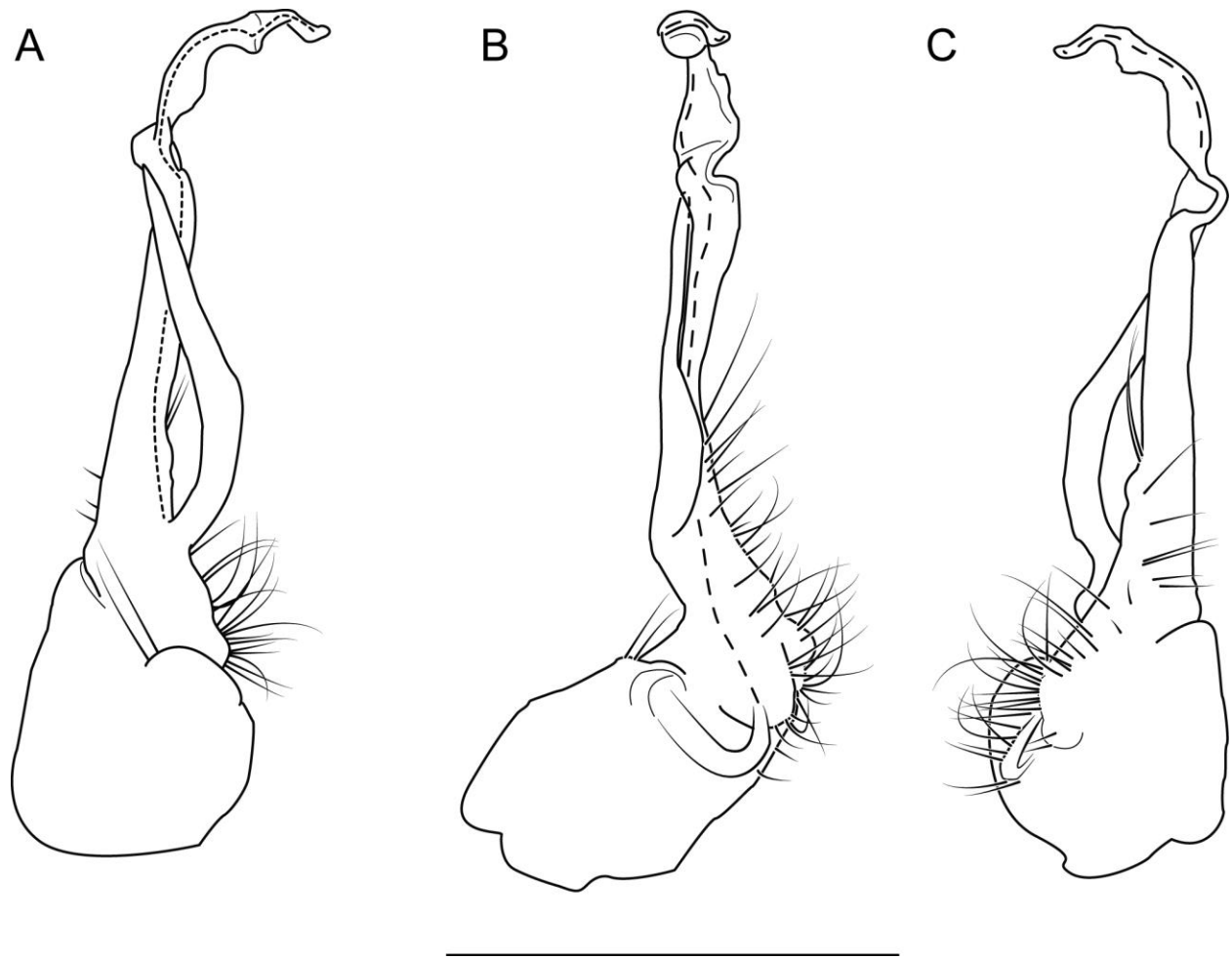


Figure 3.5. Left gonopod of *Nannaria austriicola* (NAN0072, Macon County, North Carolina). **A.** anterior view. **B.** medial view. **C.** posterior view. Scale bar: 1 mm

Material examined: Male exemplar specimen (NAN0072) from North Carolina, Macon County, 5 miles NW of Highlands (35.1038°N, -83.2594°W, ±5km), 9 July 1958, Coll: R. L. Hoffman.

Diagnosis: Adults of *Nannaria austriicola* can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal pink spots, collum outlined in pink, and tergites with background olive-brown. **Gonopods.** Male gonopod

acropodite arc straight, with an abrupt bend distally, not with a bend at midpoint as in *Nannaria* n. sp. “Juglandis” or gradually curving as in *Nannaria morrisoni*; gonopod acropodite with acute twist at anterior bend, appearing crimped and folded, twist not absent as in *Nannaria morrisoni* or in the shape of a smoothly-undulating helix, as in *Nannaria ericacea*. Acropodite medial flange lobed, not laminate as in *Nannaria wilsoni* or tooth-like as in *Nannaria scutellaria*; acropodite tip lateral flange absent, not triangular as in *Nannaria ericacea*, lobed as in *Nannaria* n. sp. “Coweeta”, hooked as in *Nannaria* n. sp. “Juglandis”, or laminate as in *Nannaria wilsoni*. Acropodite tip entire, not bifurcate as in *Nannaria morrisoni*; acropodite tip directed medially. Prefemoral process sinuous, thicker at base and tapering distally, not laminate as in *Nannaria* n. sp. “Amicalola” or simple and curving as in *Nannaria ericacea*; prefemoral process curving laterally and crossing acropodite dorsolaterally, not ventrolaterally as in *Nannaria shenandoa*; Prefemoral process tip directed cephalically, not laterally as in *Nannaria scutellaria* or dorsally as in *Nannaria shenandoa*.

Measurements: BL = 22.70, CW = 3.10, IW = 2.48, ISW = 0.75, B10W = 4.10, B10H = 2.40.

Variation: Acropodite medial flange size varies from small to large.

Distribution: *Nannaria austriicola* is only known from Macon County, North Carolina and adjacent Rabun County, Georgia.

Ecology: The type specimen was collected in a rhododendron thicket near a stream (Hoffman, 1950) on Satulah Mountain in the town of Highlands, North Carolina. Elevation notes on the labels of other specimens range from 690 meters to 1311 meters, and may indicate that *N. austriicola* is restricted to cool, high elevation habitats.

Etymology: From *L. australis*, “of the south,” named for being the most southern species of *Nannaria* known at the time (Hoffman, 1950).

***Nannaria ericacea* Hoffman, 1949**

Vernacular name: “The Heathland Twisted Claw”

Figs. 3.6, 7.



Figure 3.6. *Nannaria ericacea*, dorsal view, male exemplar MPE02263 from Roanoke County, Virginia.

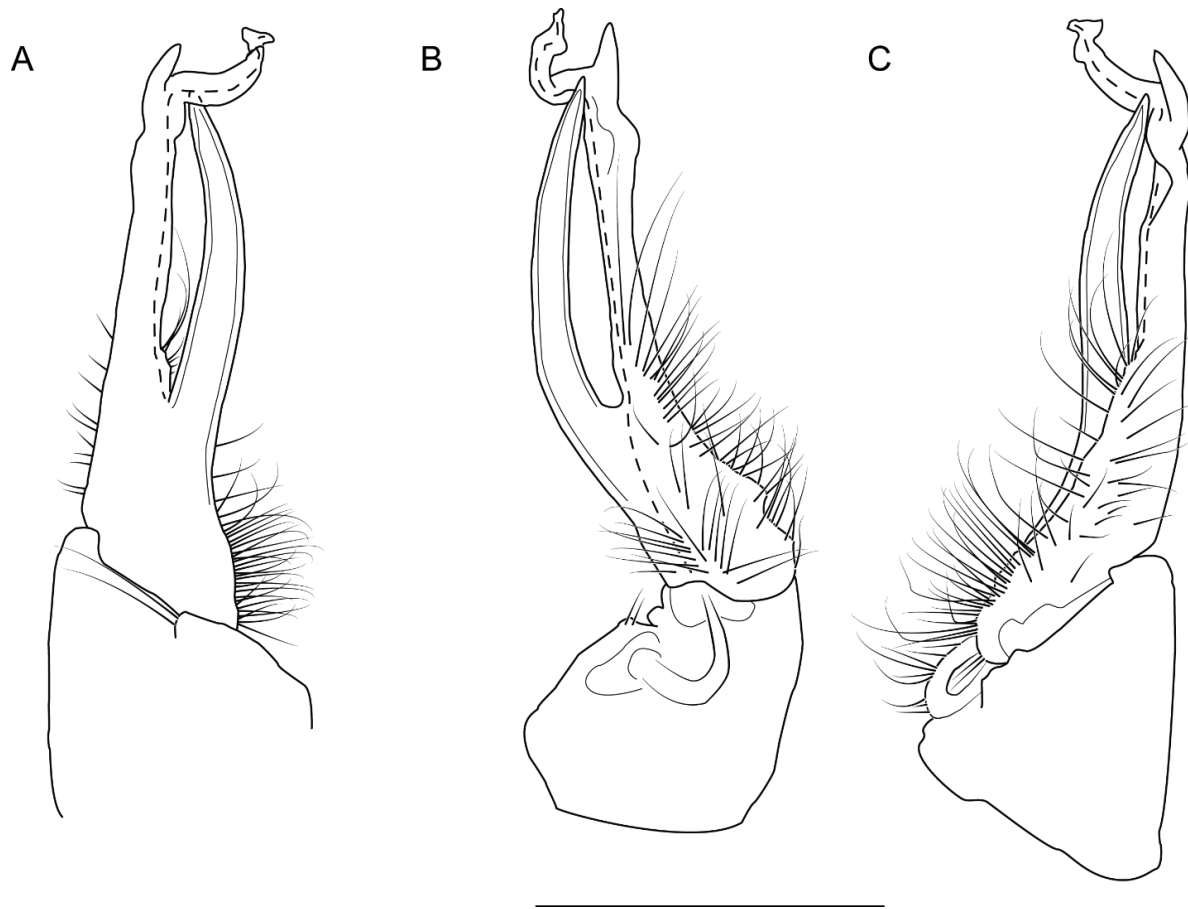


Figure 3.7. Left gonopod of *Nannaria ericacea* (MPE02263, Roanoke Co, Virginia). **A.** anterior view. **B.** medial view. **C.** posterior view. Scale bar: 1 mm

Material examined: Male exemplar specimen (MPE02263) from Virginia, Roanoke County, Salem, Carvin's Cove, down huge gully off the Trough Trail, bank of stream (37.3599°N, -79.9939°W, elevation 451 m), 20 November 2016, Coll: J. C. Means.

Diagnosis: Adults of *Nannaria ericacea* can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal orange-red spots, collum outlined in orange-red, and tergites with background black to brown. **Gonopods.** Male gonopod acropodite arc straight, with abrupt bend distally, not with a bend at midpoint as in *Nannaria* n. sp. “Juglandis” or gradually curving as in *Nannaria morrisoni*; gonopod acropodite anterior bend

with gentle twist, twist not acute as in *Nannaria austriicola* or absent as in *Nannaria morrisoni*. Acropodite medial flange absent; acropodite tip medial flange a large triangular process, slightly curved, not lobed as in *Nannaria austriicola* or laminate as in *Nannaria* n. sp. “Brandywine”; acropodite tip lateral flange triangular, not lobed as in *Nannaria* n. sp. “Coweeta”, hooked as in *Nannaria* n. sp. “Juglandis”, or laminate as in *Nannaria wilsoni*. Acropodite tip entire, slightly expanded with small lateral and medial lobes, not bifurcate as in *Nannaria morrisoni*; acropodite tip directed ventrally. Prefemoral process simple, curving, not acicular as in *Nannaria morrisoni* or stout as in *Nannaria* n. sp. “Augusta”; prefemoral process tip directed cephalically, not laterally as in *Nannaria scutellaria* or dorsally as in *Nannaria shenandoa*.

Measurements: BL = 38.48, CW = 4.35, IW = 3.12, ISW = 0.85, B10W = 5.63, B10H = 3.44

Variation: Acropodite tip sometimes lacking small distal lobes, size of acropodite medial flange typically large, but sometimes smaller.

Distribution: Restricted to Virginia, *Nannaria ericacea* has been recorded from the following counties: Alleghany, Bath, Botetourt, Craig, Giles, Montgomery, and Roanoke.

Ecology: *Nannaria ericacea* has been collected in a variety of habitats, ranging from pine-oak forests to ericaceous forests of rhododendron and hemlock. Elevation records range from 451 meters to 1097 meters.

Etymology: The species name *ericacea* derives from the Greek *ereike*, "heather," referring to the flowering plant family Ericaceae, known as the heath or heather family. The name was given based on the abundance of this species in ericaceous habitats (Hoffman, 1949).

***Nannaria morrisoni* Hoffman, 1948**

Vernacular name: “The Morrison Twisted Claw”

Figs. 3.8, 9



Figure 3.8. *Nannaria morrisoni*, dorsal view, male exemplar MPE02595 from Greene County, Virginia.

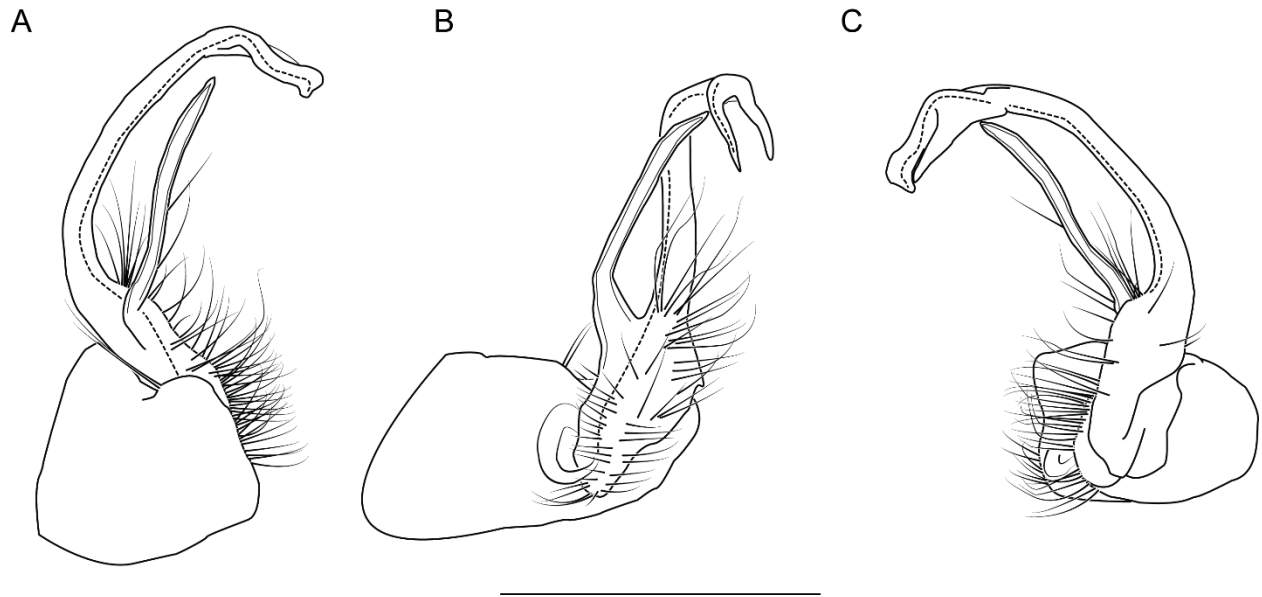


Figure 3.9. Left gonopod of *Nannaria morrisoni* (MPE02595, Greene Co, Virginia). **A.** anterior view. **B.** medial view. **C.** posterior view. Scale bar: 1 mm

Material examined: Male exemplar specimen (MPE02595) from Virginia, Greene County, Shenandoah National Park, tributary above South River Falls (38.3802°N, -78.5036°W, elevation 679 m), 29 April 2017, Coll: J. C. Means.

Diagnosis: Adults of *Nannaria morrisoni* can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal pink to orange spots, collum outlined in pink to orange, and tergites with background chestnut brown to black.

Gonopods. Male gonopod acropodite arc a gradual curve, not straight with an abrupt bend distally as in *Nannaria austriicola* or with a bend at midpoint as in *Nannaria* n. sp. "Augusta"; with medial flange extended into a large, lobed, triangular projecting process, giving the tip of the acropodite a bifurcate appearance; acropodite medial flange not laminate as in *Nannaria wilsoni* or tooth-like as in *Nannaria scutellaria*. Tips of acropodite directed medially, with a slightly ventral angle. Tip of acropodite carrying the seminal groove with slight dorsal

expansion; tip of medial flange ending in a sharp point. Prefemoral process acicular, needle-like, not stout as in *Nannaria* n. sp. “Augusta” or sinuous as in *Nannaria wilsoni*; tip directed cephalically, not laterally as in *Nannaria scutellaria* or dorsally as in *Nannaria shenandoa*.

Cyphopods. Female cyphopod receptacle much enlarged, leaf-like and laminate, not triangular as in *Nannaria austriicola* or with a finger-like projection, as in *Nannaria* n. sp. “Coweeta”.

Measurements: BL = 28.88, CW = 3.54, IW = 2.54, ISW = 0.75, B10W = 4.30, B10H = 2.80.

Variation: No appreciable variation was noted between specimens.

Distribution: Only known from Virginia, *Nannaria morrisoni* occurs in the following counties: Albemarle, Amherst, Bland, Greene, Madison, Nelson, Page, and Rockbridge. Its distribution in these counties is almost exclusively restricted to the spine of the Blue Ridge Mountains, northeast of the James River.

Ecology: Specimen label data indicates *Nannaria morrisoni* is collected in deciduous forests of oak, tulip tree, beech, and maple, rather than in areas with more rhododendron and other ericaceous plants. Many of the sites with habitat data list riparian areas as the habitat for this species. Specimens been collected from elevations as low as 339 m to as high as 1067 m.

Etymology: *Nannaria morrisoni* is named after Dr. Joseph P. E. Morrison, a mollusk curator at the Smithsonian Museum of Natural History in Washington, D.C., who collected millipedes for Richard Hoffman during his own collecting trips for land snails (Hoffman, 1948).

***Nannaria scutellaria* Causey, 1942**

Vernacular name: “The Smoky Twisted Claw”

Figs. 3.10, 11



Figure 3.10. *Nannaria scutellaria*, dorsal view, male exemplar MPE04346 from Transylvania County, North Carolina.

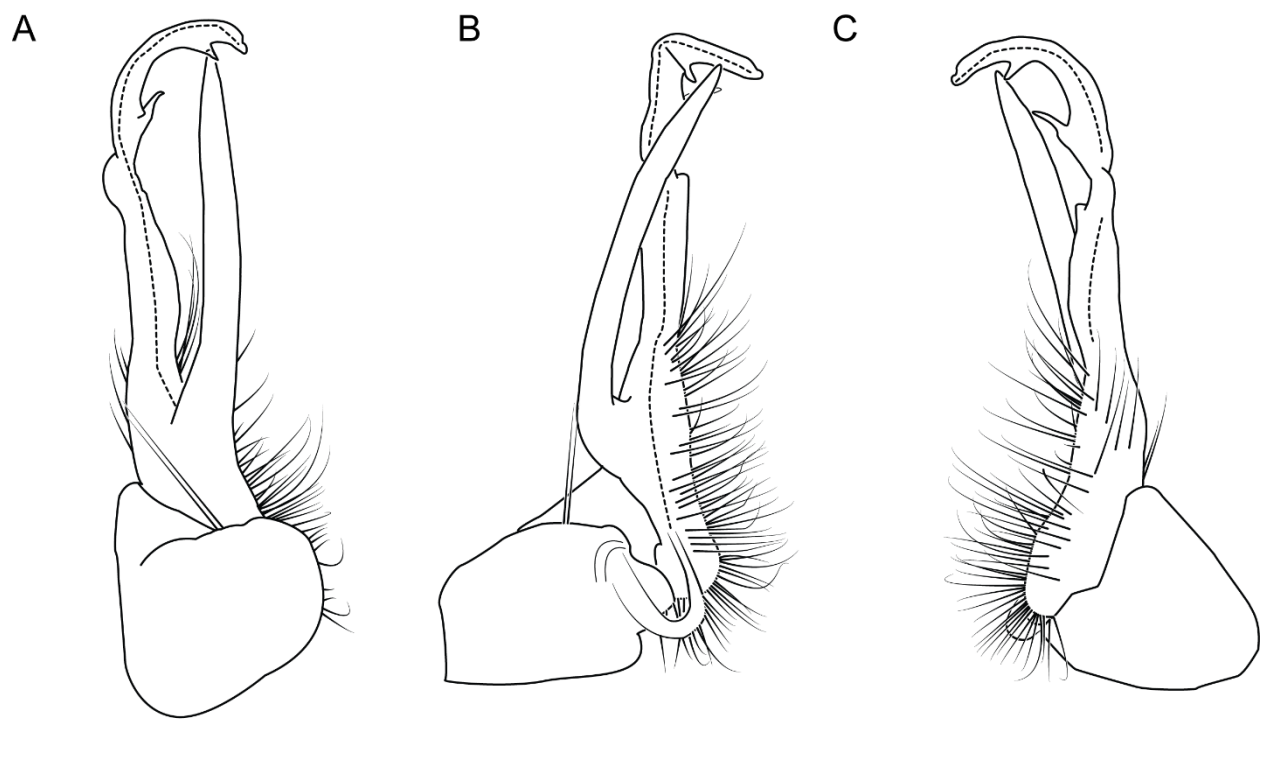


Figure 3.11. Left gonopod of *Nannaria scutellaria* (MPE01474, Sevier Co, Tennessee). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm

Material examined: Male exemplar specimen (MPE01474) from Tennessee, Sevier County, Great Smoky Mountains National Park, Chimneys Picnic Area, Cove Hardwood Trail (35.636°N, -83.4937°W, ±7m, elevation 877 m), ex along trail, moist hemlock, oak, maple, tulip poplar litter, 8 June 2016, Coll: D. A. Hennen, J. C. Means.

Diagnosis: Adults of *Nannaria scutellaria* can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal pink to red spots, collum outlined in pink, and tergites with background chestnut brown to black. **Gonopods.** Male gonopod acropodite arc straight, with abrupt bend distally, not with a bend at midpoint as in *Nannaria* n. sp. “Juglandis” or gradually curving as in *Nannaria morrisoni*; acropodite anterior bend with acute twist, a scooped-out area visible in posterior view, not absent as in *Nannaria morrisoni* or in the shape of a smoothly-undulating helix, as in *Nannaria wilsoni*. Acropodite medial flange a tooth-like, thin, triangular projection, not laminate as in *Nannaria wilsoni* or lobed as in *Nannaria austriicola*; acropodite tip medial flange absent, not triangular as in *Nannaria ericacea* or lobed as in *Nannaria shenandoa*; acropodite tip lateral flange a medium-sized triangular process, not lobed as in *Nannaria* n. sp. “BigStonyCreek” or hooked as in *Nannaria* n. sp. “Juglandis”. Acropodite tip entire, curving, not bifurcate as in *Nannaria morrisoni*; acropodite tip directed ventrally. Prefemoral process with a sinuous taper, thicker at base, not acicular as in *Nannaria morrisoni* or stout as in *Nannaria* n. sp. “Augusta”; prefemoral process curving slightly laterally, crossing acropodite ventrolaterally, not dorsolaterally as in *Nannaria austriicola*. **Cyphopods.** Female cyphopod receptacle in the shape of an enlarged and elongated club, not triangular as in *Nannaria austriicola* or an enlarged triangular hood as in *Nannaria* n. sp. “Amicalola”.

Measurements: BL = 29.70, CW = 3.08, IW = 2.54, ISW = 0.81, B10W = 3.92, B10H = 2.64.

Variation: *Nannaria scutellaria* has a large amount of gonopodal variation, as documented by Causey (1950). Her study included males from localities within Great Smoky Mountains National Park and showed variation in the acropodite medial flange and subterminal teeth near the tip of the acropodite, as well as the shape of the acropodite tip. Additionally, males examined for this study from across the distribution of *Nannaria scutellaria* exhibited a large amount of variation in the processes on the distal portion of the acropodite, and this variation may indicate that *Nannaria scutellaria* is a cryptic species complex. However, without more specimens available for genetic analysis, splitting *Nannaria scutellaria* would be ill-advised, and all of these variable forms are included within this species.

Distribution: The distribution of *Nannaria scutellaria* straddles the border between Tennessee and North Carolina in the southern Appalachian Mountains, and is known from the following counties: (Tennessee) Blount, Cocke, and Sevier; (North Carolina) Jackson, Haywood, Swain, and Transylvania.

Ecology: *Nannaria scutellaria* has been collected mostly from moist mixed oak, maple, tulip tree and hemlock, rhododendron forests. It has been found at elevations between 610 m and 1918 m.

Etymology: Causey does not specify the meaning of the name, but it derives from the Latin *scutella*, meaning a small dish or platter.

***Nannaria shenandoa* Hoffman, 1949**

Vernacular name: “The Shenandoah Twisted Claw”

Figs. 3.12, 13



Figure 3.11. *Nannaria shenandoa*, dorsal view, male exemplar MPE00231 from Marion County, West Virginia.

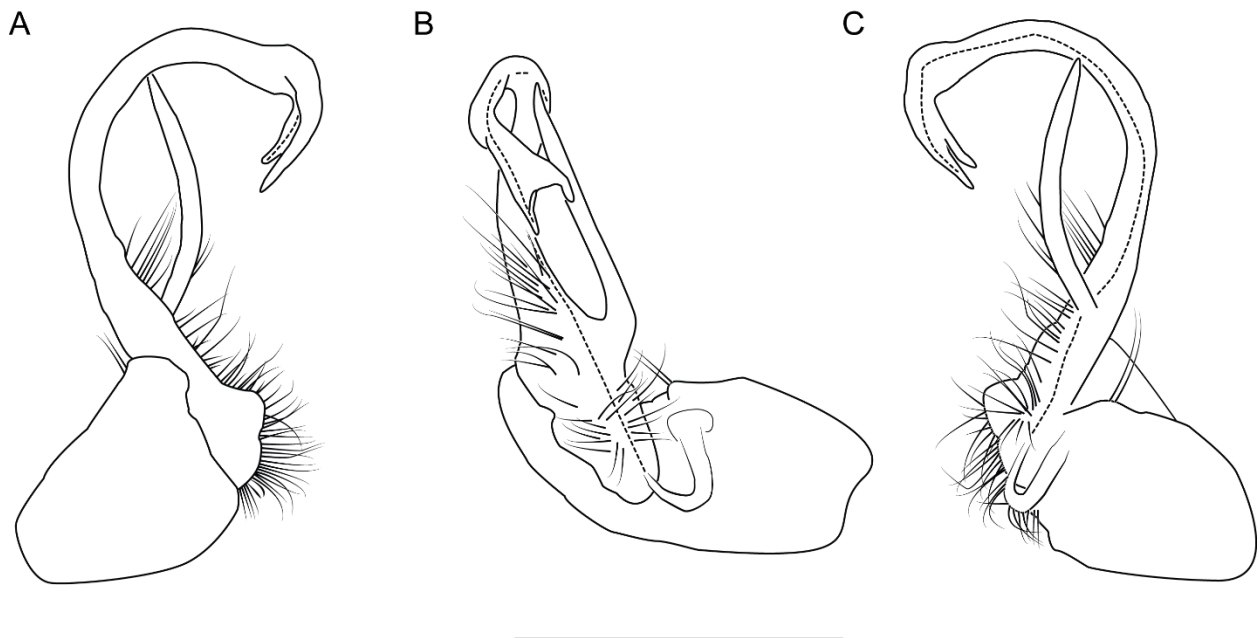


Figure 3.12. Left gonopod of *Nannaria shenandoa* (MPE03994, Shenandoah County, Virginia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm

Material examined: Male exemplar specimen (MPE03994) from Virginia, Shenandoah County, Passage Creek Gorge ~ 2 mi S jct of Rte 678 and VA-55 (38.9407°N, -78.3058°W), Under litter on steep north-facing talus slope above creek. Rhododendron thicket w/ hemlock overstory, 4 May 2018, Coll: C. W. Harden.

Diagnosis: Adults of *Nannaria shenandoa* can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal peach-pink spots, collum outlined in peach, and tergites with background olive-brown. **Gonopods.** Male gonopod acropodite arc gradually curving, not straight with an abrupt bend at tip as in *Nannaria ericacea* or a bend at midpoint as in *Nannaria* n. sp. “Augusta”; acropodite medial flange absent, not lobed as in *Nannaria morrisoni* or laminate as in *Nannaria wilsoni*. Acropodite tip medial flange lobed, giving acropodite tip a bifurcate appearance, not triangular as in *Nannaria morrisoni* or laminate as in *Nannaria* n. sp. “Nubiterrae”; acropodite tip lateral flange absent, not triangular as in *Nannaria ericacea* or laminate as in *Nannaria wilsoni*. Acropodite lacking an obvious twist at anterior bend, not present as a smoothly-undulating helix as in *Nannaria wilsoni* or acutely bent as in *Nannaria* n. sp. “Sanctimontis”. Prefemoral process simple, curving laterally, not acicular as in *Nannaria morrisoni* or sinuously tapering as in *Nannaria* n. sp. “Brandywine”; prefemoral process tip crosses acropodite ventrolaterally, not dorsolaterally as in *Nannaria* n. sp.

“Brandywine”; tip directed dorsally. **Cyphopods.** Female cyphopod receptacle enlarged into a triangular hood, covering cyphopod valves, not laminate and leaf-like as in *Nannaria morrisoni* or quadrate and curved distally as in *Nannaria* n. sp. “Brandywine”.

Measurements: BL = 27.95, CW = 3.46, IW = 2.51, ISW = 0.85, B10W = 4.25, B10H = 2.70.

Variation: No notable variation was observed between specimens.

Distribution: The distribution of *Nannaria shenandoa* extends across eastern West Virginia into northwestern Virginia and includes the following counties: (West Virginia) Marion, Preston, Pocahontas, Greenbrier; (Virginia) Augusta, Rockingham, Shenandoah. Additionally, one male specimen was collected from Natural Bridge State Park in Powell County, Kentucky, which appears morphologically identical to the specimens from West Virginia and Virginia.

Genetically, it clusters in the same clade as the other *Nannaria shenandoa* specimens, and so is included here. Further study may reveal either a larger distribution than is currently known, connecting the Kentucky population with the West Virginia population, or a cryptic species complex within *Nannaria shenandoa*.

Ecology: Label information for *Nannaria shenandoa* notes the species has been collected in mixed deciduous forests of oak, maple, and tulip tree, along with hemlock and rhododendron forests. It ranges in elevation from 325 m to 1006 m.

Etymology: Named after its type locality, Shenandoah Mountain in Rockingham County, Virginia.

***Nannaria wilsoni* Hoffman, 1949**

Vernacular name: “The Wilson Twisted Claw”

Figs. 3.14, 15



Figure 3.13. *Nannaria wilsoni*, dorsal view, male exemplar MPE02123 from Giles County, Virginia.

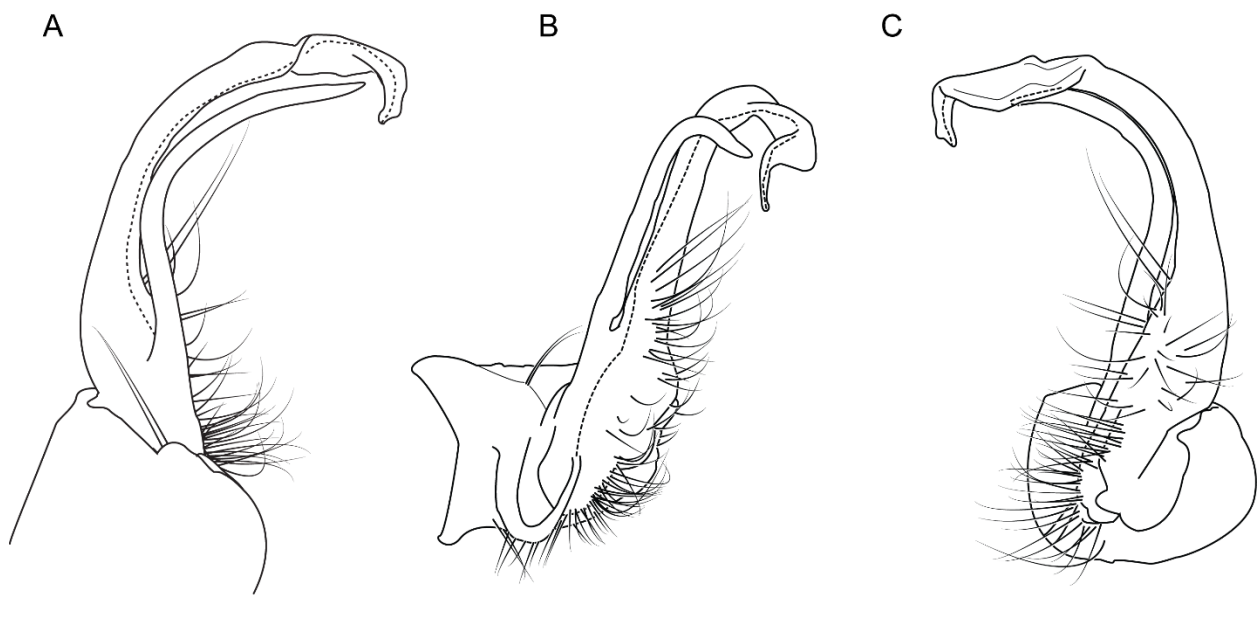


Figure 3.14. Left gonopod of *Nannaria wilsoni* (MPE02123, Giles County, Virginia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm

Material examined: Male exemplar specimen (MPE02123) from Virginia, Giles Co, Mountain Lake Wilderness, nr. Mountain Lake Biological Station. On trail to War Spur Overlook, off Rt. 613 (37.3892°N, -80.5057°W, elevation 1125 m), under moss at base of white oak tree, in deciduous woods of witch hazel, chestnut oak, white oak, 3 October 2016, Coll: J. C. Means, D. A. Hennen, V. Wong.

Diagnosis: Adults of *Nannaria wilsoni* can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal peach spots, collum outlined in peach, and tergites with background chestnut brown. **Gonopods.** Male gonopod acropodite arc gradually curving, not with an abrupt bend at tip as in *Nannaria ericacea* or a bend at midpoint as in *Nannaria* n. sp. “PottsMountain”; acropodite with a gently undulating twist at anterior bend, not acutely bent as in *Nannaria* n. sp. “Sanctimontis”. Acropodite medial flange laminate, not lobed as in *Nannaria* n. sp. “BigStonyCreek” or absent as in *Nannaria ericacea*; acropodite tip medial flange absent, not triangular as in *Nannaria ericacea* or lobed as in *Nannaria* n. sp. “LongSpringRun”; acropodite tip lateral flange laminate, in the shape of a small bump just before the tip, not triangular as in *Nannaria ericacea* or lobed as in *Nannaria* n. sp. “BigStonyCreek”. Acropodite tip directed ventrally shape blunt, not directed caudally as in *Nannaria* n. sp. “Aenigma”. Prefemoral process sinuous, not simple as in *Nannaria ericacea*; prefemoral process curving medially, not straight as in *Nannaria* n. sp. “Aenigma”; prefemoral process tip directed medially. **Cyphopods.** Female cyphopod receptacle an enlarged triangular hood, covering cyphopod valves, not triangular as in *Nannaria ericacea* and *Nannaria* n. sp. “Aenigma”.

Measurements: BL = 27.30, CW = 3.88, IW = 2.73, ISW = 0.93, B10W = 5.06, B10H = 3.00.

Variation: No notable variation observed.

Distribution: *Nannaria wilsoni* is limited to 3 southwest Virginia counties: Giles, Montgomery, and Roanoke.

Ecology: Habitat information from museum specimens is limited, but *Nannaria wilsoni* has been collected from oak, maple, and rhododendron forests.

Etymology: This species is named in honor of Charles M. Wilson, who collected the species at Mountain Lake Biological Station in Giles County (Hoffman, 1949).

***Nannaria* n. sp. “Aenigma”**

Vernacular name: “The Enigmatic Twisted Claw”

Figs. 3.16, 17



Figure 3.15. *Nannaria* n. sp. “Aenigma”, dorsal view, male holotype MPE02632 from Smyth County, Virginia.

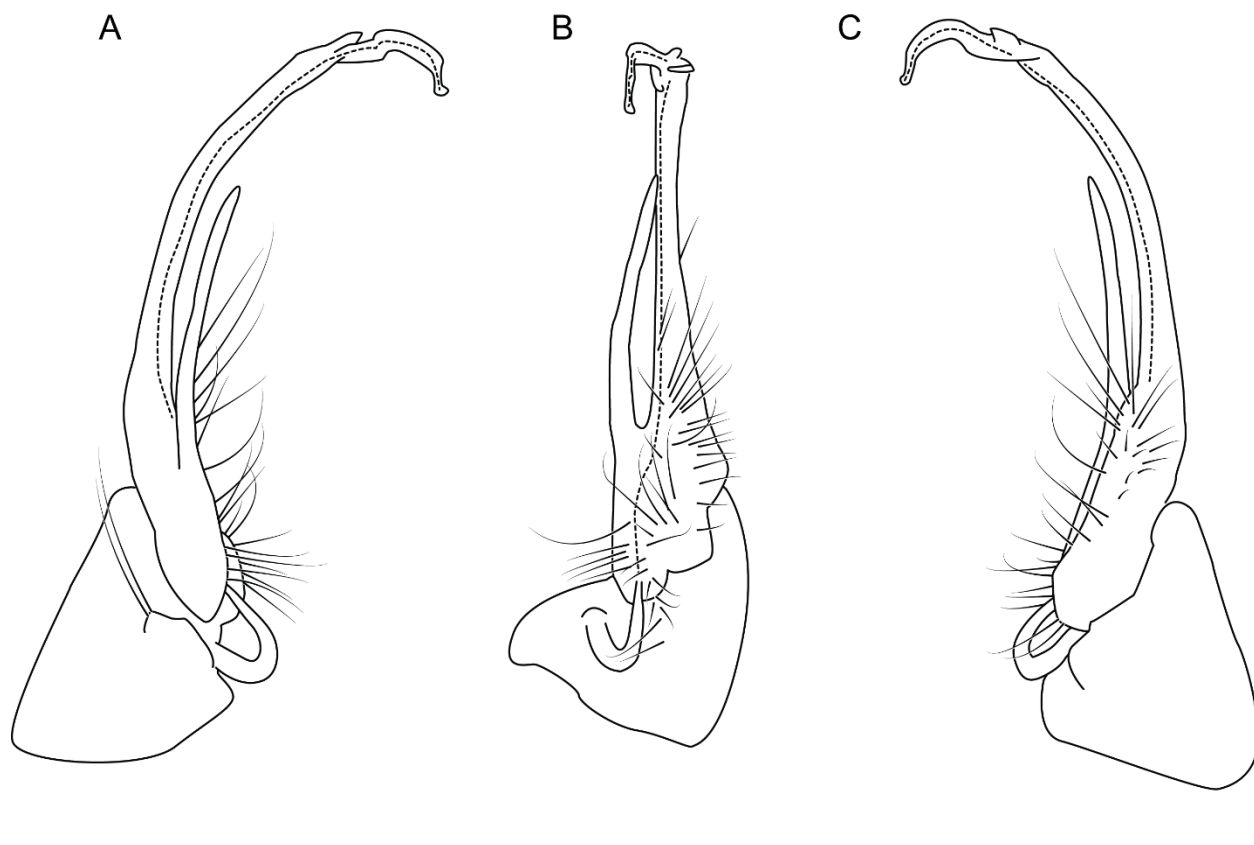


Figure 3.16. Left gonopod of *Nannaria* n. sp. “Aenigma” holotype (MPE02632, Smyth County, Virginia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm

Material examined: Male holotype specimen (MPE02632) from Virginia, Smyth County, Sugar Grove: Raccoon Branch Wilderness Campground, Jefferson National Forest, Raccoon Branch Trail (36.7454°N, -81.4259°W, ± 7 m, elevation 858 m), Moist deciduous leaf litter of rhododendron and eastern hemlock, 5 May 2017, Coll: D. A. Hennen.

Diagnosis: Adults of *Nannaria* n. sp. “Aenigma” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal white spots, collum outlined in white, and tergites with background black. **Gonopods.** Male gonopod acropodite arc gradually curving, not straight with an abrupt bend distally as in *Nannaria ericacea* or with a bend at midpoint as in *Nannaria* n. sp. “PottsMountain”; acropodite with smoothly-undulating helical twist at anterior bend, not with an acute twist as in *Nannaria* n. sp. “Coweeta”.

Acropodite medial flange laminate, and with dorsal triangular tooth, not lobed as in *Nannaria* n. sp. “Pinnacles” or tooth-like as in *Nannaria* n. sp. “Coweeta”. Acropodite tip medial and lateral flanges absent. Acropodite tip entire, slightly constricted distally, not bifurcate as in *Nannaria morrisoni*; acropodite tip directed caudally, not ventrally as in *Nannaria wilsoni* or medially as in *Nannaria* n. sp. “Trimble”. Prefemoral process acicular, straight, with only a slight curve, not sinuous as in *Nannaria wilsoni* or simple and curving as in *Nannaria* n. sp. “Pinnacles”; prefemoral process tip directed cephalically, not medially as in *Nannaria wilsoni* or laterally as in *Nannaria* n. sp. “Nubiterrae”. Prefemoral process length about half that of the acropodite.

Cyphopods. Female cyphopod receptacle triangular, not an enlarged triangular hood as in *Nannaria wilsoni*.

Measurements: BL = 31.95, CW = 4.20, IW = 2.79, ISW = 0.88, B10W = 5.19, B10H = 3.01.

Variation: The dorsal triangular tooth process on the gonopod acropodite varies in size and shape, but is generally triangular. The acropodite tip is sometimes more elaborately lobed before the distal constriction in some specimens, and the prefemoral process varies in its length, sometimes being almost as long as the acropodite. In color, *Nannaria* n. sp. “Aenigma” varies as well, with some specimens having pink to red paranotal spots instead of white.

Distribution: *Nannaria* n. sp. “Aenigma” is distributed throughout southwest Virginia, and is known from the following counties: Bland, Grayson, Pulaski, Russell, Smyth, Tazewell, Washington, Wythe. In the eastern portion of its distribution, it is limited by the course of the New River. It may eventually be found in West Virginia, Tennessee, and North Carolina, as some of its locality records are very near the borders of these states.

Ecology: This species has been found in mesic deciduous forest habitats, as well as ericaceous hemlock-rhododendron coves, and at elevations ranging from 587 m to 1219 m.

***Nannaria* n. sp. “Amicalola”**

Vernacular name: “The Amicalola Twisted Claw”

Figs. 3.18, 19



Figure 3.17. *Nannaria* n. sp. “Amicalola”, dorsal view, female exemplar MPE01230 from Lumpkin County, Georgia.

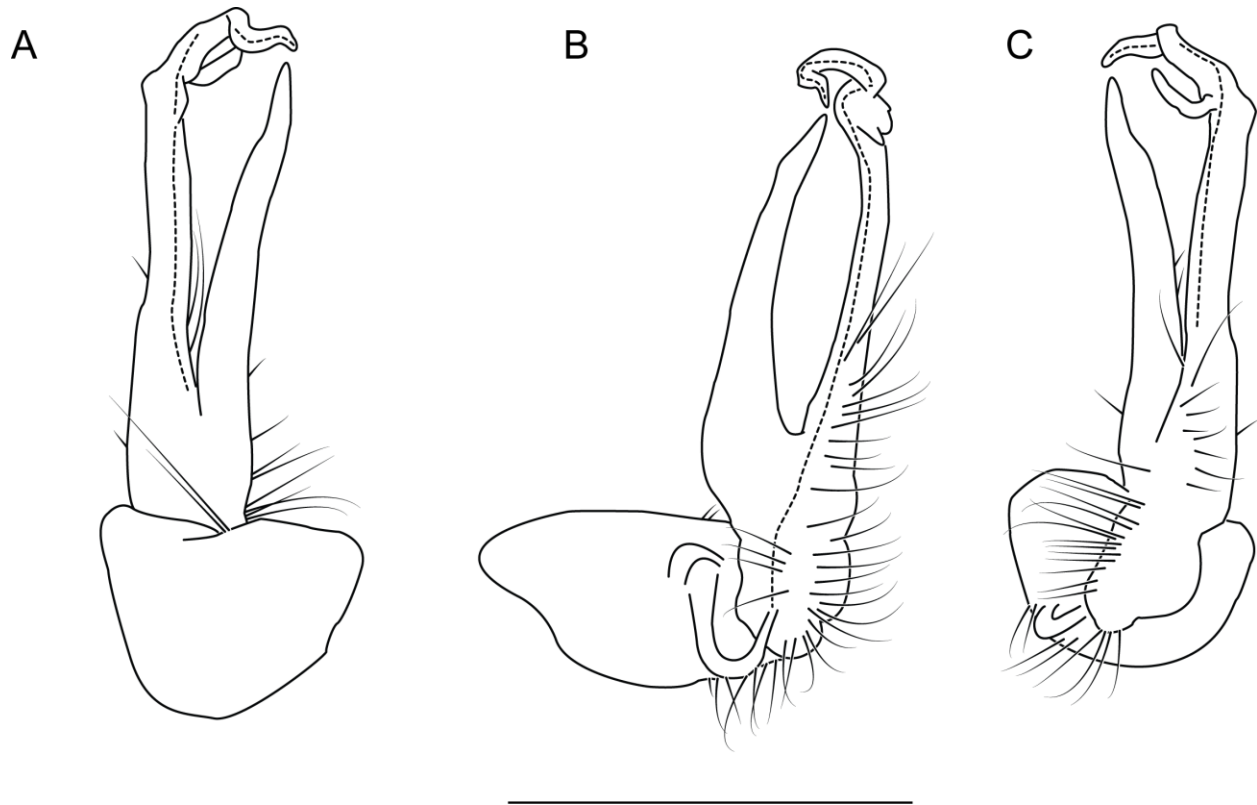


Figure 3.18. Left gonopod of *Nannaria* n. sp. “Amicalola” holotype male (NAN0319, Dawson County, GA). **A.** anterior view. **B.** medial view. **C.** posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (NAN0319) from Georgia, Dawson County, 6 miles W of Amicalola Falls (34.5681°N, -84.314°W, ±5km), ex ravine, 6 November 1960, Coll: L. Hubricht.

Diagnosis: Adults of *Nannaria* n. sp. “Amicalola” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal orange spots, collum outlined in orange, and tergites with background chestnut brown. **Gonopods.** Acropodite arc straight, with abrupt bend distally, not with a bend at midpoint as in *Nannaria* n. sp. “Juglandis”; acropodite with acute twist at anterior bend, not with a smoothly-undulating helix as in *Nannaria* n. sp. “HarrisCove”. Acropodite medial flange tooth-like, long, and hooked, curving cephalically, not lobed as in *Nannaria* n. sp. “HarrisCove”. Acropodite tip medial and lateral

flanges absent; acropodite tip entire, not bifurcate as in *Nannaria* n. sp. “Coweeta”; acropodite tip curving and directed ventrally, not medially as in *Nannaria austriicola* or caudally as in *Nannaria* n. sp. “HarrisCove”. Prefemoral process laminate, very wide at base, not a sinuous taper as in *Nannaria* n. sp. “Coweeta” or acicular as in *Nannaria* n. sp. “Cratagae”; prefemoral straight, tapering to tip with a slight curve; tip directed cephalically, not medially as in *Nannaria* n. sp. “Coweeta” or laterally as in *Nannaria* n. sp. “HarrisCove”. **Cyphopods.** Female cyphopod receptacle enlarged into a triangular hood, covering cyphopod valves, not a finger-like projection as in *Nannaria* n. sp. “Harris Cove” or a sinuous S-shape, as in *Nannaria* n. sp. “Cratagae”.

Measurements: BL = 24.40, CW = 3.56, IW = 2.82, ISW = 0.96, B10W = 4.70, B10H = 3.00.

Variation: No noticeable variation was observed.

Distribution: *Nannaria* n. sp. “Amicalola” is only known from Dawson and Lumpkin Counties in northern Georgia.

Ecology: This species inhabits ravines and riparian areas with a mix of moist oak, maple, hemlock, and rhododendron forest.

***Nannaria* n. sp. “Augusta”**

Vernacular name: “The Maple Flats Twisted Claw”

Figs. 3.20, 21



Figure 3.20. *Nannaria* n. sp. “Augusta”, dorsal view, male holotype MPE05006 from Augusta County, Virginia.

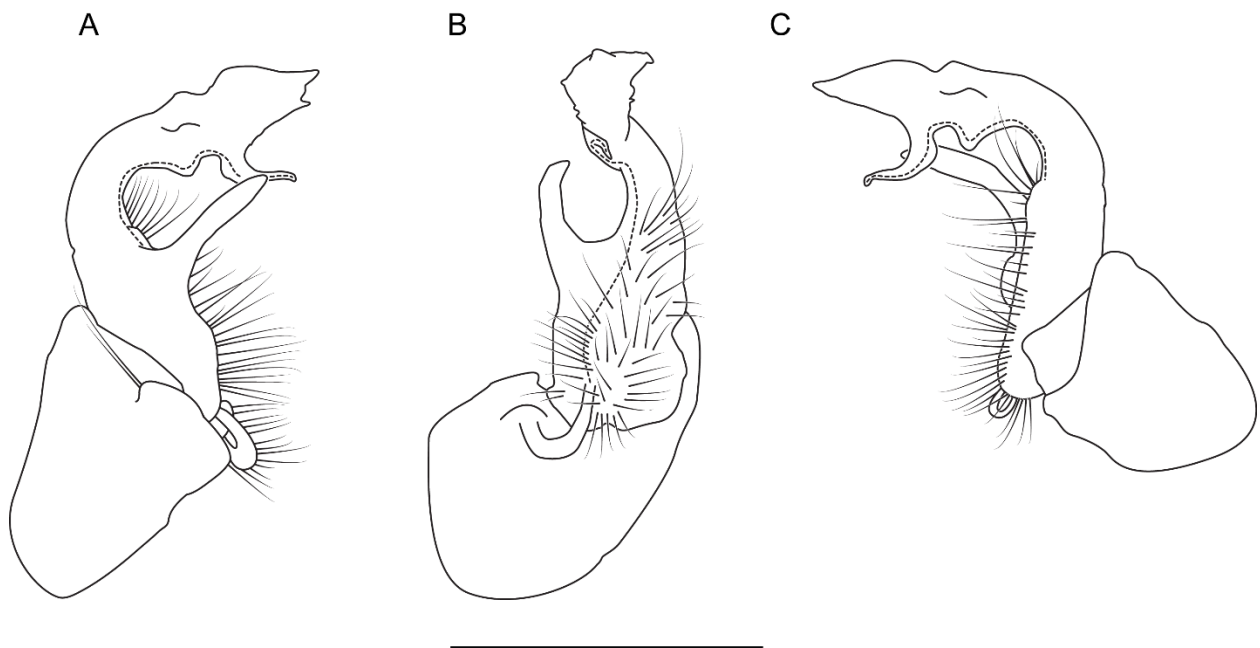


Figure 3.21. Left gonopod of *Nannaria* n. sp. “Augusta” male holotype (MPE05006, Augusta County, Virginia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (MPE05006) from Virginia, Augusta County, Sherando: George Washington National Forest, Maple Flat Ponds area (37.9776°N, -78.9938°W, ±7 m), white oak, hickory, tulip tree forest around sinkhole ponds, 12 June 2019, Coll: D. A. Hennen, J. C. Means, M. Hellier, P. E. Marek.

Diagnosis: Adults of *Nannaria* n. sp. “Augusta” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal orange spots, collum outlined in orange, and tergites with background chestnut brown. **Gonopods.** Male gonopod acropodite arc straight, with acute bend at midpoint, not gradually curving as in *Nannaria morrisoni* or with abrupt bend at tip as in *Nannaria* n. sp. “Sanctimontis”; acropodite anterior bend twist lacking or almost imperceptible, not in the shape of a smoothly-undulating helix as in *Nannaria* n. sp. “Lutra” or acutely bent as in *Nannaria* n. sp. “Sanctimontis”.

Acropodite medial flange lobed, not absent as in *Nannaria* n. sp. “Brandywine”; acropodite tip medial flange absent, not triangular as in *Nannaria morrisoni* or laminate as in *Nannaria* n. sp. “Sanctimontis”; acropodite tip lateral flange laminate and greatly enlarged, with various small teeth along its edges and culminating in a broad, triangular tip, acropodite tip lateral flange not lobed as in *Nannaria* n. sp. “Lutra”. Acropodite tip sinuous, bifurcate, directed medially, not caudally as in *Nannaria morrisoni*. Prefemoral process stout, thick; projecting straight at a 45° angle from the acropodite, dissimilar to any other *Nannaria wilsoni* species group prefemoral process, not acicular as in *Nannaria morrisoni* or sinuous as in *Nannaria* n. sp. “Sanctimontis”.

Cyphopods. Female cyphopod receptacle enlarged into a triangular hood, covering cyphopod valves, not laminate and leaf-like as in *Nannaria morrisoni* or triangular as in *Nannaria* n. sp. “Sanctimontis”.

Measurements: BL = 29.60, CW = 3.60, IW = 2.57, ISW = 0.82, B10W = 4.35, B10H = 2.67.

Variation: The number and shape of the teeth on the laminate acropodite tip lateral flange varies, but are generally quite jagged.

Distribution: *Nannaria* n. sp. “Augusta” is known from a small area at the base of the Blue Ridge Mountains in southern Augusta County, Virginia. Males have only been collected at the Maple Flats Sinkhole Ponds complex near Sherando, but genetic analysis places female *Nannaria* specimens collected in the St. Mary’s Wilderness area with the males.

Ecology: This species has been collected in mesic white oak, hickory, and tulip tree forest and a mesic oak, hemlock, and mountain laurel forest along a stream. The Maple Flat Ponds area is known to harbor disjunct plant species. Elevation for these sites ranges from 480 m to 624 m.

***Nannaria* n. sp. “BigStonyCreek”**

Vernacular name: “The Stony Creek Twisted Claw”

Figs. 3.22, 23



Figure 3.22. *Nannaria* n. sp. “BigStonyCreek”, dorsal view, male holotype MPE02500 from Giles County, Virginia.

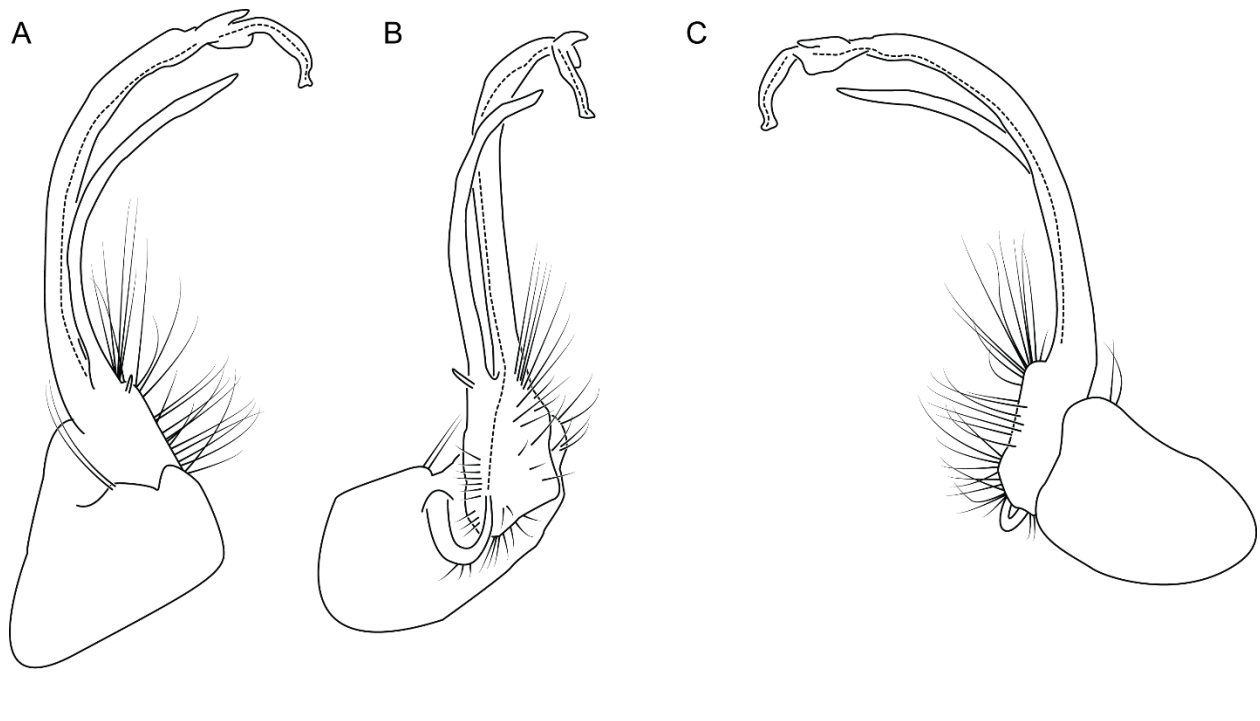


Figure 3.22. Left gonopod of *Nannaria* n. sp. “BigStonyCreek” male holotype (MPE02500, Giles County, Virginia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (MPE02500) from Virginia, Giles County, Jefferson National Forest, in gully beside North Fork Mountain Rd (37.4493°N, -80.5201°W, ± 6m, elevation 876 m), gully with small stream, litter moderately moist, rhododendron, oak, maple, mountain laurel, some pine; rocky, with large logs, 27 April 2017, Coll: D. A. Hennen, J. C. Means, P. E. Marek, P. Shorter, V. Wong.

Diagnosis: Adults of *Nannaria* n. sp. “BigStonyCreek” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal red spots, collum outlined in red, and tergites with background black. **Gonopods.** Male gonopod acropodite arc gradually curving, not straight with abrupt bend at tip as in *Nannaria ericacea*; acropodite with smoothly undulating twist at anterior bend. Acropodite medial flange lobed and with a large dorsal, triangular tooth, not laminate as in *Nannaria wilsoni* or absent as in *Nannaria* n. sp. “LongSpringRun”. Acropodite tip medial flange absent, not triangular as in *Nannaria ericacea*; acropodite tip lateral flange lobed, not laminate as in *Nannaria wilsoni* or triangular as in *Nannaria* n. sp. “LongSpringRun”. Acropodite tip entire; directed ventrally, not caudally as in *Nannaria* n. sp. “Aenigma”. Prefemoral process sinuous, not acicular as in *Nannaria* n. sp. “Aenigma” or simple and curving as in *Nannaria* n. sp. “Pinnacles”; prefemoral process curving medially, not straight as in *Nannaria* n. sp. “Aenigma”; prefemoral process tip directed medially, length almost as long as acropodite. Prefemoral process beset at base with a short, thin triangular process. **Cyphopods.** Female cyphopod receptacle triangular, not an enlarged triangular hood as in *Nannaria wilsoni*.

Measurements: BL = 27.80, CW = 3.96, IW = 2.76, ISW = 0.81, B10W = 4.75, B10H = 2.68.

Variation: No notable variation observed.

Distribution: Only known from the type locality in Giles County, Virginia.

Ecology: The type locality is a rocky mesic mixed forest with oak, maple, rhododendron, mountain laurel, and pine, bisected by a stream. Elevation is 876 m.

***Nannaria* n. sp. “Brandywine”**

Vernacular name: “The Pickled Twisted Claw”

Figs. 3.23, 24



Figure 3.23. *Nannaria* n. sp. “Brandywine”, dorsal view, male holotype MPE02109 from Pendleton County, West Virginia.

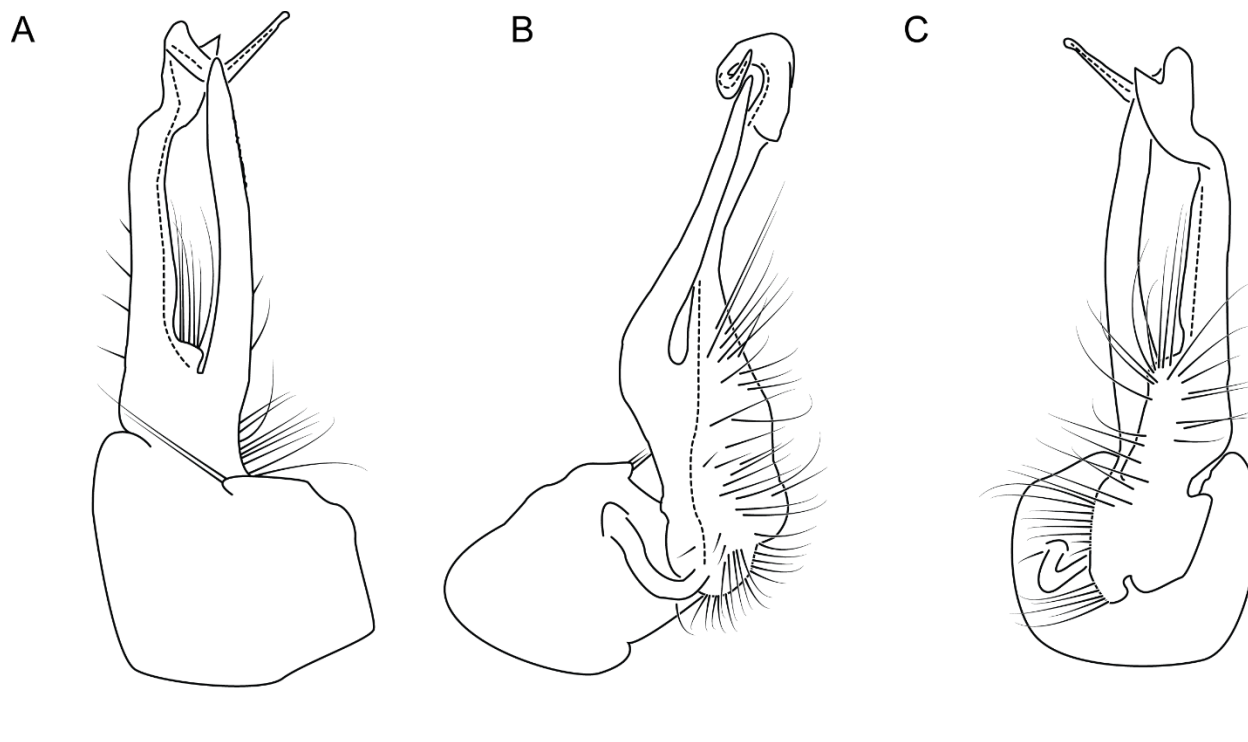


Figure 3.24. Left gonopod of *Nannaria* n. sp. “Brandywine” (MPE02111, Pendleton County, West Virginia). **A.** anterior view. **B.** medial view. **C.** posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (MPE02109) from West Virginia, Pendleton County, Brandywine Recreation Area, behind campsite 20, George Washington National Forest (38.5948°N, -79.1982°W, ± 5 m, elevation 619 m), moist deciduous litter (oak, maple, hickory) near stream, Coll: D. A. Hennen, J. C. Means

Diagnosis: Adults of *Nannaria* n. sp. “Brandywine” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal orange spots, collum outlined in orange, and tergites with background chestnut brown. **Gonopods.** Male gonopod acropodite arc straight, with abrupt bend distally, not gradually curving as in *Nannaria shenandoa*; acropodite with gently undulating twist at anterior bend, not absent as in *Nannaria shenandoa*. Acropodite medial flange absent; acropodite tip medial flange broad and laminate,

produced into a sharp corner, not triangular as in *Nannaria* n. sp. “Trimble” or lobed as in *Nannaria* n. sp. “LongSpringRun”; acropodite tip lateral flange absent. Acropodite tip thin and pointed, acutely recurved; tip directed antero-medially, not caudally as in *Nannaria shenandoa* or ventrally as in *Nannaria ericacea*. Acropodite with basomedial quadrate process. Prefemoral process sinuously tapered, not simple and curving as in *Nannaria ericacea*; prefemoral process crossing acropodite dorsolaterally; tip directed cephalically, not dorsally as in *Nannaria shenandoa* or laterally as in *Nannaria* n. sp. “Trimble”. **Cyphopods.** Female cyphopod receptacle quadrate, recurved distally, not an enlarged triangular hood as in *Nannaria shenandoa* or triangular as in *Nannaria* n. sp. “Trimble”.

Measurements: BL = 27.30, CW = 4.10, IW = 2.85, ISW = 0.96, B10W = 4.85, B10H = 2.70.

Variation: No noticeable variation was observed.

Distribution: *Nannaria* n. sp. “Brandywine” is known from Pendleton County, West Virginia and the following adjacent Virginia counties: Augusta, Highland, Rockingham.

Ecology: This species has been found in mesic forests of oak, hickory, and maple, as well as in a rhododendron grove in a rhododendron, hemlock, and oak forest near a stream.

***Nannaria* n. sp. “Coweeta”**

Vernacular name: “The Chilled Twisted Claw”

Figs. 3.25, 26



Figure 3.25. *Nannaria* n. sp. “Coweeta”, dorsal view, male exemplar MPE03317 from Towns County, Georgia.

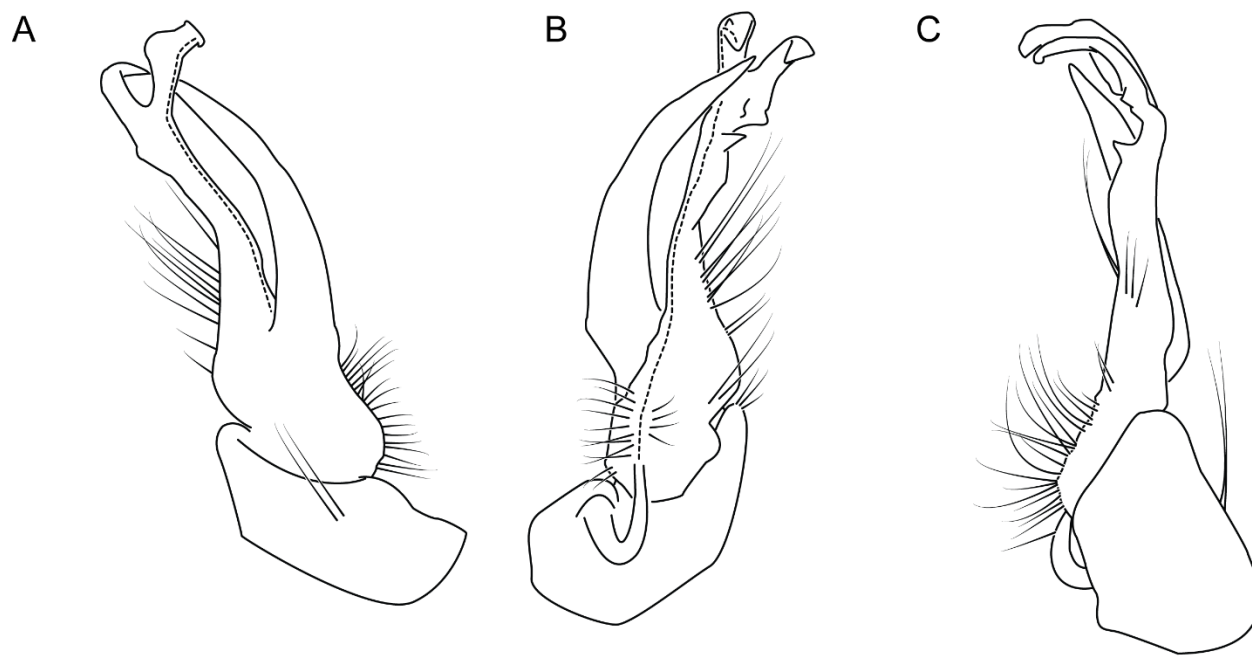


Figure 3.26. Left gonopod of *Nannaria* n. sp. “Coweeta” male holotype (MPE03311, Towns County, Georgia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (MPE03311) from North Carolina, Macon County, 9.79 mi WSW of Franklin, at scenic view spot along US 64 beside Poplar Cove Creek (35.1241°N, -83.5392°W, ±10m, elevation 1082 m), steep hillside with moist dark crumbly soil in tulip poplar, buckeye, birch, cherry woods beside mountain stream, 26 October 2017, Coll: D. A. Hennen, J. C. Means.

Diagnosis: Adults of *Nannaria* n. sp. “Coweeta” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal orange spots, collum outlined in orange, and tergites with background chestnut brown. **Gonopods.** Male gonopod acropodite arc straight, with abrupt bend distally, not bend at midpoint as in *Nannaria* n. sp. “Juglandis”; acropodite with acute twist at anterior bend, appearing somewhat scooped and folded, not with a smoothly-undulating helix as in *Nannaria* n. sp. “HarrisCove”. Acropodite medial flange tooth-like, ending in a sharp point, not lobed as in *Nannaria austriicola*.

Acropodite tip medial flange laminate, long and curving, terminating in a distal sharp point, not lobed as in *Nannaria austriicola*. Acropodite tip lateral flange lobed, and with a short tooth-like projection dorsally, lateral flange not triangular as in *Nannaria scutellaria*. Acropodite tip bifurcate, each branch hooked at tip and directed ventrally. Prefemoral process sinuously tapered and quite thick at base, not laminate as in *Nannaria* n. sp. “Dell”; prefemoral process curving laterally, not ventrally as in *Nannaria* n. sp. “Amicalola”; prefemoral process crossing acropodite ventrolaterally, not dorsolaterally as in *Nannaria austriicola*; prefemoral process tip directed medially. **Cyphopods.** Female cyphopod receptacle in the shape of a finger-like projection, curving over cyphopod valves and tapering distally, not triangular as in *Nannaria austriicola*.

Measurements: BL = 23.50, CW = 3.20, IW = 2.48, ISW = 0.78, B10W = 3.92, B10H = 2.32.

Variation: No noticeable variation observed.

Distribution: *Nannaria* n. sp. “Coweeta” is known from Dawson and Towns Counties, Georgia, and Macon County, North Carolina.

Ecology: This species has been collected in typical *Nannaria wilsoni* species group habitat: mesic deciduous forest (oak, maple, tulip tree, birch, cherry, buckeye) and rhododendron and hemlock coves. It ranges in elevation from 690 m to 1189 m.

***Nannaria* n. sp. “Cratagae”**

Vernacular name: “The Hawthorn Twisted Claw”

Figs. 3.27, 28



Figure 3.27. *Nannaria* n. sp. “Cratagae”, dorsal view, male holotype MPE01222 from Van Buren County, Tennessee.

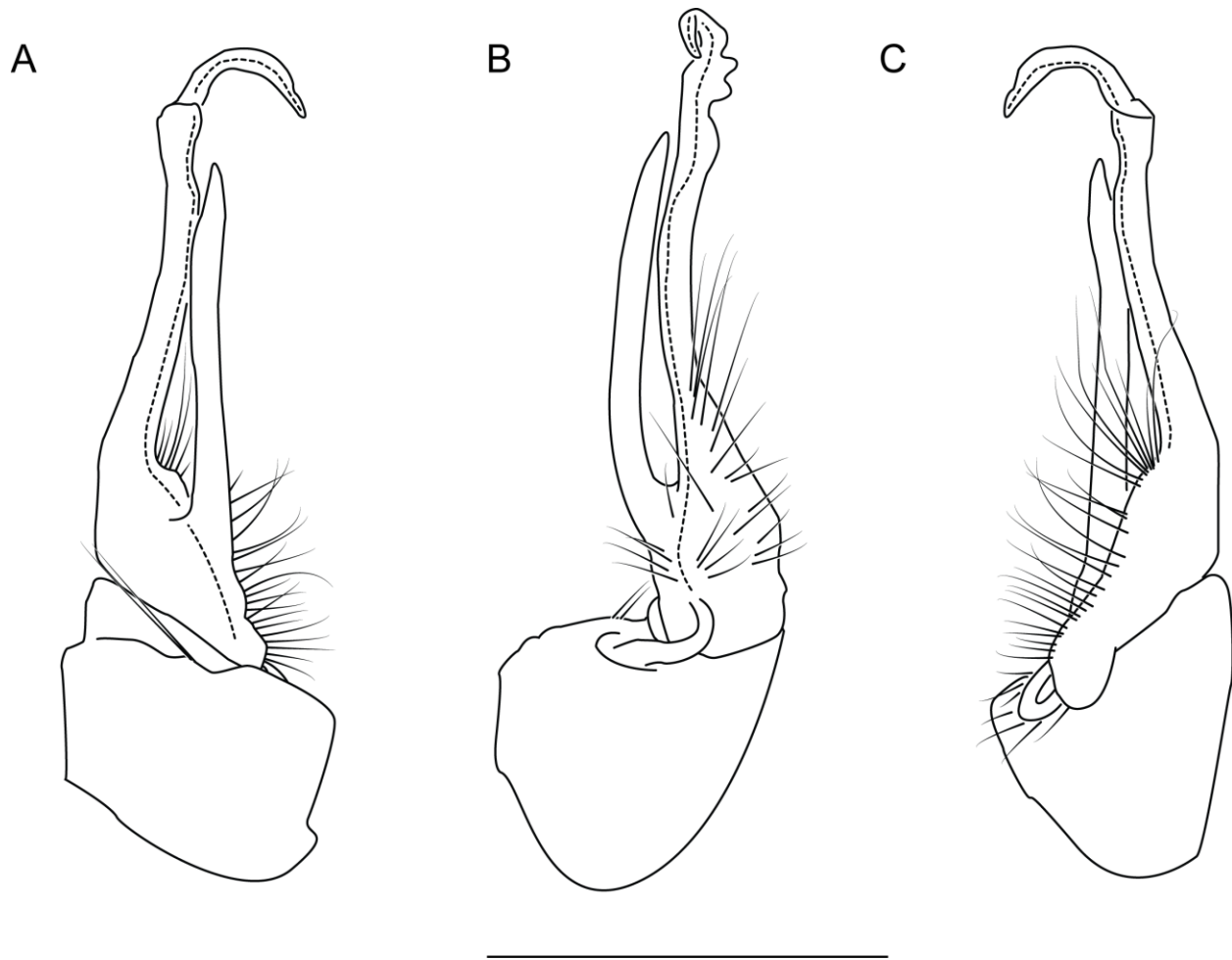


Figure 3.28. Left gonopod of *Nannaria* n. sp. “Cratagae” male holotype (MPE01222, Van Buren County, Tennessee). **A.** anterior view. **B.** medial view. **C.** posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (MPE01222) from Tennessee, Van Buren County, Falls Creek Falls State Park, Crane Creek Falls overlook (35.6628°N, -85.3498°W, ±5m, elevation 483 m), at base of witch hazel, in deciduous forest, 22 May 2016, Coll: J. C. Means, D. A. Hennen.

Diagnosis: Adults of *Nannaria* n. sp. “Cratagae” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal orange spots, collum outlined in orange, and tergites with background chestnut brown. **Gonopods.** Male

gonopod acropodite arc straight, with abrupt bend distally, not bent at midpoint as in *Nannaria* n. sp. “Juglandis”; acropodite with acute twist at anterior bend, folding distal zone of acropodite, not in the shape of a smoothly-undulating helix as in *Nannaria* n. sp. “HarrisCove”. Acropodite medial flange lobed, with two small rounded bumps visible in medial view, not tooth-like as in *Nannaria scutellaria*. Acropodite tip medial and lateral flanges absent; acropodite tip slightly tapering distally and directed medially, not ventrally as in *Nannaria scutellaria*. Gonopod acropodite with small basomedial quadrate process. Prefemoral process acicular, not sinuously tapered as in *Nannaria austriicola*; prefemoral process straight, not curving laterally as in *Nannaria scutellaria*; prefemoral process tip directed cephalically, not laterally as in *Nannaria scutellaria*. **Cyphopods.** Female cyphopod receptacle sinuous and S-shaped, blunt and rounded distally, not in the shape of an elongated club as in *Nannaria scutellaria*.

Measurements: BL = 22.40, CW = 3.50, IW = 2.50, ISW = 0.88, B10W = 4.35, B10H = 2.55.

Variation: No noticeable variation observed.

Distribution: *Nannaria* n. sp. “Cratagae” is only known from Tennessee and has been collected in the following counties: Cumberland, Monroe, and Van Buren.

Ecology: This species has been collected in mesic forests with hemlock, maple, oak, tulip tree, witch hazel, and pine, at elevations ranging from 481 m to 1539 m.

***Nannaria* n. sp. “Dell”**

Vernacular name: “The Unicorn Twisted Claw”

Figs. 3.29, 30



Figure 3.29. *Nannaria* n. sp. "Dell", dorsal view, male holotype MPE04377 from Rabun County, Georgia.

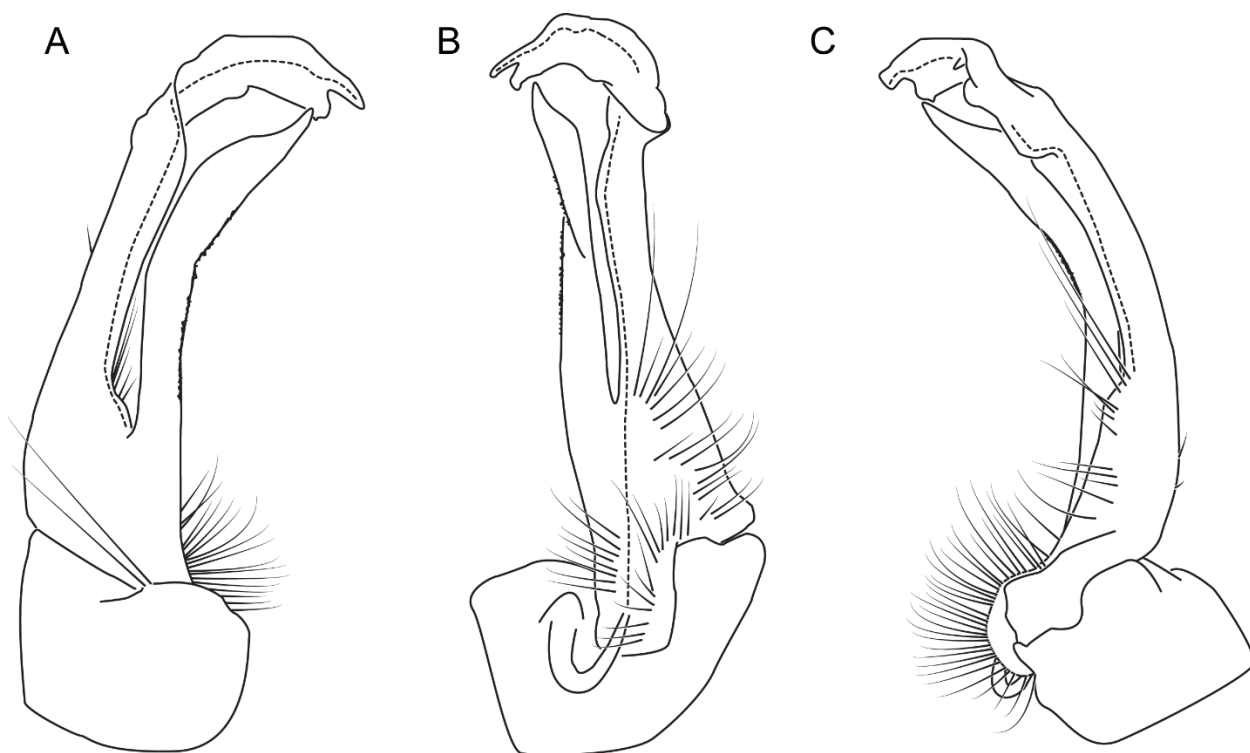


Figure 3.30. Left gonopod of *Nannaria* n. sp. “Dell” male holotype (MPE04377, Rabun County, Georgia). **A.** anterior view. **B.** medial view. **C.** posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (MPE04377) from Georgia, Rabun County, Clayton: Chattahoochee NF, Warwoman Dell Rec Area, near picnic area (34.8813°N, - 83.3539°W, ±8m, elevation 644 m), buried in dark sandy soil in patch of beech, tuliptree, rhododendron woods, 13 October 2018, Coll: D. A. Hennen.

Diagnosis: Adults of *Nannaria* n. sp. “Dell” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal red spots, collum outlined in red, and tergites with background black. **Gonopods.** Male gonopod acropodite arc straight, with abrupt bend distally, not bent at midpoint as in *Nannaria* n. sp. “Juglandis”; acropodite with wide, acute twist at anterior bend, giving the acropodite a folded appearance, not a smoothly-undulating helix as in *Nannaria* n. sp. “HarrisCove”. Acropodite medial flange lobed

and slightly undulating, not tooth-like as in *Nannaria* n. sp. “Coweeta”. Acropodite tip medial flange a short, triangular projection, not lobed as in *Nannaria austriicola*; acropodite tip lateral flange lobed and projecting, not triangular as in *Nannaria scutellaria*. Acropodite tip directed medially, not caudally as in *Nannaria* n. sp. “PucPuggy”; acropodite tip bifurcated distally, giving it a unicorn-like appearance. Prefemoral process laminate, not sinuously tapered as in *Nannaria austriicola*; prefemoral process curving medially, not laterally as in *Nannaria* n. sp. “Coweeta”; prefemoral process with a slight taper distally, tip directed cephalically, not medially as in *Nannaria* n. sp. “Coweeta”. Medial margin of prefemoral process somewhat jagged.

Measurements: BL = 26.30, CW = 3.70, IW = 2.88, ISW = 0.92, B10W = 4.50, B10H = 2.40.

Variation: Acropodite medial flange size varies from small to large. No other noticeable variation observed.

Distribution: *Nannaria* n. sp. “Dell” is only known from the type locality in Rabun County, Georgia.

Ecology: Specimens were found buried 3-5 cm in sandy soil near a stream in a cove forest of beech, tulip tree, and rhododendron.

***Nannaria* n. sp. “HarrisCove”**

Vernacular name: “The Rabbit Twisted Claw”

Figs. 3.31, 32



Figure 3.31. *Nannaria* n. sp. “HarrisCove”, dorsal view, female (MPE04399, Towns County, Georgia).

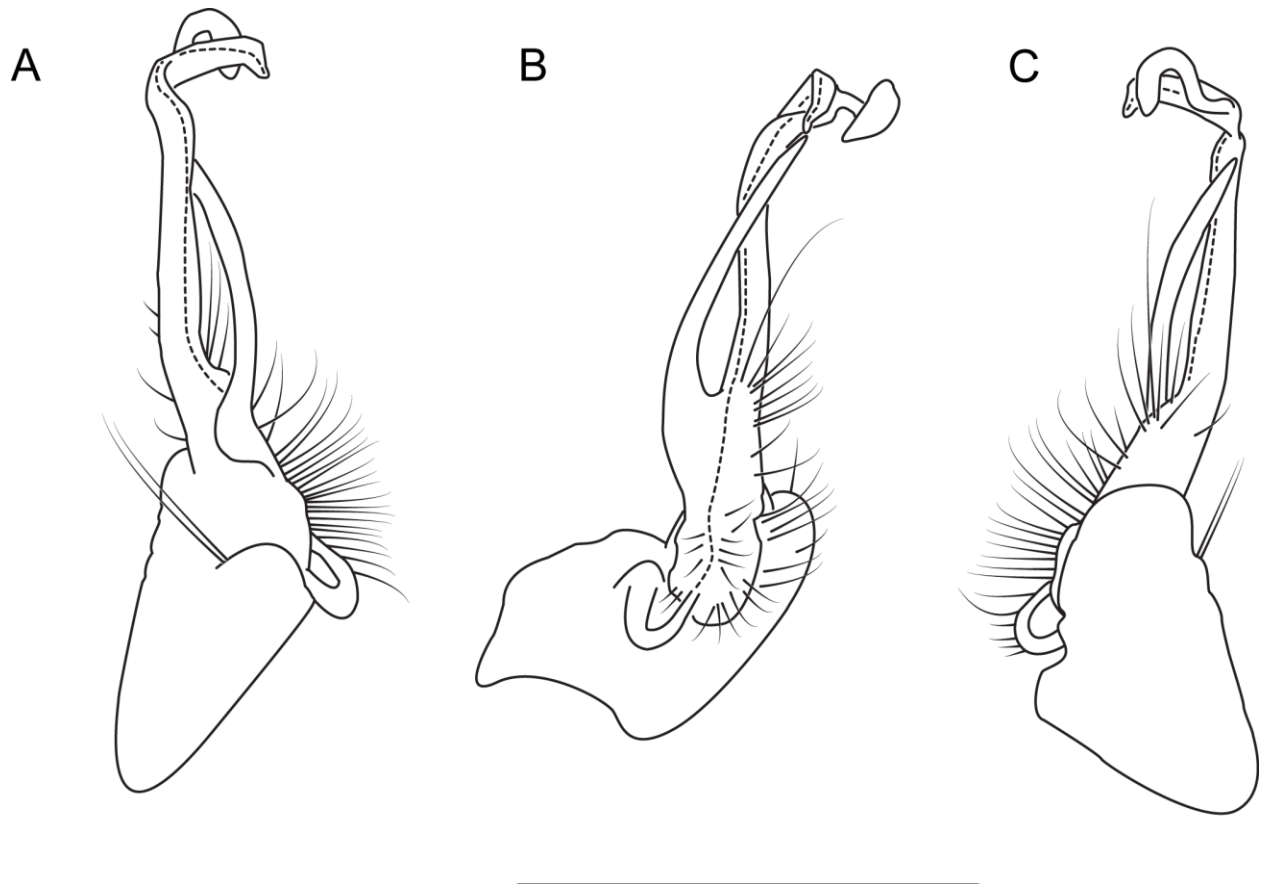


Figure 3.32. Left gonopod of *Nannaria* n. sp. “HarrisCove” male holotype (MPE04401, Towns County, Georgia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (MPE04401) from Georgia, Towns County, Hiawasse: Long Ridge Campground, Harris Cove (34.9256°N, -83.7776°W, ±6m, elevation 647 m), moist litter and soil in rhododendron, mountain laurel, tulip tree, beech forest near stream, 15 October 2018, Coll: D. A. Hennen.

Diagnosis: Adults of *Nannaria* n. sp. “HarrisCove” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal red spots, collum outlined in red, and tergites with background black. **Gonopods.** Male gonopod acropodite arc straight, with abrupt bend distally, not bent at midpoint as in *Nannaria* n. sp. “Juglandis”; acropodite with a smoothly undulating twist at acropodite bend, not acutely twisted as in *Nannaria* n. sp. “Coweeta”. Acropodite medial flange slightly lobed, not tooth-like as in *Nannaria* n. sp. “Coweeta”. Acropodite tip medial flange produced into a recurved lobe with a rounded tip and shaped like a rabbit ear, not laminate as in *Nannaria* n. sp. “Coweeta”. Acropodite tip lateral flange slightly lobed before acropodite, not triangular as in *Nannaria scutellaria*. Acropodite tip blunt, tapering; directed caudally, not medially as in *Nannaria austriicola*. Prefemoral process sinuously tapered, thicker at base, not laminate as in *Nannaria* n. sp. “Amicalola”; prefemoral process curving laterally, not ventrally as in *Nannaria* n. sp. “Amicalola”; prefemoral process crossing acropodite ventrolaterally; tip directed laterally, not medially as in *Nannaria* n. sp. “Coweeta”. **Cyphopods.** Female cyphopod receptacle in the shape of a finger-like projection, curving over cyphopod valves and tapering distally, not an enlarged triangular hood as in *Nannaria* n. sp. “Amicalola”.

Measurements: BL = 20.20, CW = 3.10, IW = 2.38, ISW = 0.78, B10W = 3.80, B10H = 2.63.

Variation: No noticeable variation observed.

Distribution: *Nannaria* n. sp. “HarrisCove” is known from two counties in northern Georgia: Towns and Lumpkin.

Ecology: This species has been collected in both dry and moist situations, ranging from upland oak-hickory forest to riparian cove forests of rhododendron and hemlock. Elevations at collecting localities are between 610 and 977 m.

***Nannaria* n. sp. “Juglandis”**

Vernacular name: “The Walnut Twisted Claw”

Figs. 3.33, 34



Figure 3.33. *Nannaria* n. sp. “Juglandis”, dorsal view, female (MPE03296, Rutherford County, North Carolina).

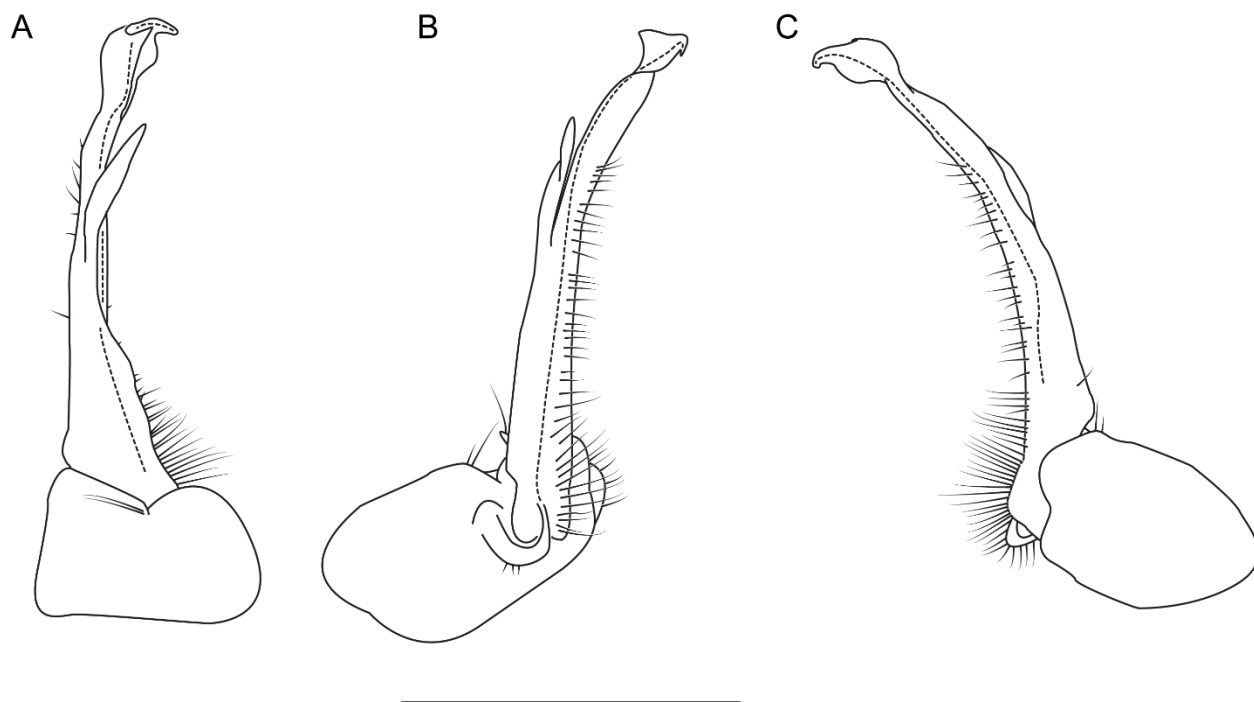


Figure 3.34. Left gonopod of *Nannaria* n. sp. “Juglandis” male holotype (MPE03440, Rutherford County, North Carolina). **A.** anterior view. **B.** medial view. **C.** posterior view. **Scale bar: 1 mm.**

Material examined: Male holotype specimen (MPE03440) from North Carolina, Rutherford County, Chimney Rock State Park, gully on Four Seasons Trail (35.4315°N, -82.2435°W, ±8m, elevation 457 m), moist sandy soil in rhododendron thicket, in rhododendron, oak, maple litter near rocks, 21 October 2017, Coll: D. A. Hennen, J. C. Means.

Diagnosis: Adults of *Nannaria* n. sp. “Juglandis” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal red spots, collum outlined in red, and tergites with background black. **Gonopods.** Male gonopod acropodite arc straight, with bend at midpoint, not straight with an abrupt bend distally as in *Nannaria scutellaria*; acropodite anterior bend with gently undulating twist, not acutely twisted as in *Nannaria scutellaria*. Acropodite medial flange absent. Acropodite tip medial flange laminate, smooth, not lobed as in *Nannaria austriicola*; acropodite tip lateral flange hooked and produced

into a sharp point and curving dorsally, not triangular as in *Nannaria scutellaria*. Acropodite tip with a hood-like appearance, distally tapering and blunt. Prefemoral process spear-shaped, arising from halfway up the lateral side of the acropodite, laminate, not sinuously tapered as in *Nannaria scutellaria*; prefemoral process straight, with slight medial curve distally, not curving laterally as in *Nannaria austriicola*; prefemoral process tip directed medially, not laterally as in *Nannaria scutellaria*. Setae present from base of acropodite to three-quarters up the full acropodite length, not restricted to the basal half of the acropodite length as in the rest of the *Nannaria wilsoni* species group. **Cyphopods.** Female cyphopod receptacle capsule-shaped; blunt, rounded distally, not an elongated club as in *Nannaria scutellaria*.

Measurements: BL = 26.20, CW = 3.68, IW = 2.76, ISW = 0.90, B10W = 4.88, B10H = 2.81.

Variation: The length of the acropodite tip lateral flange and acropodite tip both vary, and the curve of the acropodite tip varies from medial to ventral.

Distribution: *Nannaria* n. sp. “Juglandis” is known from Buncombe and Rutherford Counties in southwest North Carolina, and adjacent Greenville County, South Carolina.

Ecology: This species has been collected from rhododendron coves in mesic deciduous and ericaceous forests.

***Nannaria* n. sp. “LongSpringRun”**

Vernacular name: “The Streamside Twisted Claw”

Fig. 3.35

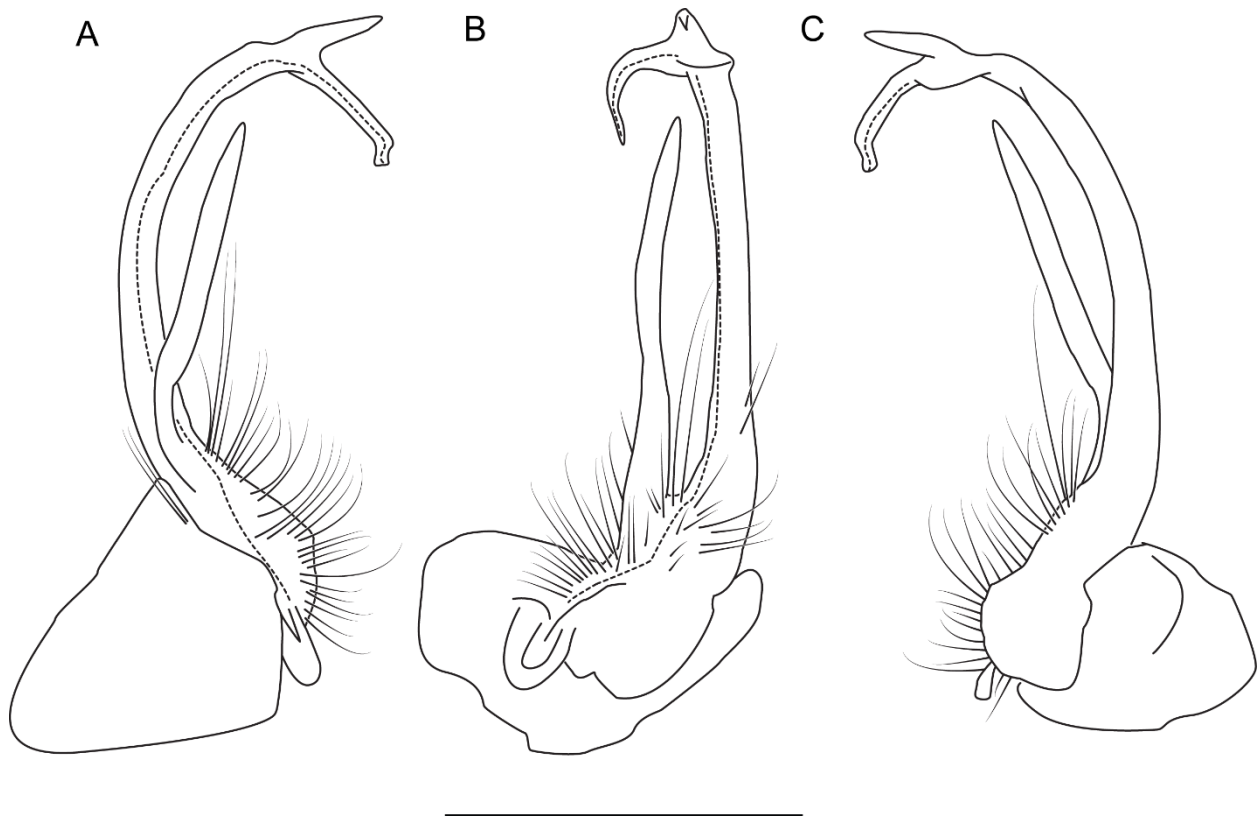


Figure 3.35. Left gonopod of *Nannaria* n. sp. “LongSpringRun” male holotype (NAN0650, Bath County, Virginia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (NAN0650) from Virginia, Bath County, George Washington National Forest, Long Spring Run, above Little Back Creek (38.2213°N, -79.8387°W, ±2km), pitfall trap, 24 July 1992, Coll: J. Pagels, D. Kobuszewski.

Diagnosis: Adults of *Nannaria* n. sp. “LongSpringRun” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Color in life unknown, but specimen with typical bimaculate pattern, faded in alcohol. **Gonopods.** Male gonopod acropodite arc gradually curving, not straight with abrupt bend distally as in *Nannaria ericacea*; acropodite with gently undulating twist at apical bend, not acutely twisted as in *Nannaria* n. sp. “Sanctimontis”. Acropodite medial flange absent; acropodite tip medial flange lobed, shelf-like, not triangular as

in *Nannaria ericacea*; acropodite tip lateral flange large, triangular, projecting anteromedially, not lobed as in *Nannaria* n. sp. “BigStonyCreek”. Acropodite tip entire, with a small preapical bump, not bifurcate as in *Nannaria shenandoa*; acropodite tip directed caudally, not ventrally as in *Nannaria ericacea*. Prefemoral process acicular, mostly straight but with a slight curve basally, not simple and curving laterally as in *Nannaria shenandoa*; prefemoral process tip directed cephalically, not dorsally as in *Nannaria shenandoa*.

Measurements: BL = 27.80, CW = 4.31, IW = 2.95, ISW = 1.02, B10W = 5.06, B10H = 2.69.

Variation: No noticeable variation observed.

Distribution: *Nannaria* n. sp. “LongSpringRun” is only known from a small area within Pocahontas County, West Virginia and Bath County, Virginia.

Ecology: The label data from the collected specimens does not provide any notes on the habitat, but on a collecting trip to the type locality to search for live individuals by D. A. Hennen and P. E. Marek, the habitat was found to be a mesic oak, hickory, tulip poplar, and hemlock forest bisected by streams down in a holler, with an elevation of 840 m.

***Nannaria* n. sp. “Lutra”**

Vernacular name: “The Otter Twisted Claw”

Figs. 3.36, 37



Figure 3.36. *Nannaria* n. sp. “Lutra”, dorsal view, male holotype MPE02147 from Rockbridge County, Virginia.

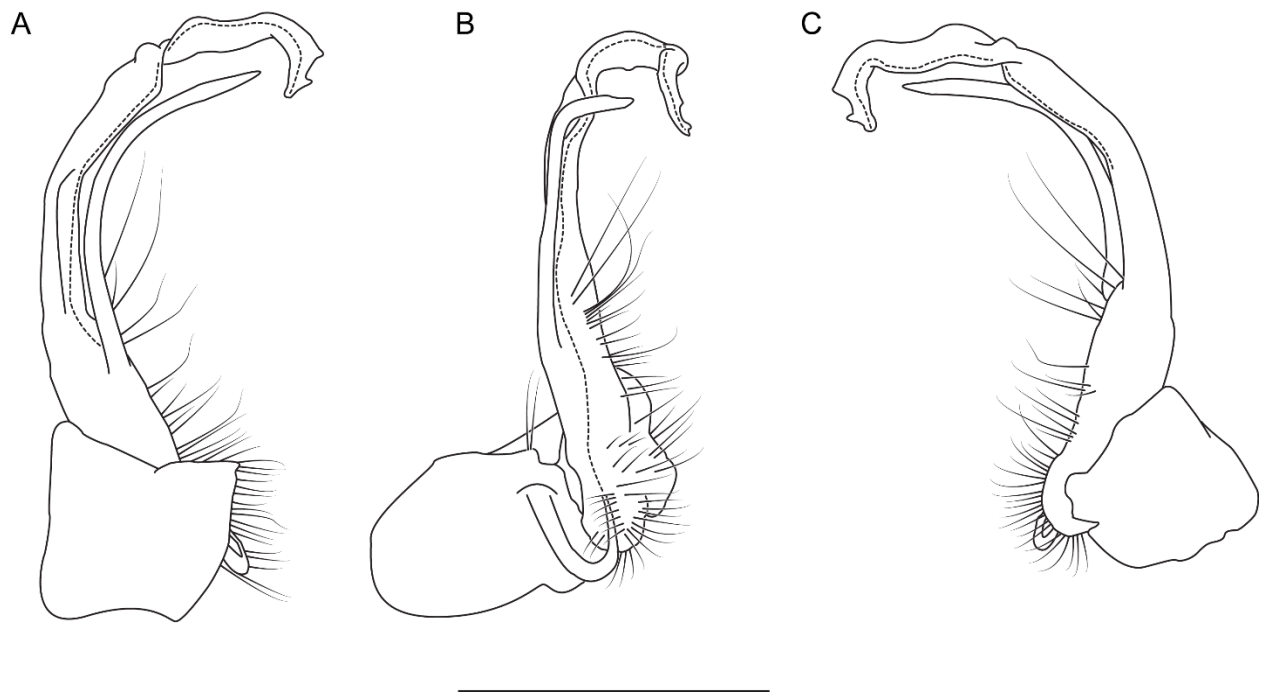


Figure 3.37. Left gonopod of *Nannaria* n. sp. “Lutra” male holotype (MPE02147, Rockbridge County, Virginia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (MPE02147) from Virginia, Rockbridge County, Glasgow: Jefferson National Forest, James River Face Wilderness, Balcony Falls Trailhead, at end of CR 782 off 759 (37.6185°N, -79.4709°W, ±9m, elevation 250 m), riparian area near trail in sandy soil, moist oak, maple litter, forest with pawpaw, sour wood also, 18 August 2016, Coll: D. A. Hennen, J. C. Means, V. Wong.

Diagnosis: Adults of *Nannaria* n. sp. “Lutra” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal orange spots, collum outlined in orange, and tergites with background chestnut brown. **Gonopods.** Male gonopod acropodite arc gradually curving, not straight up with an abrupt bend distally as in *Nannaria ericacea*; acropodite with an undulating twist at anterior bend, not acutely twisted as in *Nannaria* n. sp. “Sanctimontis”. Acropodite entire, not bifurcate as in *Nannaria morrisoni*. Acropodite medial flange slightly lobed, with a median dome-like projection dorsally, not laminate as in *Nannaria wilsoni*. Acropodite tip medial flange absent; acropodite tip lateral flange bilobed, the more distal lobe slightly smaller, not laminate as in *Nannaria wilsoni*. Acropodite tip directed caudally, not ventrally as in *Nannaria ericacea*. Prefemoral process simple, curving medially, not sinuous as in *Nannaria wilsoni*; tip directed medially, not cephalically as in *Nannaria ericacea*. **Cyphopods.** Female cyphopod receptacle triangular, not an enlarged triangular hood as in *Nannaria wilsoni*.

Measurements: BL = 25.60, CW = 3.80, IW = 2.76, ISW = 0.87, B10W = 4.75, B10H = 2.69.

Variation: Size and shape of the median dome-like projection varies and is sometimes more pointed than rounded. Acropodite tip lateral flange sometimes with lobes smaller and rounder, rather than pointed.

Distribution: *Nannaria* n. sp. “Lutra” is known from the following Virginia counties: Bedford, Botetourt, Rockbridge.

Ecology: This species is known from mixed hardwood forests at elevations ranging from 250 m to 1219 m.

***Nannaria* n. sp. “Nubiterrae”**

Vernacular name: “The Hazel Twisted Claw”

Figs. 3.38, 39



Figure 3.38. *Nannaria* n. sp. “Nubiterrae”, dorsal view, male (MPE02451, Allegheny County, Virginia).

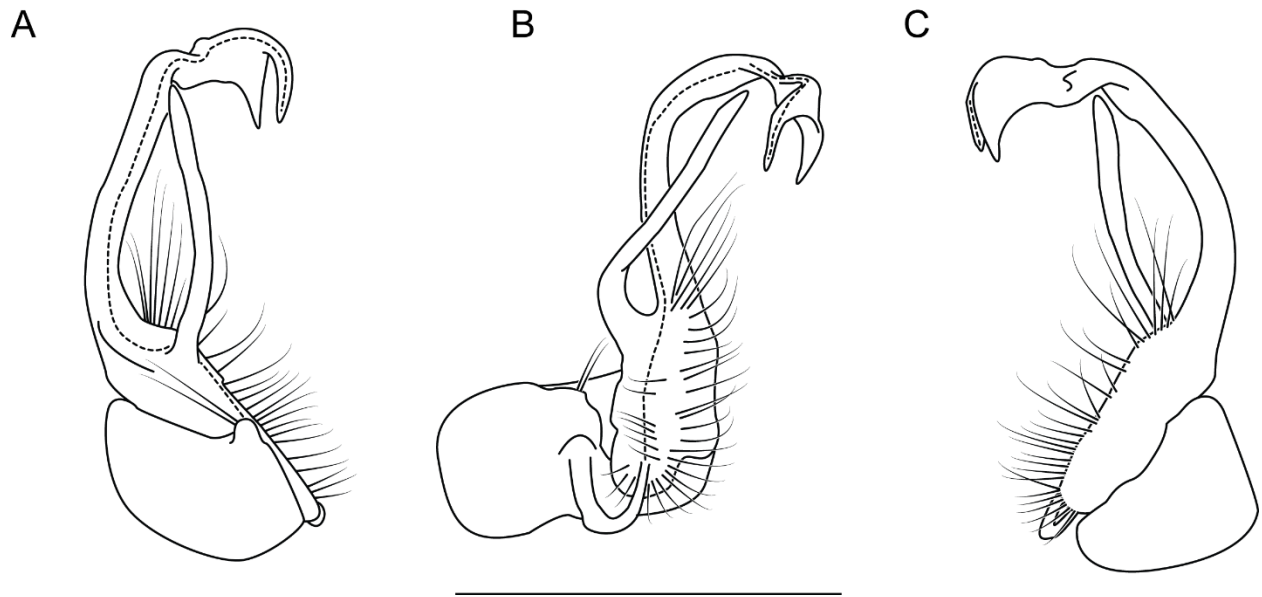


Figure 3.39. Left gonopod of *Nannaria* n. sp. “Nubiterrae” male holotype (MPE02110, Allegheny County, Virginia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (MPE02110) from Virginia, Allegheny County, McGraw’s Gap, NW of town on RT 606 at fishing area in George Washington National Forest along Smith Creek (37.8526°N, -79.8512°W, ±9 m, elevation 428 m), dry deciduous litter near stream in forest of oak, rhododendron, maple, mountain laurel, and witch hazel, soil a bit sandy, 21 June 2016, Coll: J. C. Means.

Diagnosis: Adults of *Nannaria* n. sp. “Nubiterrae” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal red-orange spots, collum outlined in red-orange, and tergites with background chestnut brown. **Gonopods.** Male gonopod acropodite arc a gradual curve, not straight with an abrupt bend distally as in *Nannaria ericacea*; acropodite with a smooth helical twist at apical bend, not acutely twisted as in *Nannaria* n. sp. “Sanctimontis”. Acropodite medial flange slightly lobed, not laminate as in *Nannaria wilsoni*. Acropodite tip medial flange large, laminate and produced into a caudally-

directed pointed tip larger than acropodite tip, not lobed as in *Nannaria* n. sp. “LongSpringRun”. Acropodite tip lateral flange absent; acropodite tip directed caudally, not ventrally as in *Nannaria ericacea*. Prefemoral process sinuous, curving laterally, not simple as in *Nannaria* n. sp. “Trimble”; prefemoral process crossing acropodite ventrolaterally, instead of not crossing as in *Nannaria* n. sp. “Sanctimontis” or crossing dorsolaterally as in *Nannaria* n. sp. “Brandywine”; prefemoral process tip directed laterally, not cephalically as in *Nannaria ericacea*. **Cyphopods.** Female cyphopod receptacle enlarged into a triangular hood, covering cyphopod valves, not triangular as in *Nannaria* n. sp. “Sanctimontis”.

Measurements: BL = 18.90, CW = 3.25, IW = 2.25, ISW = 0.78, B10W = 3.56, B10H = 2.19.

Variation: No noticeable variation observed.

Distribution: Only known from a small area in Allegheny County, Virginia.

Ecology: Label data indicates this species is found in mesic deciduous forests and rhododendron coves from 428 m to 1241 m in elevation.

***Nannaria* n. sp. “Pinnacles”**

Vernacular name: “The Mountain Twisted Claw”

Figs. 3.40, 41



Figure 3.40. *Nannaria* n. sp. “Pinnacles”, lateral view, male holotype MPE00460 from Floyd County, Virginia.

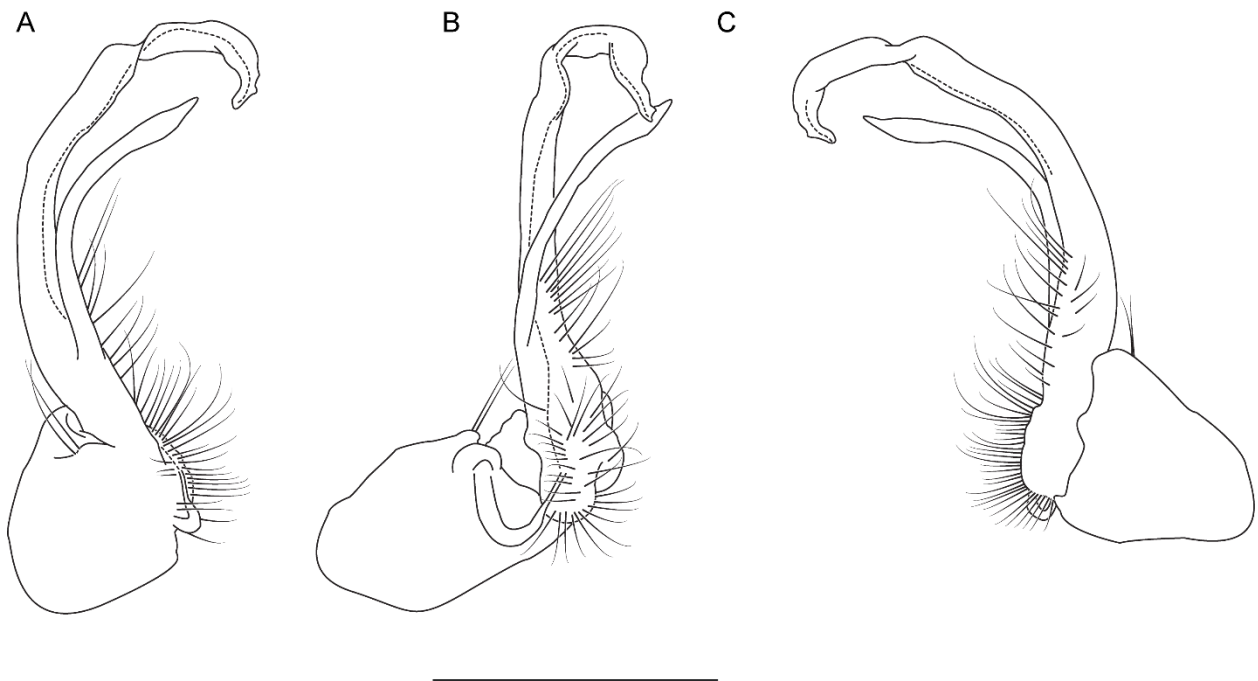


Figure 3.41. Left gonopod of *Nannaria* n. sp. “Pinnacles” male holotype (MPE00460, Floyd County, Virginia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (MPE00460) from Virginia, Floyd County, bottomland in Rocky Knob Recreation Area near creek (36.7856°N, -80.3726°W, ±2m, elevation 830 m), ex forest with ferns, yam, witch-hazel, oak, magnolia, maple, 27 May 2015, Coll: J. C. Means, A. Chazal.

Diagnosis: Adults of *Nannaria* n. sp. “Pinnacles” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal red spots, collum outlined in red, and tergites with background black. **Gonopods.** Male gonopod acropodite arc gradually curving, not straight with an abrupt bend distally, as in *Nannaria ericacea*; acropodite gently twisting at anterior bend, not acutely twisted as in *Nannaria* n. sp. “Sanctimontis”.

Acropodite medial flange slightly lobed, not laminate as in *Nannaria wilsoni*. Acropodite tip medial flange absent; acropodite tip lateral flange slightly bilobed, both projections rounded and small, not triangular as in *Nannaria ericacea*. Acropodite tip blunt, entire, directed ventrally, not caudally as in *Nannaria* n. sp. “Aenigma”. Prefemoral process simple, curving medially, not sinuous as in *Nannaria wilsoni*; prefemoral process slightly spear-shaped distally, tip directed medially, not cephalically as in *Nannaria* n. sp. “Aenigma”. **Cyphopods.** Female cyphopod receptacle triangular, not an enlarged triangular hood as in *Nannaria wilsoni*.

Measurements: BL = 27.60, CW = 3.72, IW = 2.70, ISW = 0.99, B10W = 4.60, B10H = 2.85.

Variation: The size of the acropodite tip lateral flanges differs slightly among specimens.

Distribution: Known from the following Virginia Counties: Carroll, Floyd, Montgomery, Patrick.

Ecology: This species has been collected most often from rhododendron cove habitats, but is also sometimes found in mesic mixed forests.

***Nannaria* n. sp. “PottsMountain”**

Vernacular name: “The Potts Twisted Claw”

Figs. 3.42, 43



Figure 3.42. *Nannaria* n. sp. “PottsMountain”, dorsal view, holotype male MPE04200 from Craig County, Virginia.

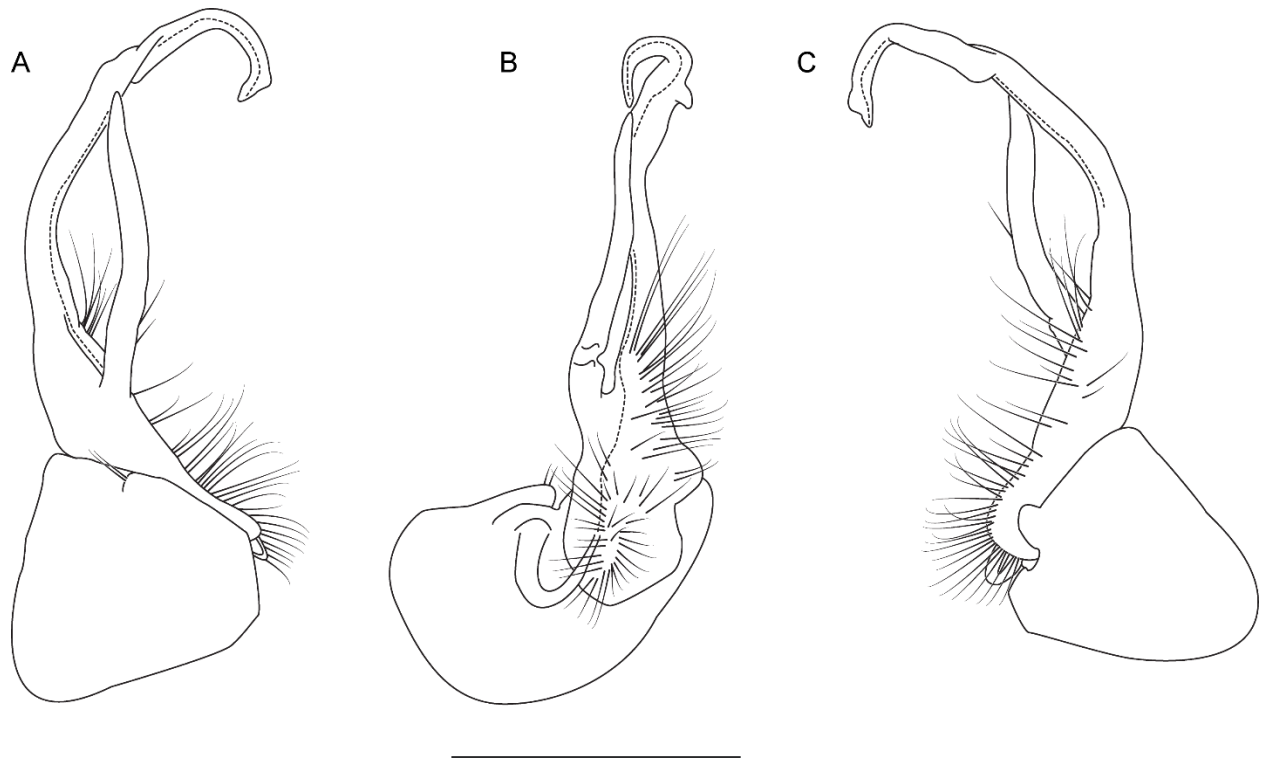


Figure 3.43. Left gonopod of *Nannaria* n. sp. “PottsMountain” male holotype (MPE04200, Craig County, Virginia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm. (Note: the tip of the gonopod acropodite is broken in the holotype specimen, so the tip of a topotypic specimen, NAN0057 was used to estimate its shape.)

Material examined: Male holotype specimen (MPE04200) from Virginia, Craig County, Potts Cove, Cove Branch, next to jeep trail (37.5833°N, -80.1604°W, ±6m, elevation 772 m), forest of tulip tree, oak, persimmon, 25 June 2018, Coll: D. A. Hennen, J. C. Means, K. Williamson, P. E. Marek.

Diagnosis: Adults of *Nannaria* n. sp. “PottsMountain” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal red spots, collum outlined in red, and tergites with background black. **Gonopods.** Male gonopod acropodite arc straight, with bend at midpoint, not abruptly bent distally as in *Nannaria ericacea*; acropodite anterior bend with gentle twist, not acutely twisted as in *Nannaria* n. sp.

“Sanctimontis”. Acropodite medial flange lobed, somewhat triangular in medial view, not laminate as in *Nannaria wilsoni*. Acropodite tip medial flange absent; acropodite tip lateral flange lobed, rounded, not triangular as in *Nannaria ericacea*. Acropodite tip directed caudally, not ventrally as in *Nannaria wilsoni*. Prefemoral process slightly sinuous, not simple and curving as in *Nannaria ericacea*; prefemoral process crossing acropodite dorsolaterally, not ventrolaterally as in *Nannaria shenandoa*; prefemoral process with slight indentation near base, tip directed cephalically, not medially as in *Nannaria wilsoni*. **Cyphopods.** Female cyphopod receptacle an enlarged triangular hood, covering cyphopod valves, not triangular as in *Nannaria ericacea*.

Measurements: BL = 35.70, CW = 4.70, IW = 3.32, ISW = 1.12, B10W = 5.75, B10H = 3.13.

Variation: No noticeable variation observed.

Distribution: Only known from the type locality, Potts Mountain, in Craig County, Virginia.

Ecology: The forest at the type locality is a deciduous oak-tulip tree forest surrounding a stream.

***Nannaria* n. sp. “PucPuggy”**

Vernacular name: “The Overlook Twisted Claw”

Figs. 3.44, 45



Figure 3.44. *Nannaria* n. sp. “PucPuggy”, dorsal view, holotype male MPE01465 from Macon County, North Carolina.

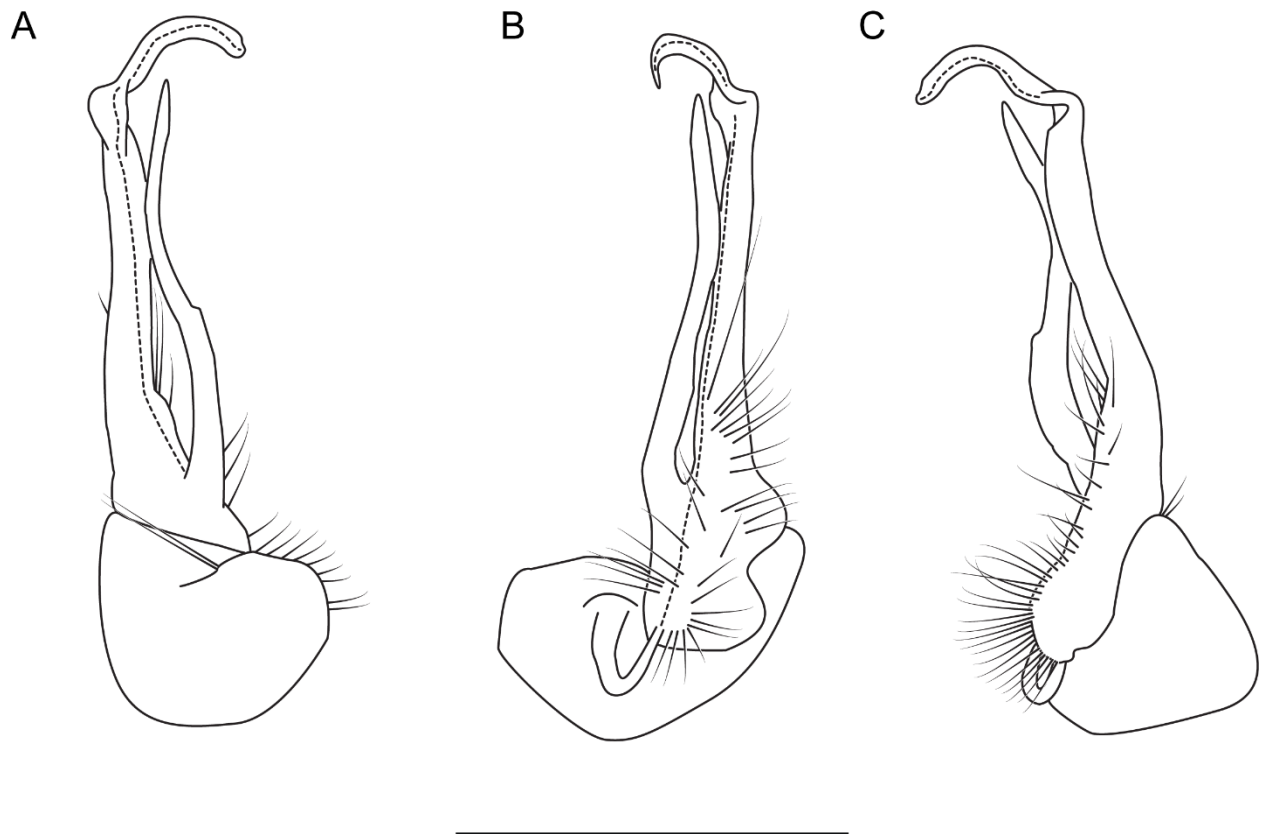


Figure 3.45. Left gonopod of *Nannaria* n. sp. “PucPuggy” male holotype (MPE01465, Macon County, North Carolina). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (MPE01464) from North Carolina, Macon County, South of Highlands, Nantahala National Forest, Orange Mountain, near start of Puc Puggy Trail (35.0288°N, -83.2823°W, ±7m, elevation 1124 m), on hillside along path, moist oak, tulip poplar, rhododendron forest, litter of medium depth, 7 June 2016, Coll: D. A. Hennen, J. C. Means.

Diagnosis: Adults of *Nannaria* n. sp. “PucPuggy” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal red spots, collum outlined in red, and tergites with background black. **Gonopods.** Male gonopod acropodite arc straight, with abrupt bend at tip, not bent at midpoint as in *Nannaria* n. sp. “Juglandis”; acropodite with acute twist at anterior bend, giving the acropodite a pinched appearance, not smoothly-undulating as in *Nannaria* n. sp. “HarrisCove”. Acropodite medial flange lobed, not tooth-like as in *Nannaria scutellaria*; acropodite tip medial flange absent, not lobed as in *Nannaria austriicola*; acropodite tip lateral flange absent, not triangular as in *Nannaria scutellaria* or lobed as in *Nannaria* n. sp. “Coweeta”. Acropodite tip blunt, directed caudally, not medially as in *Nannaria austriicola*. Prefemoral process sinuously tapered, with basal jutting shelf medially, not laminate as in *Nannaria* n. sp. “Amicalola”; prefemoral process tip directed cephalically, not laterally as in *Nannaria scutellaria*. **Cyphopods.** Female cyphopod receptacle triangular, not an elongated club as in *Nannaria scutellaria*.

Measurements: BL = 22.10, CW = 3.28, IW = 2.73, ISW = 0.99, B10W = 3.95, B10H = 2.75.

Variation: No noticeable variation observed.

Distribution: *Nannaria* n. sp. “PucPuggy” is only known from Macon County, North Carolina and adjacent Rabun County, Georgia.

Ecology: This species is found in deciduous and rhododendron cove habitats, within leaf litter and under rocks.

***Nannaria* n. sp. “Sanctimontis”**

Vernacular name: “The Raptor Twisted Claw”

Figs. 3.46, 47



Figure 3.46. *Nannaria* n. sp. “Sanctimontis”, dorsal view, holotype male MPE03810 from Rockbridge County, Virginia.

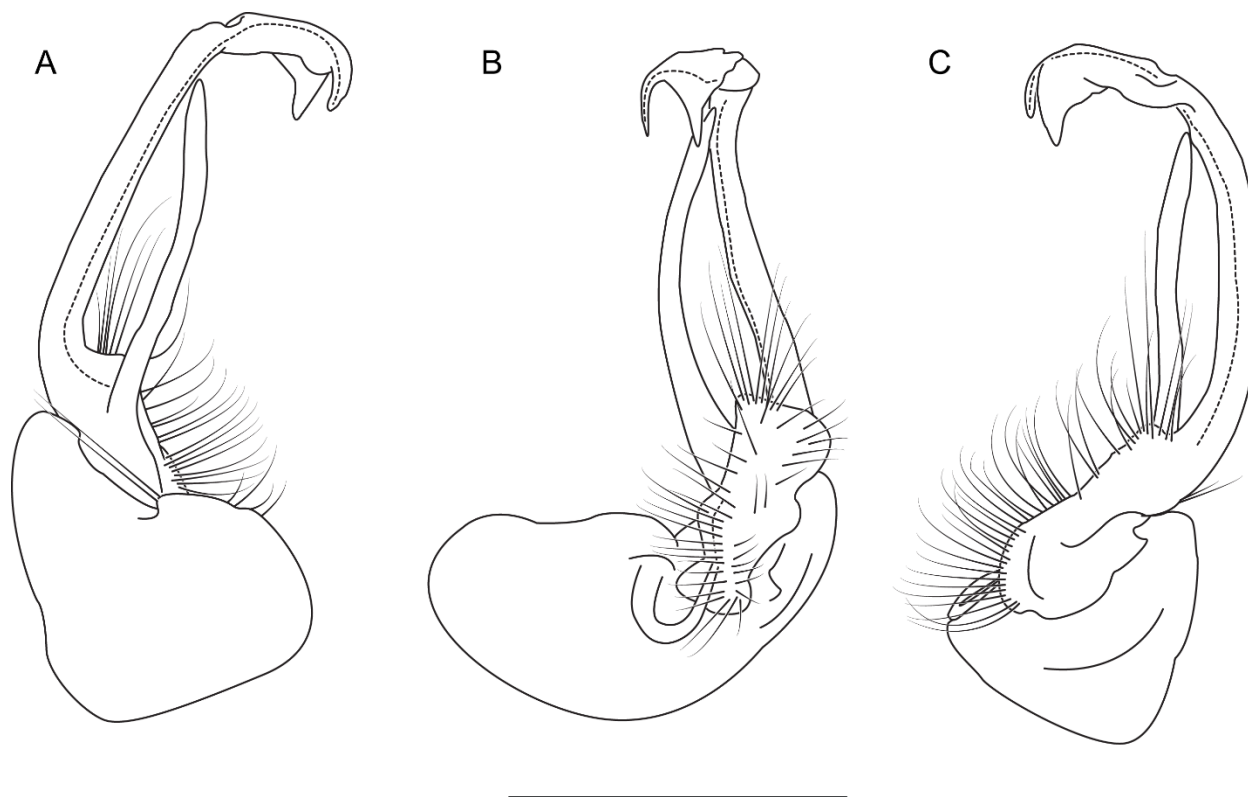


Figure 3.47. Left gonopod of *Nannaria* n. sp. “Sanctimontis” male holotype (MPE03810 Rockbridge County, Virginia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (MPE03810) from Virginia, Rockbridge County, 1.5 air miles NW of Collierstown: Lake Robertson Recreation Area, Mountain Trail. Hillside near Hawks Creek stream crossing (37.8065°N, -79.6152°W, ±10m, elevation 457 m), tulip tree, maple, sycamore woods; moist, dark soil, 20 Feb 2018, Coll: D. A. Hennen, J. C. Means.

Diagnosis: Adults of *Nannaria* n. sp. “Sanctimontis” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal white spots, collum outlined in white, and tergites with background black. **Gonopods.** Male gonopod acropodite arc straight, with abrupt bend distally, not gradually curving as in *Nannaria morrisoni*; acropodite anterior bend with acute twist, appearing folded over, not a smoothly-

undulating helix as in *Nannaria* n. sp. “Trimble”. Acropodite medial flange lobed and concave dorsally, not laminate as in *Nannaria wilsoni*. Acropodite tip medial flange greatly enlarged and laminate, directed caudally with a pointed tip, not triangular as in *Nannaria ericacea* or lobed as in *Nannaria shenandoa*; acropodite tip lateral flange absent. Acropodite tip directed caudally, not medially as in *Nannaria* n. sp. “Trimble”. Gonopod acropodite with quadrate basomedial process, not absent as in *Nannaria* n. sp. “Nubiterrae”. Prefemoral process sinuous, curving laterally, not simple as in *Nannaria* n. sp. “Trimble”; prefemoral process tip directed laterally, not cephalically as in *Nannaria ericacea*. **Cyphopods.** Female cyphopod receptacle triangular, not an enlarged triangular hood as in *Nannaria* n. sp. “Nubiterrae”.

Measurements: BL = 28.30, CW = 4.00, IW = 2.88, ISW = 0.90, B10W = 4.90, B10H = 3.00.

Variation: The color of the paranotal spots in *Nannaria* n. sp. “Sanctimontis” may be either white or red. The male gonopod twist at the anterior bend ranges from acutely twisted to strongly crimped, almost recurving the distal portion of the acropodite backwards.

Distribution: This species is known from the following Virginia Counties, where it mainly inhabits the Blue Ridge Mountains: Amherst, Augusta, Nelson, Rockbridge, Rockingham.

Ecology: Label data indicates this species inhabits mesic mixed deciduous woods, at elevations ranging from 330 m to 1189 m.

***Nannaria* n. sp. “Trimble”**

Vernacular name: “The Rainy Twisted Claw”

Figs. 3.48, 49



Figure 3.48. *Nannaria* n. sp. “Trimble”, dorsal view, holotype male MPE02060 from Augusta County, Virginia.

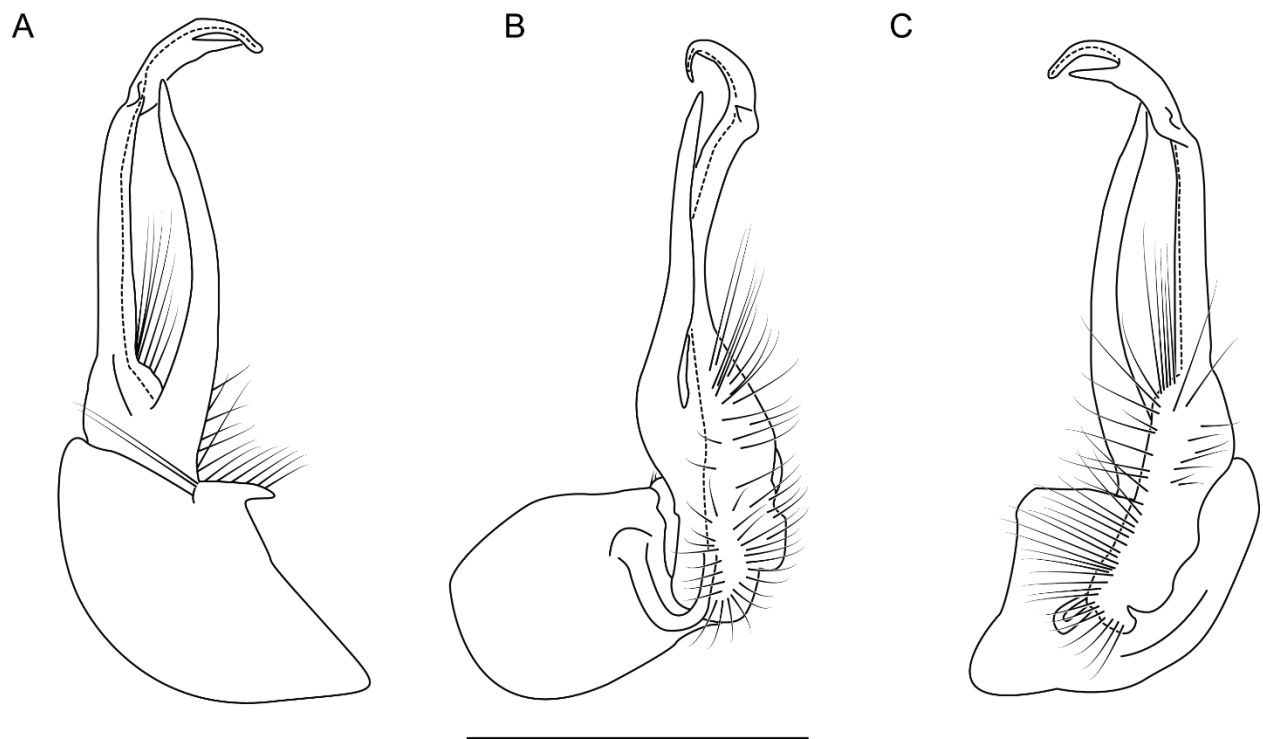


Figure 3.49. Left gonopod of *Nannaria* n. sp. “Trimble” male holotype (MPE02060, Augusta County, Virginia). A. anterior view. B. medial view. C. posterior view. Scale bar: 1 mm.

Material examined: Male holotype specimen (MPE02060) from Virginia, Augusta County, George Washington National Forest, Trimble Mountain Trail off Forest Road 95, SE of Todd Lake, NE West Augusta (38.3605°N, -79.2048°W, ±9m, elevation 602 m), moist oak, maple forest with scattered pines, on hillside by path, 24 June 2016, Coll: D. A. Hennen, J. C. Means.

Diagnosis: Adults of *Nannaria* n. sp. “Trimble” can be separated from other *Nannaria* species by the following combination of characters: **Color.** Tergites with 2 paranotal orange spots, collum outlined in orange, and tergites with background chestnut brown. **Gonopods.** Male gonopod acropodite arc straight, with abrupt bend distally, not gradually curving as in *Nannaria shenandoa*; acropodite anterior bend with gentle twist, not acutely twisted as in *Nannaria* n. sp. “Sanctimontis”. Acropodite medial flange lobed, not laminate as in *Nannaria wilsoni*.

Acropodite tip medial flange triangular, extended and with a laminate base, not lobed as in *Nannaria shenandoa*; acropodite tip entire, directed medially slightly curved ventrally, not caudally as in *Nannaria* n. sp. “Nubiterrae”; acropodite tip lateral flange absent. Prefemoral process simple, curving laterally, not sinuously tapered and straight as in *Nannaria* n. sp.

“Brandywine”; prefemoral process tip directed laterally, not cephalically as in *Nannaria* n. sp.

“Brandywine”. **Cyphopods.** Female cyphopod receptacle triangular, not quadrate and recurved distally as in *Nannaria* n. sp. “Brandywine”.

Measurements: BL = 23.50, CW = 4.20, IW = 2.82, ISW = 1.02, B10W = 5.00, B10H = 2.75.

Variation: No noticeable variation observed.

Distribution: *Nannaria* n. sp. “Trimble” is known from three counties in Virginia: Alleghany, Augusta, and Rockbridge.

Ecology: This species has been collected from both rhododendron-hemlock cove habitats and general mesic deciduous forests, at elevations between 571 m and 602 m.

Distributional Notes.

The distribution of the Central Appalachian species of the *Nannaria wilsoni* species group extends from southwest Virginia and adjacent Kentucky north to West Virginia and the Maryland border (Fig. 3.50).

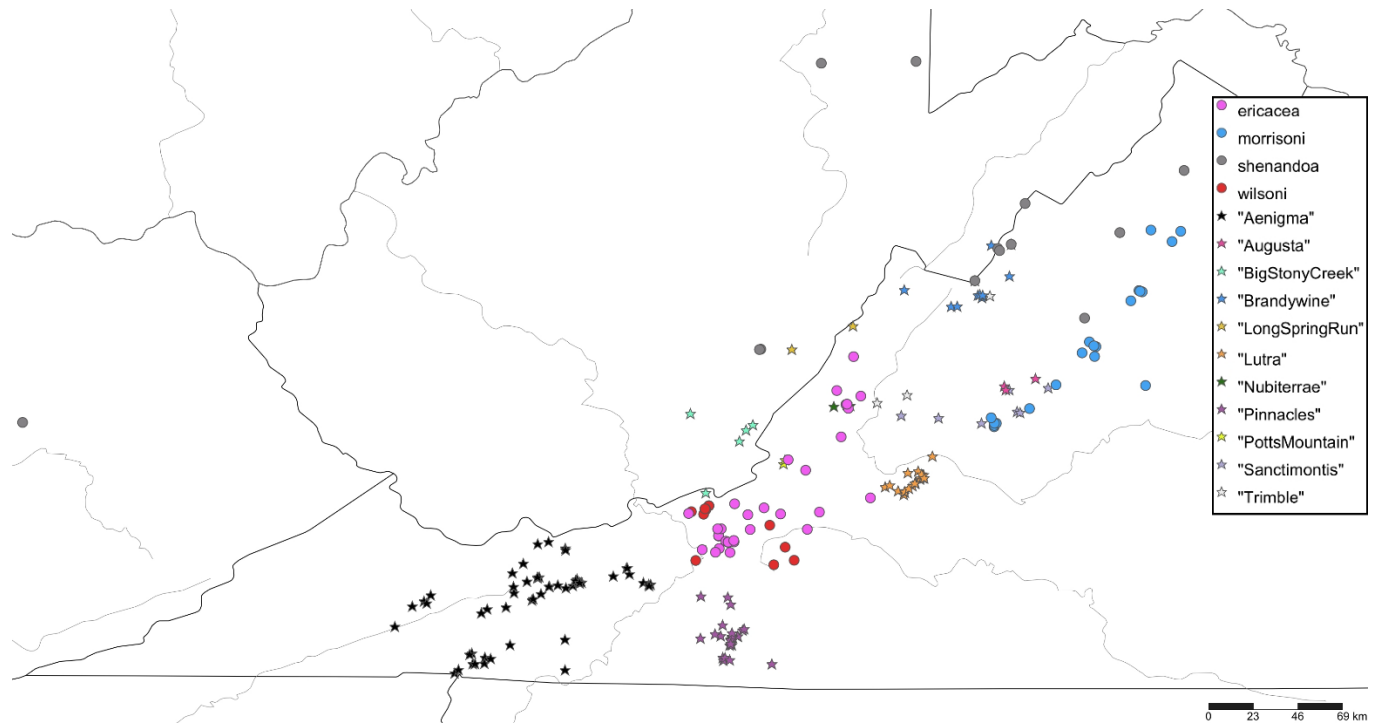


Figure 3.50. Distribution of the Central Appalachian species of the *Nannaria wilsoni* species group. Dots = described species, stars = new species.

The distribution of the Southern Appalachian species extends from western North Carolina and adjacent Tennessee south through northern Georgia (Fig. 3.51)

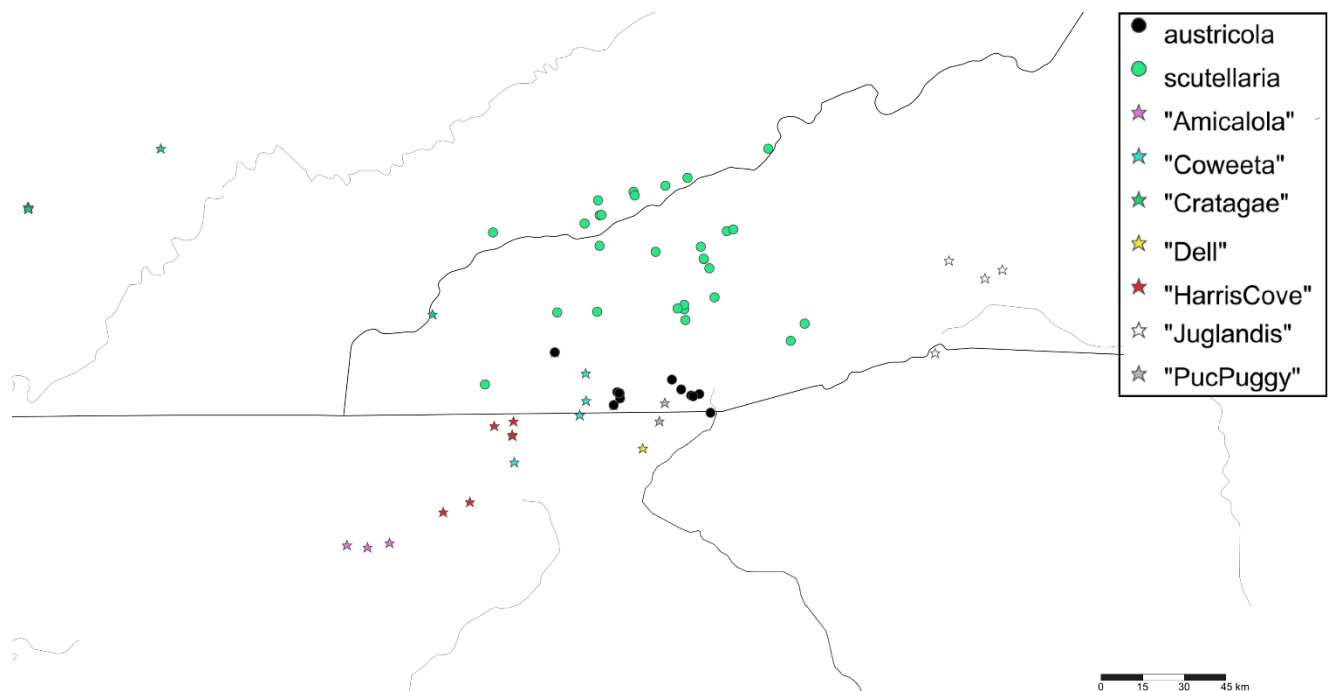


Figure 3.51. Distribution of the Southern Appalachian species of the *Nannaria wilsoni* species group. Dots = described species, stars = new species.

3.5 Discussion

With the description of 18 new species, the species diversity of the *Nannaria wilsoni* species group has increased fourfold. The Southern Appalachian Mountains in particular held a plethora of new species, and only two of the species in that region had been previously described. It is almost certain that more *Nannaria* species will be found in the region in the future, particularly in the moist cove habitats of northern Georgia and adjacent South Carolina. Both the Central Appalachian and Southern Appalachian Regions likely harbor new species. In Central Appalachia, large portions of West Virginia have not been sampled for *Nannaria* and other millipedes. Revisionary work on the genus *Brachoria* Chamberlin, 1939 revealed ten new species in the Appalachian Mountains, eight of which were described from extreme southwestern Virginia, eastern Kentucky, and southern West Virginia (Marek, 2010). Millipedes in the genus

Brachoria are large-bodied (40-60 mm in length), brightly colored, and comparatively easier to collect in the field than *Nannaria*, so it is likely new *Nannaria* species may have been missed during previous collecting in this area.

The well-delineated clades of the *Nannaria minor* and *Nannaria wilsoni* species groups suggest that the distinction between the two may be better encapsulated at the generic level, rather than as species groups, and further study is being done to determine if this groups should indeed be split. The differences between them are supported by genetic, morphological, and biogeographical data. The splits between the *Nannaria minor* and *Nannaria wilsoni* species groups, as well as between *Oenomaea pulchella* and the outgroups are all well-supported, with bootstrap values greater than 70. Morphologically, *O. pulchella* can be separated from *Nannaria* based on its gonopod morphology (a strongly curving acropodite with bifurcate tip and stout prefemoral process projecting at a 45° angle from the acropodite in *O. pulchella*), but also based on somatic characters: the paranota of *O. pulchella* are caudally produced into projecting processes, while in *Nannaria*, the paranota lack such processes (though they may be sharp caudally); the pregonopodal tarsi in *O. pulchella* are not strongly twisted as they are in *Nannaria* (Hoffman, 1964, see figs. 1, 4, 5, 7, and 10).

The *Nannaria minor* and *Nannaria wilsoni* species groups are also easily separable morphologically, based on the structure of the gonopods (see Genus diagnosis, above). The origin of the prefemoral process at the base of the acropodite in the *wilsoni* species group, lack of an elongation between coxa and telopodite, presence of a twist at the anterior bend, and lack of setae past more than three-quarters the length of the acropodite separate it from the *minor* species group, which have the origin of the prefemoral process off an acropodital shelf, an elongation between the coxa and telopodite, lack a twist at the anterior bend, and often have setae present at

the anterior bend of the acropodite. Additionally, an unexpected result of this study was the observation that the receptacle of the female cyphopods differs greatly in size and shape, and may allow identification of females to species in the *wilsoni* group. The valves of the cyphopods appear to vary intraspecifically, but the epigyne (cyphopodal rim) appears to have utility for female species identification and is intraspecifically static. The *minor* species group is not known to possess such modifications to its cyphopod receptacle, though in *Oenomaea pulchella*, the cyphopod receptacle is a large, quadrate, erect, laminate and leaf-like structure that entirely covers the cyphopod valves. The morphology of the cyphopod receptacles are noted in the morphological matrix and species accounts for each species for which female specimens were available to allow for future comparison of female specimens and other investigations into the utility of the female genitalia for species identification in the Nannariini. The most common shape of the cyphopod receptacle in the *Nannaria wilsoni* species group is for the receptacle to be small and triangular, but many modifications to this configuration are found, particularly in the Southern Appalachian Mountains species cluster.

The utility of characters of the female genitalia for identification of genera or species in Polydesmida is not as well-investigated as the characters of the male genitalia. However, previous workers have documented the utility of female genitalic characters for separating genera in the Xystodesmidae, such as with *Euryurus* and *Auturus* (Hoffman, 1978a; Shelley, 1982). Female genitalic characters are also useful for species identification in the genera *Euryurus* and *Eurymerodesmus* (Hoffman, 1978a; Shelley, 1990), and form the defining characters of the subfamily Parafontariinae Hoffman, 1978 (Hoffman, 1978b). Other variations within the Xystodesmidae are known, such as in the genus *Cherokia* Chamberlin, 1949, which lacks a receptacle entirely (Hoffman, 1960) and the genus *Pachydesmus* Cook, 1895, in which

females have modified cyphopodal apertures and receptacles (Hoffman, 1958). Other families of Polydesmida are also recorded to have modified female genitalia, such as the Chelodesmidae, with the species *Plectrogonodesmus gounellei* (Brölemann, 1902) having an enlarged epigyne and recurved receptacles with tubular projections (Bouzan et al., 2019). In the Polydesmidae, *Pseudopolydesmus* Attems, 1898 has recently-identified cyphopod structures that may be useful for species identification (Zahnle et al., 2020).

Two regional distributions were observed within the *Nannaria wilsoni* species group: one cluster in the Central Appalachian Mountains, with species known from Kentucky, West Virginia, and Virginia, and the second cluster in the Southern Appalachian Mountains, with species known from Georgia, North Carolina, South Carolina, and Tennessee (Fig 3.2). These clusters are separated by a gap of 108 km, in which no specimens of the *Nannaria wilsoni* species group have been collected, despite repeated collecting events that revealed multiple species of the *Nannaria minor* species group living in this area. Due to the presence of other *Nannaria* in this area and no lack of collecting, this gap was determined to be a reflection of the biological reality of the *Nannaria wilsoni* species group, rather than an artifact of a lack of collecting effort in this area. The gap extends from near Abingdon, Virginia south to the vicinity of Asheville, North Carolina, and no known biogeographical barriers exist to limit the distribution of each cluster, as evidenced by the presence of species from the *Nannaria minor* species group. Future collecting in the intervening area may clarify the exact boundaries of this gap.

The low bootstrap support (average 5.8) for higher level relationships within the *wilsoni* species group phylogeny (Fig. 3.4) precludes specific discussion about which clades are most closely related, but in general the species in each geographic cluster are more closely related to

each other than to species from the other geographic cluster. *Nannaria* n. sp. “Juglandis” is sister to all other species in the *wilsoni* species group, an unsurprising result considering its divergent gonopod anatomy, with the presence of acropodital setae up to three-quarters the length of the acropodite, and its spear-shaped prefemoral process originating halfway up the acropodite, two morphological characters not present in any other species in the group.

Central Appalachian Mountains Species Cluster

In the Central Appalachian Mountains cluster, 15 species of the *wilsoni* group are known, and 11 of those species are newly described. In this cluster, species typically have a gradually curving acropodite or a straight acropodite with an abrupt bend distally. In the cases of *Nannaria* n. sp. “Augusta” and *Nannaria* n. sp. “PottsMountain,” the acropodite is bent at the midpoint. The acropodite anterior bend twist is generally absent or in the shape of a smoothly-undulating helix, except for *Nannaria* n. sp. “Sanctimontis”, which is acutely twisted at the anterior bend of the acropodite. Species from the Central Appalachian Mountains cluster form eight clades in the phylogeny. The higher level relationships among these clades are not well-supported, but most of the species clades are highly supported by bootstrap values greater than 70.

In the first clade from this cluster, *Nannaria* n. sp. “Augusta” was found to be sister to a clade containing *Nannaria* n. sp. “Trimble” and *Nannaria* n. sp. “Brandywine”, with all relationships highly supported. *Nannaria* n. sp. “Trimble” and *Nannaria* n. sp. “Brandywine” have similar gonopod morphology, with the acropodite tip medial flange being a large produced structure: laminate in *Nannaria* n. sp. “Brandywine” (Fig. 3.24) and triangular in *Nannaria* n. sp. “Trimble” (Fig. 3.49). These two species are restricted to the Allegheny Mountains on the western edge of the Shenandoah Valley. The distribution of *Nannaria* n. sp. “Trimble” extends further south to the James River near Clifton Forge in Alleghany County, and is only recorded

from Alleghany, Augusta, and Rockbridge Counties, with a sizeable gap between collecting sites. All known specimens look morphologically identical, but the Rockbridge County specimen (MPE03492) falls into a separate clade with low support values. Opposed to these two species, *Nannaria* n. sp. “Augusta” diverges greatly in gonopod morphology. Found in the Blue Ridge Mountains on the eastern edge of the Shenandoah Valley, its prefemoral process extends at a 45° angle from the acropodite, and the acropodite distal region of the acropodite is greatly expanded, with the acropodite tip lateral flange laminate and jagged (Fig. 3.21). This morphology immediately separates it from any other known *Nannaria* and is more similar to the gonopod morphology of *Oenomaea pulchella*. The cyphopod receptacle of *Nannaria* n. sp. “Trimble” is triangular, while in *N. n. sp. “Augusta”*, it is an enlarged triangular hood that covers the cyphopod valves, and is quadrate and distally recurved in *N. n. sp. “Brandywine”*. *Nannaria* n. sp. “Augusta” is only known from the Big Levels-Maple Flats region in southern Augusta County, an area with many sinkhole ponds that are known to harbor rare and disjunct populations of a number of plant and animal species (Buhlmann et al., 1999).

The second clade in the Central Appalachians species cluster contains only *Nannaria morrisoni*, with all branches highly supported. This species has a bifurcate acropodite and is restricted mainly to the Blue Ridge Mountains on the eastern edge of the Shenandoah Valley, though it is also known from one site in the Shenandoah Valley between Massanutten Mountain and the Blue Ridge Mountains and from another locality near Charlottesville, Virginia. The cyphopod receptacle of *N. morrisoni* is laminate and leaf-like, a shape unlike any other known *wilsoni* group species.

The third clade includes *Nannaria shenandoa* and *Nannaria* n. sp. “Sanctimontis”. Both these species have bifurcate acropodites and have similar gonopod morphologies, but *Nannaria*

shenandoa has a gradually curving acropodite, while *Nannaria* n. sp. “Sanctimontis” has a straight acropodite with an abrupt bend distally. The cyphopod receptacle in *Nannaria shenandoa* is an enlarged triangular hood, while in *N. n. sp. “Sanctimontis”*, it is simply triangular. *Nannaria shenandoa* has the largest distribution of any *wilsoni* group species, extending from eastern Kentucky through West Virginia and Virginia. One male specimen (MPE03104) was found at Natural Bridge State Resort Park in Powell County, Kentucky, greatly extending its known range by approximately 300 km west of the closest locality for the species. This specimen matches the morphology observed in other specimens of this species, and has a well-supported branch placing it in the *N. shenandoa* species clade. *Nannaria* n. sp. “Sanctimontis” is restricted mainly to the Blue Ridge Mountains near Waynesboro, Virginia south to the vicinity of Lexington. Its gonopod morphology is very similar to that of *Nannaria* n. sp. “Nubiterrae”, but differs mainly in having a more acute twist at the anterior bend of the acropodite. *Nannaria* n. sp. “Sanctimontis” is the only species of the Central Appalachian Mountains cluster that has an acute twist on the acropodite, all the other species either lack a twist or have a smoothly-undulating helical twist. The two species fall out far from each other genetically, but *Nannaria* n. sp. “Sanctimontis” has poor branch support, and also has a specimen of *Nannaria* n. sp. “Trimble” falling out within its clade. Due to this poor branch support, however, *Nannaria* n. sp. “Trimble” is included in the first clade of the Central Appalachian Mountains cluster, in which it has high branch support and similar gonopod morphology to its sister species *Nannaria* n. sp. “Brandywine”.

The fourth clade contains one species, *Nannaria* n. sp. “Aenigma”. In Virginia, this is the only known *wilsoni* group species west of the New River and is quite abundant. Its gonopod morphology is similar to that of *Nannaria wilsoni*, *Nannaria* n. sp. “BigStonyCreek”, and

Nannaria n. sp. “Pinnacles”, but differs from these species by having a shorter prefemoral process, a lobed acropodite medial flange, and lacking an acropodite tip lateral flange and basal triangular spine. *Nannaria* n. sp. “Aenigma” has a triangular cyphopod receptacle. Its distribution occupies an area from the west bank of the New River west to the vicinity of Abingdon, Virginia. Its eastern distribution is limited by the New River, as it has not been collected east of this river, and none of the *wilsoni* group species that occur to the east of the river (*N. wilsoni*, *N. ericacea*, *N. n. sp. “Pinnacles”*) have been found west of the river. Therefore, the New River acts as a geographical barrier for these species, and has resulted in the vicariance of these species.

The fifth clade includes three species: *Nannaria wilsoni*, *Nannaria ericacea*, and *Nannaria* n. sp. “PottsMountain”. The branch support values in this clade are poorly supported, except for many of the *Nannaria ericacea* specimens, therefore gonopod morphology was used to delineate species in this clade. *Nannaria wilsoni* occupies a small area of three counties: Giles, Montgomery, and Roanoke Counties in southwest Virginia. Its gonopod morphology is very similar to *Nannaria* n. sp. “Lutra” and *Nannaria* n. sp. “Pinnacles”, but interestingly, is not closely related to them. It is separated from these species by having its acropodite medial flange large and laminate and lacking the dorsal dome-shaped tubercle near this flange that is present in *Nannaria* n. sp. “Lutra”. It is more closely related genetically to *Nannaria* n. sp. “PottsMountain”, which has a similar gonopod morphology (but this species lacks the stronger twist in the anterior bend and larger, laminate acropodite medial flange) and *Nannaria ericacea*, which has a very different gonopod morphology, particularly in its acropodite, which is straight with an abrupt bend distally. *Nannaria* n. sp. “PottsMountain” is known only from its type locality of Potts Mountain in Craig County, Virginia, and also falls out in clade seven of the

Central Appalachians cluster, but this is likely due to long branch attraction. *Nannaria ericacea* is the most widespread of the three species in this clade, occurring from the vicinity of Blacksburg, Virginia north to Bath County, Virginia. Both *Nannaria wilsoni* and *N. n. sp. "PottsMountain"* have cyphopod receptacles in the shape of enlarged triangular hoods that cover the cyphopod valves, while *N. ericacea* has a triangular receptacle not enlarged into a hood.

The sixth clade includes one species, *Nannaria n. sp. "Nubiterrae"*. This species is only known from Alleghany County, Virginia in the vicinity of Clifton Forge within the Alleghany Mountains region. As previously mentioned, its gonopod morphology is very similar to that of *Nannaria n. sp. "Sanctimontis"*, but is described as a distinct species due to the high bootstrap support of its clade in the molecular phylogeny. Its cyphopod receptacle is in the shape of an enlarged triangular hood that covers the cyphopod valves.

The seventh clade includes two species, *Nannaria n. sp. "BigStonyCreek"* and *Nannaria n. sp. "PottsMountain"*, as well as a specimen of the *Nannaria minor* species group, *Nannaria n. sp. "Lambsburg"*. This is likely due to long branch attraction from *Nannaria n. sp. "Lambsburg"*, and none of the branches in the clade are well-supported, therefore these species are evaluated based on their gonopod morphology and are placed in other clades.

The eight clade includes three species: *Nannaria n. sp. "BigStonyCreek"*, *Nannaria n. sp. "Lutra"*, and *Nannaria n. sp. "Pinnacles"*. *Nannaria n. sp. "BigStonyCreek"* does not have high support values in this clade, but based on its gonopod morphology, is similar to the other two included species, differing from these in having a thinner acropodite tip, the presence of a dorsal triangular tubercle near its acropodite medial flange, the presence of a thicker and shorter acropodite tip lateral flange, and the presence of a triangular spine medially at the base of its prefemoral process. *Nannaria n. sp. "BigStonyCreek"* is known only from a small area of Giles

County, Virginia, and Greenbrier and Monroe Counties in adjacent West Virginia. Both *Nannaria* n. sp. “Lutra” and *N. n. sp. “Pinnacles”* are known from the Blue Ridge Mountains of Virginia. *Nannaria* n. sp. “Lutra” lives in the mountains bounded in the north by the James River and in the south by the Roanoke River, while *N. n. sp. “Pinnacles”* is found in the Blue Ridge Mountains of the south and has its distribution limited by the transition to the Piedmont region of Virginia. The two species can be distinguished by the presence of the dorsal dome-shaped tubercle near the acropodite medial flange in *Nannaria* n. sp. “Lutra” (which *Nannaria* n. sp. “Pinnacles” lacks) and the presence of large triangular tubercles near the acropodite tip in *Nannaria* n. sp. “Lutra” (which are smaller, rounded bumps in *Nannaria* n. sp. “Pinnacles”). All three species in this clade have triangular cyphopod receptacles.

No specimens of *Nannaria* n. sp. “LongSpringRun” suitable for genetic analyses were collected, as all available specimens were preserved in alcohol for at least 30 years. Unfortunately, multiple collecting trips to the type locality did not result in the discovery of any additional specimens. However, I hypothesize that when the genetics of freshly-collected specimens are analyzed, this species will be most similar to the species in clade eight. Its most likely sister species appears to be *Nannaria* n. sp. “BigStonyCreek”. I base this on its similar gonopod morphology, as *N. n. sp. “LongSpringRun”* has a small but lobed acropodite medial flange and a large, dorsal triangular projection near this flange. These structures are similar to those observed in *Nannaria* n. sp. “BigStonyCreek”, and may represent homologous structures. No female specimens of *Nannaria* n. sp. “LongSpringRun” are known, therefore the characteristics of the cyphopods are unknown.

Southern Appalachian Mountains Species Cluster

In the Southern Appalachian Mountains cluster, 9 species of the *wilsoni* group are known, and 7 of those species are newly described. In this cluster, all but one species have a straight acropodite with an abrupt bend distally, and only *Nannaria* n. sp. “Juglandis” is different, having an acropodite bent at its midpoint. The acropodite anterior bend twist is typically acutely twisted and appearing almost crimped or pinched, except in *Nannaria* n. sp. “HarrisCove” and *N. n. sp. “Juglandis”*, in which the twist is in the shape of a smoothly-undulating helix. The acute, crimped twists of the acropodite anterior bend are most common in the Southern Appalachian Mountains species cluster, and are only known from one species in the Central Appalachians, *Nannaria* n. sp. “Sanctimontis”. The acropodite tip is mostly smooth and entire, though it is bifurcated in a few species. The cyphopod receptacle is typically modified in this cluster, rather than being small and triangular. The most interesting modification is observed in *Nannaria* n. sp. “Coweeta” and *N. n. sp. “HarrisCove”*, in which the receptacle is modified into a long, finger-like structure that projects outside of the cyphopod aperture and can be observed without dissecting the millipede. Species from the Southern Appalachian Mountains cluster form eight clades in the phylogeny. The higher level relationships among these clades are not well-supported, nor are most of the species clades. Due to the low support values, the morphology of the gonopods was used to assess species boundaries and formed the basis of the species hypotheses.

The first clade in the Southern Appalachian Mountains species cluster contains one species, *Nannaria* n. sp. “Juglandis”. As previously discussed, *Nannaria* n. sp. “Juglandis” is sister to all other species in the *wilsoni* species group, with gonopod morphology that is divergent from the other *wilsoni* group species. Its cyphopod receptacle is shaped like an oblong rounded capsule, and no other *wilsoni* group species shares this morphology. This species is

known from a small area in the southern Blue Ridge Mountains in Buncombe and Rutherford Counties, North Carolina and adjacent Greenville County, South Carolina. It is bounded on the eastern edge of its distribution by the transition to the Piedmont physiographic region of the Carolinas. On the western edge of its distribution, it may be sympatric with *Nannaria scutellaria*.

The second clade also includes only one species, *Nannaria* n. sp. “PucPuggy”. This species is quite similar in gonopod morphology to *Nannaria austriicola*, but differs in lacking the lobed acropodite medial tip flange that *N. austriicola* has, and has a smoother curve between the anterior bend twist and the tip of the acropodite. Additionally, the prefemoral process in *Nannaria* n. sp. “PucPuggy” has a notch about halfway up its length at which it abruptly becomes much thinner. The cyphopod receptacle of *Nannaria* n. sp. “PucPuggy” is simple and triangular. This species is known from only a few localities in Macon County, North Carolina and Rabun County, Georgia.

The third clade includes two species: *Nannaria austriicola* and *N.* n. sp. “Dell”. *Nannaria austriicola* occupies a medium-sized range in the southern Blue Ridge Mountains in Macon County, North Carolina and Rabun County, Georgia. Its acropodite has a crimped look at its anterior bend, and its acropodite tip is wavy and has a prominent lobe at its tip medial flange. Its cyphopod receptacle is simple and triangular. *Nannaria* n. sp. “Dell”, on the other hand, is known only from its type locality of Warwoman Dell Recreation Area near Clayton, Rabun County, Georgia in the Warwoman Shear fault zone. Its gonopods are distinct from others in the Southern Appalachians cluster, with a thick acropodite terminating in a bifurcation due to a shallow indentation distally, giving it a unicorn-like appearance. The inner edge of the prefemoral process is jagged instead of smooth as soon in all other *Nannaria wilsoni* group species. No female specimens are known, so its cyphopod characters are unknown.

The fourth clade includes one species, *Nannaria scutellaria*. This species is separated into two clades (four and six in the Southern Appalachians cluster) that are not closely related and do not have high bootstrap support. To avoid establishing new species hypotheses based on inconclusive genetic evidence that may later be revealed to be synonymous with *N. scutellaria*, I am including under *N. scutellaria* many specimens with variable gonopod morphology. This somewhat follows the precedent set by Causey, who showed that this species has large variation in its gonopods, even between specimens collected from the same locality (Causey, 1950b). Specimens ascribed to this species typically have a tooth-like acropodite medial flange, a triangular acropodite tip medial flange, and a prefemoral process that is quite wide basally. The gonopod characters may vary in the size of the medial flange, the distance between the tip medial flange and acropodite tip, the size and position of the tip medial flange, the direction the acropodite tip is pointed, and the distal curve of the acropodite. In some specimens, the acropodite tip medial flange is absent. A specimen (NAN0369) collected near Nantahala, North Carolina even has the tip of the acropodite expanded and laminate, forming the shape of a distal cup. With such morphological variation, more specimens are needed for genetic analyses to determine if any cryptic species exist within *Nannaria scutellaria*. The specimens in clade four were collected in Transylvania and Clay Counties, North Carolina, at the southern edge of the species distribution in the Blue Ridge Mountains.

The fifth clade includes three species: *Nannaria* n. sp. “HarrisCove”, *Nannaria* n. sp. “Amicalola”, and *Nannaria* n. sp. “Coweeta”. Bootstrap support for this clade is low, except for a portion of the clade including *Nannaria* n. sp. “Coweeta” and some specimens of *Nannaria* n. sp. “HarrisCove”, which have bootstrap support greater than 70. *Nannaria* n. sp. “HarrisCove” is not cohesive genetically, but the morphology of the gonopods match among all the specimens,

with little appreciable variation. *Nannaria* n. sp. “HarrisCove” can be separated from the other species in this clade by the presence of an acropodite medial tip flange in the shape of a sinuous lobe, best seen in posterior view (Fig. 3.32). This lobe gives the appearance of rabbit ears at the tip of the gonopod acropodite, easily separating this species from the erect, bifurcate acropodite of *Nannaria* n. sp. “Coweeta” and the acropodite of *N. n. sp. “Amicalola”*, which has a tooth-like medial flange which underhangs the distal portion of the acropodite. The cyphopod receptacles of both *Nannaria* n. sp. “HarrisCove” and *N. n. sp. “Coweeta”* are elongated, finger-like structures projecting beyond the cyphopod aperture, while that of *N. n. sp. “Amicalola”* is an enlarged triangular hood that covers the cyphopod valves. *Nannaria* n. sp. “HarrisCove” is limited to the Blue Ridge Mountains in Towns and Lumpkin Counties in northern Georgia. *Nannaria* n. sp. “Amicalola” is known from only three localities in Dawson and Lumpkin Counties, Georgia. It is the southernmost species in the *Nannaria wilsoni* species group, and its distribution may be limited to the southern extent of the Blue Ridge Mountains in northern Georgia. *Nannaria* n. sp. “Coweeta” is the most widespread species in this clade and is found in the southern Blue Ridge Mountains of Macon County, North Carolina south through Towns and Dawson County, Georgia.

The sixth clade includes specimens of *Nannaria scutellaria* from the more northern portion of its distribution. This includes a specimen (MPE01474) from the type locality at Chimneys Picnic Area in Great Smoky Mountains National Park, which matches closely with the gonopod morphology of the holotype (Causey, 1942). The other specimens of *Nannaria scutellaria* in this clade vary in the size and position of the acropodite medial flange tooth and in the curve of the acropodite tip. Overall, *Nannaria scutellaria* has the largest distribution of any of the Southern Appalachians cluster, and is found throughout Great Smoky Mountains National

Park and its periphery in southeast Tennessee and southwest North Carolina southward to the edge of the distribution of *Nannaria austriicola* in Macon County, North Carolina. With other specimens of this species falling into a separate clade (the fourth clade of the Southern Appalachians cluster), further genetic analyses from a wider array of specimens are desirable for determining whether or not *Nannaria scutellaria* is a cryptic species complex or simply one highly morphologically variable species. Within the *Nannaria wilsoni* species group, this species is by far the most highly variable in its gonopod morphology.

The seventh clade contains one species, *Nannaria* n. sp. “Cratagae”. It is separable from other *wilsoni* group species based on its straight acropodite with distal bend, acute twist at its anterior bend, and its doubly-lobed acropodite medial flange (best observed in medial view, Fig. 3.28). It is quite simple compared to other species in the group, lacking any acropodite tip flanges or other processes distally on the acropodite. Its cyphopod receptacle is a sinuously-curving structure with a blunt end, a character not observed in any other *Nannaria wilsoni* group species. This is the westernmost species of the group, and has been recorded from the Cumberland Plateau in Van Buren and Cumberland Counties in east-central Tennessee and in the Blue Ridge Mountains in Monroe County, Tennessee.

Much remains to be learned about the ecology of the *Nannaria wilsoni* species group, but its species are typically limited to mesic deciduous forests or rhododendron coves in the Appalachians, with few exceptions. The Blue Ridge Mountains are home to many of these species, as are the Alleghany Mountains in the more northern parts of their distribution. A fungal pathogen specializing on millipedes was recently found to infect the genus (Hodge et al, 2017), but little is known about its effects at a population level. More undescribed species in the genus surely await description, particularly in little-collected areas of northern Georgia, eastern

Tennessee and Kentucky, and eastern West Virginia. The introduction of the forest pest *Adelges tsugae* Annand, 1924, the hemlock wooly adelgid, (Hemiptera: Adelgidae), into the United States from Japan poses a threat to habitat commonly favored by *Nannaria* species. In some areas, this pest can kill up to 92% of hemlock trees (Rohr et al., 2009), which could greatly impact these millipedes if forest succession stages transition the formerly shady, cool hemlock forest microhabitats *Nannaria* prefers into drier habitats less amenable to their preferences. The small, highly endemic distributions of *Nannaria* are susceptible to habitat loss and fragmentation, a cause for concern as the hemlock wooly adelgid spreads and invades new areas of forest.

In this study, I targeted three main objectives: (1) test the monophyly of the genus *Nannaria* using molecular phylogenetics, (2) describe new species within the *Nannaria wilsoni* species group, and (3) investigate the ecology of the genus. The genus *Nannaria* was found to be monophyletic, but has two large clades with species that are morphologically, genetically, and biogeographically separable from each other, and may be different genera. I described 18 new species in the *Nannaria wilsoni* species group, quadrupling the number of known species from 6 to 24. Based on numerous collecting events and data from museum collections, I discovered that individual *Nannaria* tend to prefer riparian habitats in rhododendron-hemlock cove forests and mesic deciduous forests in North America. The *Nannaria wilsoni* species group in particular prefer mesic forests of the Appalachian Mountains, and are more fossorial in their habits, being found buried in the soil or at the soil-leaf litter interface. The discovery of so much additional species diversity in the *Nannaria wilsoni* species group and in the *Nannaria minor* species group reveals the Nannariini to be one of the most successful species radiations within the Xystodesmidae, rivaling the species diversity of the Apheloriini. This may be due in part to the

highly endemic habits of *Nannaria* and repeated vicariance of their distributions by the physiographic history of the Appalachian Mountains in eastern North America.

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Chapter 4 - Integrative taxonomy of the North American pink flat-backed millipedes

(Polydesmidae: *Pseudopolydesmus* Attems, 1898)

4.1 Abstract

The genus *Pseudopolydesmus* Attems, 1898 is the largest and most wide-ranging polydesmid genus in North America. Eight species have been delimited based on the morphology of the genitalia, but it has not been investigated genetically. Here, we take an integrated taxonomic approach, using five genes to infer a molecular phylogeny of all *Pseudopolydesmus* species, and analyzing a dataset of 15 morphological characters in an effort to objectively delimit species in the genus. 1,148 *Pseudopolydesmus* specimens were collected for this study, and all known species were collected. These specimens were used for genetic analysis, and were combined with museum specimens and literature records into a database of 3,462 unique specimen records. This database was used to produce distribution maps for each species in the genus. The maximum likelihood phylogeny inferred by RAxML found support for eight species in the genus, supporting previous morphological hypotheses. However, previous morphology-based hypotheses regarding relationships between the species were not supported by the analysis. A species tree inferred by *BEAST did not find the same relationships as the RAxML-inferred phylogeny, but the *BEAST tree had low ESS values. The morphological analysis, performed with a modified PCA, failed to delimit species clusters as well as genitalic and genetic differences did, due to comparatively uniform somatic morphology in the genus. However, our results show that genitalic morphology, combined with genetic analysis, provide congruent species delimitation hypotheses for *Pseudopolydesmus*, and these lines of evidence may prove useful in delimiting species and generic boundaries in other Polydesmidae worldwide.

4.2 Introduction

The millipede genus *Pseudopolydesmus* (Attems, 1898) is one of the most commonly-encountered millipedes in eastern North America. With eight species, the genus covers a large geographic area (Fig. 4.1) from North Dakota south through Texas, east to Florida, and north to Québec. They are typically pink or brown in color, but one species, *P. canadensis* (Newport, 1844), often has deep red paranota (Fig. 4.2). *Pseudopolydesmus* species range between 7 – 32 mm in length, and are found under logs and in leaf litter in deciduous forests (Withrow 1988).

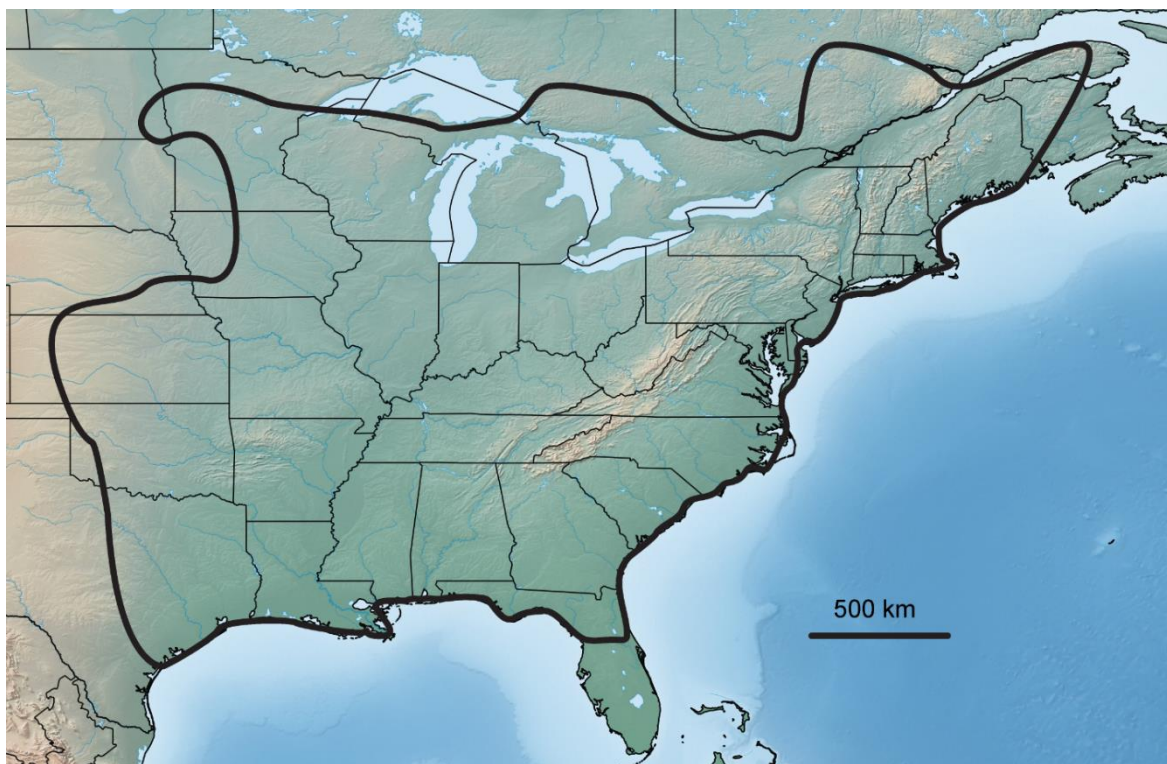


Figure 4.9. Distribution of the genus *Pseudopolydesmus* in eastern North America, outlined in black.

A historical review of the genus, synthesizing available taxonomic information up to 2019, has recently been provided by Sierwald et al. (2019); this provided a key and lists of synonymies for each species, photographs and scanning electron micrographs of the gonopods of each species, and comments on morphological terminology in the genus. Hence, only a brief summary of the taxonomic history of the genus is given here. Eight species of *Pseudopolydesmus* are currently recognized, based purely on morphological characters: *Pseudopolydesmus serratus* (Say, 1821), *P. canadensis*, *P. minor* (Bollman, 1888), *P. pinetorum* (Bollman, 1888), *P. erasus* (Loomis, 1943), *P. caddo* Chamberlin, 1949, *P. paludicolus* Hoffman, 1950, and *P. collinus* Hoffman, 1974. The first species to be described, *P. serratus*, was placed in the genus *Polydesmus* Latreille, 1802, and was collected on the eastern shore of Virginia (Sierwald et al. 2019). Most names in the genus were described under the genera *Polydesmus* or *Dixidesmus* Chamberlin, 1943 (the latter now recognized as a junior subjective synonym of *Pseudopolydesmus*), and 32 species names have been ascribed to *Pseudopolydesmus*, with 18 of those names coined by Chamberlin. Most work on *Pseudopolydesmus* consisted of short taxonomic descriptions of a single species or a few species. Hoffman (1974) established a useful terminology for the gonopod processes in his description of *P. collinus*, which was subsequently adopted by taxonomists working on the genus. An unpublished Ph.D. dissertation by Withrow (1988) summarized the distribution of the genus, was the first work to provide a key to species, provided ecological information, and hypothesized relationships within the genus and with other Polydesmidae. However, it was never published, limiting its usefulness, and preventing its changes to the classification.



Figure 4.2. General habitus of *Pseudopolydesmus canadensis*, dorsal view. Male specimen MPE02435 from Craig Co., Virginia.

Sporadic interest in the genus during the early 21st century provided useful information on its natural history from laboratory observations (Youngsteadt 2009), and a litany of piecemeal distributional records from across its range (Shelley & Snyder 2012). The review by Sierwald et al. (2019) postulated species concepts within the genus and provided clear morphological images for species identification. This resolved confusion that has continually trailed the genus since the description of its first species. Most recently, the genital morphology of female *Pseudopolydesmus* millipedes and the hypothesized mechanics of mating in the genus were investigated by Zahnle et al. (2020). This investigation augmented our understanding of a poorly understood area of study in millipedes.

Despite these advances, phylogenetic relationships within the genus *Pseudopolydesmus* and the family Polydesmidae remain unclear. The family is mainly Holarctic, with about 30 described genera and over 240 species (Enghoff et al. 2015). The group is not well-delimited

taxonomically, never has been analyzed with molecular phylogenetics, and has been described as a wastebasket (Shear 2012) due to large species-rich genera such as *Polydesmus* and *Epanerchodus* Attems, 1901; these genera have many ill-defined species and subgenera. A subfamilial classification was proposed by Withrow (1988) and referenced by later workers (Simonsen 1990; Golovatch 1991), but has not been widely adopted (Shelley 1996a). Much synthetic work remains to be done—preferably driven by molecular phylogenetics—particularly with the European and Asian genera. While historically challenging, the genera of Polydesmidae present an excellent opportunity for using molecular phylogenetics to bring order to the historical chaos of the genus.

In North America, the taxonomy of the family has received more recent work, with seven precinctive genera: *Pseudopolydesmus*, *Scytonotus* Koch, 1847, *Bidentogon* Buckett & Gardner, 1968, *Calianotus* Shelley, 1997, *Retrorsia* Shelley, 2003, *Snoqualmia* Shear, 2012, and *Utadesmus* Chamberlin & Hoffman, 1950. There are two introduced genera: *Polydesmus* and *Brachydesmus* Heller, 1858. Shear (2012) and Shear & Reddell (2017) provided keys to the genera. The native North American genera are comparably well-delineated and have clear illustrations of their morphology, allowing for confident species identification (Chamberlin 1930; Chamberlin & Hoffman 1950; Buckett & Gardner 1968; Shelley 1993, 1997, 2003; Shear 2012). Little is known about the ecology of these genera, and most information on them is taxonomic and distributional in nature, but *Scytonotus* has been revised (Shelley 1993); this genus is the only native polydesmid genus found in both western and eastern North America. New genera and species from western North America await description (Shear & Reddell 2017), presenting another opportunity for the use of molecular phylogenetics to clarify intergeneric relationships.

Within *Pseudopolydesmus*, hypothesized relationships between species have been based on morphological characters, especially those of the gonopods (Withrow 1988). Two main species groups have been proposed: the *canadensis* group and the *serratus* group. The *canadensis* group is composed of *Pseudopolydesmus canadensis*, *P. collinus*, *P. erasus*, and *P. pinetorum*, while the *serratus* group contains *Pseudopolydesmus serratus*, *P. caddo*, *P. minor*, and *P. paludicolus*. Withrow summarized his hypothesized relationships as follows: ((*pinetorum*, ((*tallulanus*, *erasus*), (*canadensis*, *collinus*))), ((*serratus*, *paludicolus*), (*minor*, *caddo*))) (1988, his figure 121). *Pseudopolydesmus tallulanus* (Chamberlin, 1943) was determined to be a junior subjective synonym of *P. erasus* by Sierwald et al. (2019). These relationships have not been tested by molecular phylogenetics, and remain the sole hypothesis for species delimitation within the genus.

Species delimitation in millipedes has historically placed sole emphasis on gonopod morphology, but genetic investigations have revealed cryptic species complexes in juliformian taxa with uniform gonopod morphology (Bond & Sierwald 2002, Walker et al. 2009, Mwabvu et al. 2013, 2015). Within the Polydesmidae, a proliferation of inconsistently-applied terms for the various processes of the gonopods has emerged, causing difficulties in direct comparison of species. As a result, homologies of the gonopod structures are uncertain, challenging to efficiently compare, despite efforts to standardize the terminology. The polydesmid gonopod ranges from a relatively simple, singly-branched structure with a few triangular or quadrate processes (such as in *Pseudopolydesmus* or *Pacidesmus* Golovatch, 1991) to a complex, multi-branched structure with many differently-shaped processes (such as in *Snoqualmia*). A few terms, such as coxa and cannula, have been consistently applied throughout the family. Other terms, such as endomerite and acropodite, are not as consistently applied. The acropodite, for

example, has also been called the tibiotarsus (Shelley, 2003), endomere (Anh, 2009), and distofemoral part (Djursvoll, 2008) Thus, the current suite of descriptive terms is large and open to interpretation, and species delimitation and description within the Polydesmidae (and within the suborder Polydesmidea) remains subjective.

The use of objective and repeatable methods for species delimitation provides a path for solving this problem. Recent studies have highlighted the usefulness of genetic data for species delimitation and identification, with the cytochrome c oxidase subunit I (COI) gene proving useful for identifying polydesmid species (Spelda et al. 2011; Angyal et al. 2017). A modified principal components analysis (PCA) for species delimitation based on morphological measurements has recently been developed (Ezard et al. 2010) and used in an integrative framework with genetic data to revise the Opiliones genus *Sclerobunus* in western North America (Derkarabetian & Hedin 2014). Like millipedes, Opiliones are low-vagility organisms, not able to fly or disperse widely, and with many species distributions corresponding to the physiography of their region.

In this study, my aim is to delimit species of *Pseudopolydesmus* in a framework integrating molecular phylogenetics and morphology. Here, I test the monophyly of *Pseudopolydesmus*, provide distributions for each species, photographs of living individuals, and ecological information for each species. Useful morphological characters for delimiting species in the genus, and possible characters for use throughout the family are also discussed.

4.3 Materials and Methods

Specimens of *Pseudopolydesmus* were collected in 20 states in the eastern United States from 2013-2019, according to methods outlined in Means et al. (2015). Mesic deciduous forests

were the primary habitats targeted for field collection, and specimens were searched for under leaf litter and logs. Each putative species in the genus was targeted for recollecting, and historical holotype localities were visited when possible. Old notes from Richard Hoffman were examined for information on any possible new species in the genus, and one potential new species was noted by Hoffman from southwest Virginia. This possible new species was also targeted during field collecting. Specimens were collected throughout the range of each species in order to obtain enough specimens for population analyses.

After field collection, specimens were kept alive for processing in the lab. When living specimens were returned to the lab, representative specimens were photographed with a Canon 6D dSLR camera with a 50 mm or 65 mm Canon MP-E macro lens. The 50 mm lens was used for medium to large-sized individuals, while the 65 mm lens was used for small individuals. Specimens were subsequently given a catalog number beginning with the prefix MPE, and 24 legs were removed from the right side of the body and preserved in 100% ethanol or RNAlater (Qiagen) for molecular analysis, while the rest of the body was preserved in 70% isopropanol for morphological analysis and deposited in the Virginia Tech Insect Collection (abbreviation VTEC, <https://www.collection.ento.vt.edu>).

In addition to field-collected specimens, museum material and literature records were also examined. Museum specimens were borrowed from the Virginia Museum of Natural History (VMNH, Martinsville, Virginia), the North Carolina Museum of Natural Sciences (NCSM, Raleigh, North Carolina), and the Illinois Natural History Survey (INHS, Champaign, Illinois). Specimens from the VMNH and NCSM were georeferenced with the program GEOLocate (<http://www.museum.tulane.edu/geolocate/web/WebGeoref.aspx>), and databased with catalog numbers beginning with the prefix PSE (e.g. PSE0001). Additional specimen

records were downloaded from the Symbiota Collections of Arthropods Network (SCAN) database (<https://scan-bugs.org/>). Literature records from published works on *Pseudopolydesmus* (Shelley & Snyder 2012, Withrow 1988) were digitized, georeferenced as above, and included with museum records into a distribution database of the genus. Distribution maps for each species were produced with the website SimpleMappr (Shorthouse, 2010).

Field-collected specimens were processed for molecular phylogenetics according to previously published methods (Means & Marek, 2017). Outgroup taxa were selected from within the Polydesmidae and from additional families in the suborder Polydesmidea. Six genera from the family Polydesmidae were included: ten species of *Epanerchodus*, 3 species of *Polydesmus*, 1 species of *Brachydesmus*, 2 species of *Schizoturanus* Verhoeff, 1931, 1 species of *Scytonotus*, and 1 unknown genus and species of Polydesmidae. Five families from the suborder Polydesmidea were included: 4 species of Macrosternodesmidae, 2 species of Opisotretidae, 2 species of Cryptodesmidae, 2 species of Haplodesmidae, and 1 species of Pyrgodesmidae. An unidentified species from the suborder Dalodesmidea, family Dalodesmidae was used to root the phylogeny. Genomic DNA from 4-6 legs was extracted and purified with a Qiagen DNeasy kit, according to instructions of the manufacturer. DNA was amplified with polymerase chain reaction, and bands visualized with an agarose gel to confirm product amplification. Amplified DNA was cleaned, concentrations quantified and normalized, and then sequenced with an Applied Biosystems 3730 DNA Analyzer at the University of Arizona Genetics Core. Five gene regions were sequenced: two mitochondrial genes, cytochrome c oxidase subunit I (COI) and large subunit ribosomal RNA (16S); and three nuclear genes: 28S ribosomal RNA (28S), elongation factor-1 alpha (EF1-a), and Enolase, with amplification protocols found in Means & Marek (2017). Enolase was chosen due to its successful use in Coleoptera phylogenetics (Wild &

Maddison, 2008). The 16S primer set used for other Polydesmida were not consistently successful for *Pseudopolydesmus*, and a primer set for Enolase in Diplopoda was not available, therefore primer sets for each gene were designed. This was accomplished by using a published transcriptome of *Pseudopolydesmus* (Rodriguez et al., 2018) and performing a local nucleotide BLAST search in the program Geneious to identify the 16S and Enolase gene regions. Primers were then developed for these regions. For 16S, a new primer set was designed (both listed in 5' to 3' orientation): a forward primer J-P16S (CTTTTCTCGAATAATTTTCGGGCTAA) and a reverse primer N-P16S (CGCTGTTATCCCTACAGTAGTT). Polymerase chain reaction procedures followed that of Means & Marek (2017) for 12S - 16S (5' end), with an annealing temperature of 52 °C for 30 seconds. For Enolase, the following primer pair was designed: LR-J-PseEno (GCTGTTCCGAGTGGCGCTTC) and SR-N-PseEno (GGCACACTTGCCGTGAAAGC), with an annealing temperature of 53 °C for 30 seconds. This annealing temperature was determined after calculating an estimated melting temperature of the primers and empirically determining the optimal annealing temperature by using a gradient block to test temperature regimes between 51°C and 61°C.

Chromatograms were trimmed, bases called, and overlapping fragments assembled into contiguous sequences with the program Mesquite version 3.6 using phred and phrap in the Chromaseq version 1.51 module (Maddison and Maddison, 2010; Ewing et al., 1998). The program Prank version 170427 (Löytynoja and Goldman, 2005) was used for sequence alignment using the default gap opening and extension probabilities. Aligned gene sequences were subsequently partitioned by gene, codon position, and intron and exon locations in Mesquite. Partitions were evaluated in PartitionFinder2 to determine a best-fit partitioning model and identify appropriate models of nucleotide evolution (Lanfear et al., 2017). A concatenated

matrix of the sequences was exported and used in the program RAxML version 8.2.12 (Stamatakis, 2014) to infer a phylogeny from 296 specimens using the GTRGAMMA model with the rapid bootstrap algorithm and 1,000 bootstraps. Single gene RAxML trees were inferred using the same methodology.

An integrative framework combining morphological and phylogenetic data for species delimitation in *Pseudopolydesmus* was used, according to the methods outlined in Derkarabetian & Hedin (2014, see their fig. 1). These methods involve using morphological data to delimit species clusters by using a modified PCA, and assessing these clusters with genetic data to subsequently use these clusters as *a priori* species hypotheses in a species tree, which is then validated with the program BPP. A series of 15 morphological characters were measured for males and females of each species (Appendix B) for analysis with a modified PCA described in Ezard et al. (2010): body length, head width, collum width, collum length, left 3rd antennomere length, gnathochilarium width, gnathochilarium length, clypeus width, interantennal space width, metazonite 6 width, metazonite 6 length, left leg 6 tarsus length, epiproct width, epiproct length, gonopod aperture width (males only), and cyphopod aperture width (females only). Specimens were chosen from across the distribution of each species, with preference given to specimens with all chosen characters intact. The selected specimens were chosen from collection localities that maximized geographic coverage for each species, with a maximum of 12 specimens used for each species, depending on available specimens. Analysis was done in R version 3.6.2 (Filzmoser et al., 2018; Filzmoser & Gschwandtner, 2018; Oksanen et al., 2019; R Core Team, 2019; Scrucca et al., 2016; Warnes et al., 2020), and the Ezard et al. (2010) protocol was implemented with a script written by Derkarabetian (2018). Species hypotheses informed by the RAxML-inferred phylogeny and the modified PCA results were then input into BEAST v2.6.2

(Bouckaert et al., 2019) using the *BEAST algorithm (Barido-Sottani et al., 2017, Heled & Drummond, 2010), and two species in the genus *Polydesmus* were used as outgroups to root the tree. Analyses were run for 350 million generations and logged every 10,000 generations. Resulting runs were assessed for stationarity and effective sample size (ESS) values above 200 with Tracer 1.5 (Rambaut et al., 2018). TreeAnnotator was used to reconstruct species trees from 350,000 trees (10% burnin).

4.4 Results

A total of 1,148 *Pseudopolydesmus* specimens were collected during field work in 20 states. All known species of *Pseudopolydesmus* were collected, and these specimens were the basis of the genetic analysis. The two most commonly collected species were *P. serratus* and *P. canadensis*, which are also the most widespread species in the genus. The new specimen records were included with 2,314 unique records from museum collections and literature sources into a Darwin Core standard database of 3,462 records covering all described *Pseudopolydesmus* species.

The phylogeny inferred by the RAxML analysis with all five genes is given in Fig. 4.3, and a pruned phylogeny including only the *Pseudopolydesmus* specimens is given in Fig. 4.4. Each species of *Pseudopolydesmus* had bootstrap support greater than or equal to 90, and higher relationships within the genus had bootstrap support of at least 77. Within each species clade, bootstrap support among specimens varied widely: almost all specimens in the *Pseudopolydesmus paludicolus*, *P. minor*, and *P. caddo* had highly supported branches over 70, while in the *P. serratus* clade, branch support ranged from 2 to 100. *Pseudopolydesmus pinetorum*, *P. erasus*, *P. collinus*, and *P. canadensis* also had widely variable branch support.

The most closely-related polydesmid genus to *Pseudopolydesmus* was found to be *Epanerchodus*, a species-rich east Asian genus, but had low bootstrap support of 41. Within *Pseudopolydesmus*, the species taxonomy of Sierwald et al. (2019) was supported, with each species clade having bootstrap support of over 70%. *Pseudopolydesmus paludicolus* and *P. serratus* were found to be sister species and formed a separate clade from the rest of the species. The other species formed two smaller clades. One, composed of *P. caddo*, *P. minor*, and *P. pinetorum* had *P. caddo* and *P. minor* as sister species, with *P. pinetorum* outside the *P. caddo* and *P. minor* clade. The second clade was composed of *P. erasus*, *P. canadensis*, and *P. collinus*. The species *P. canadensis* and *P. collinus* were found to be sister species, with *P. erasus* most closely related to them. Monophyly of *Pseudopolydesmus* had high branch support of 100. The included *Epanerchodus* species had branch support values ranging between 30 and 100, and the genus was not found to be monophyletic. *Epanerchodus* was split into two clades, one comprised of mostly species found in Japan and one with species found in China eastern Russia.

Outside of the *Pseudopolydesmus* and *Epanerchodus* clades was a well-supported clade (bootstrap value of 98) including species of *Polydesmus* and *Brachydesmus*. *Polydesmus inconstans* Latzel, 1883 and *P. angustus* Latzel, 1884 were found to be sister taxa, and these species were sister to *Brachydesmus furcatus* Lohmander, 1936 (all bootstrap values of 100). Sister to this clade was *Polydesmus vietnamicus* Anh, 2009 (bootstrap value of 68). Sister to this clade and the *Pseudopolydesmus* and *Epanerchodus* clade was an unidentified species of Polydesmidae from Japan (bootstrap value of 87). Sister to the rest of the Polydesmidae was a clade with *Scytonotus granulatus* (Say, 1821) sister to *Schizoturanius clavatipes* (Stuxberg, 1876) and *Schizoturanius tabescens* (Stuxberg, 1876) (all bootstrap values greater than or equal to 77).

The other Polydesmidea families included in the analysis had generally high support values within each family, but higher relationships among the families were not well supported. Macrosternodesmidae was the sister group to the Polydesmidae, but with a bootstrap value of 42, while bootstrap values between species in the family were 99 and 100. Macrosternodesmidae and Polydesmidae were sister to the rest of the Polydesmidea families, with bootstrap support of 43. Opisetretidae was sister to an unidentified species of *Peridontodesmus* Silvestri, 1896 in the Cryptodesmidae (bootstrap support of 75). This clade was sister to a clade of the Haplodesmidae and Pyrgodesmidae (bootstrap support of 40 and bootstrap support of 53 for Haplodesmidae + Pyrgodesmidae). An unidentified species of *Kiusiunum* Verhoeff, 1942 was found to be sister to the rest of these clades, therefore this analysis did not find the Cryptodesmidae to be monophyletic.

For the modified PCA, a total of 69 male *Pseudopolydesmus* specimens were measured for 15 characters. Six morphological clusters were identified, and two principal components were retained (Fig. 4.5). Visualization of the eigenvalues with a scree plot showed that the first principal component (body length) explained about 96% of the variance, while the second principal component explained only about 2% of the variance. The second component (and the other included measurements) is highly positively correlated with body length. The identified morphological clusters differed from the species clades identified by the genetic analysis, and species were subsequently separated using the genetic clades to formulate the *a priori* species hypotheses for the species tree analysis.

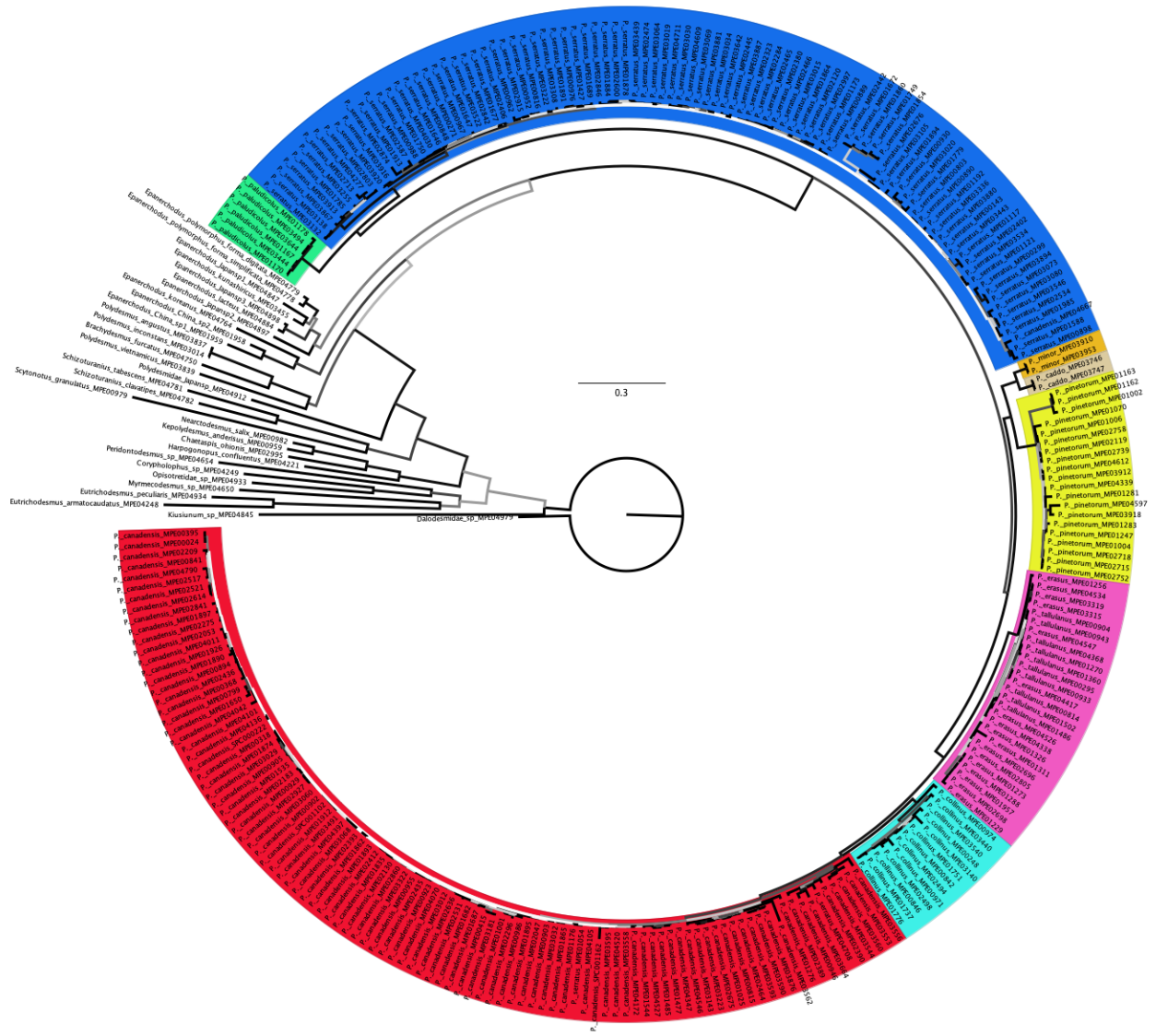


Figure 4.3. Phylogeny inferred by the RAxML analysis. Black branches indicate bootstrap support ≥ 70 . Highlighted areas indicate *Pseudopolydesmus* species: green, *P. paludicolus*; blue, *P. serratus*; gold, *P. minor*; tan, *P. caddo*; yellow, *P. pinetorum*; magenta, *P. erasus*; cyan, *P. collinus*; red, *P. canadensis*. Scale: 0.3 site substitutions.

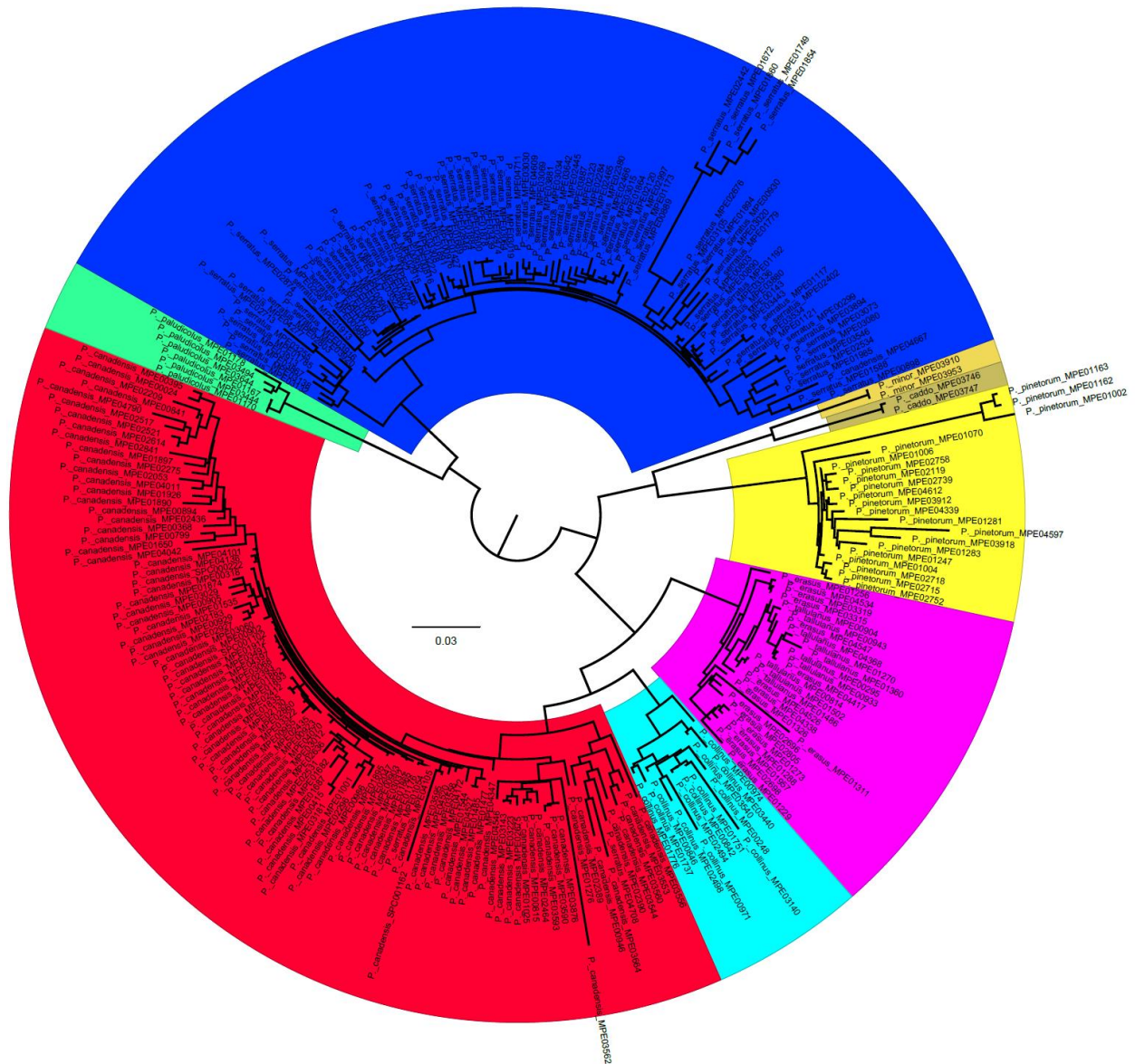


Figure 4.4. Phylogeny inferred by the RAxML analysis, only *Pseudopolydesmus* included. Highlighted areas indicate species: green, *P. paludicolus*; blue, *P. serratus*; gold, *P. minor*; tan, *P. caddo*; yellow, *P. pinetorum*; magenta, *P. erasus*; cyan, *P. collinus*; red, *P. canadensis*. Scale: 0.3 site substitutions.

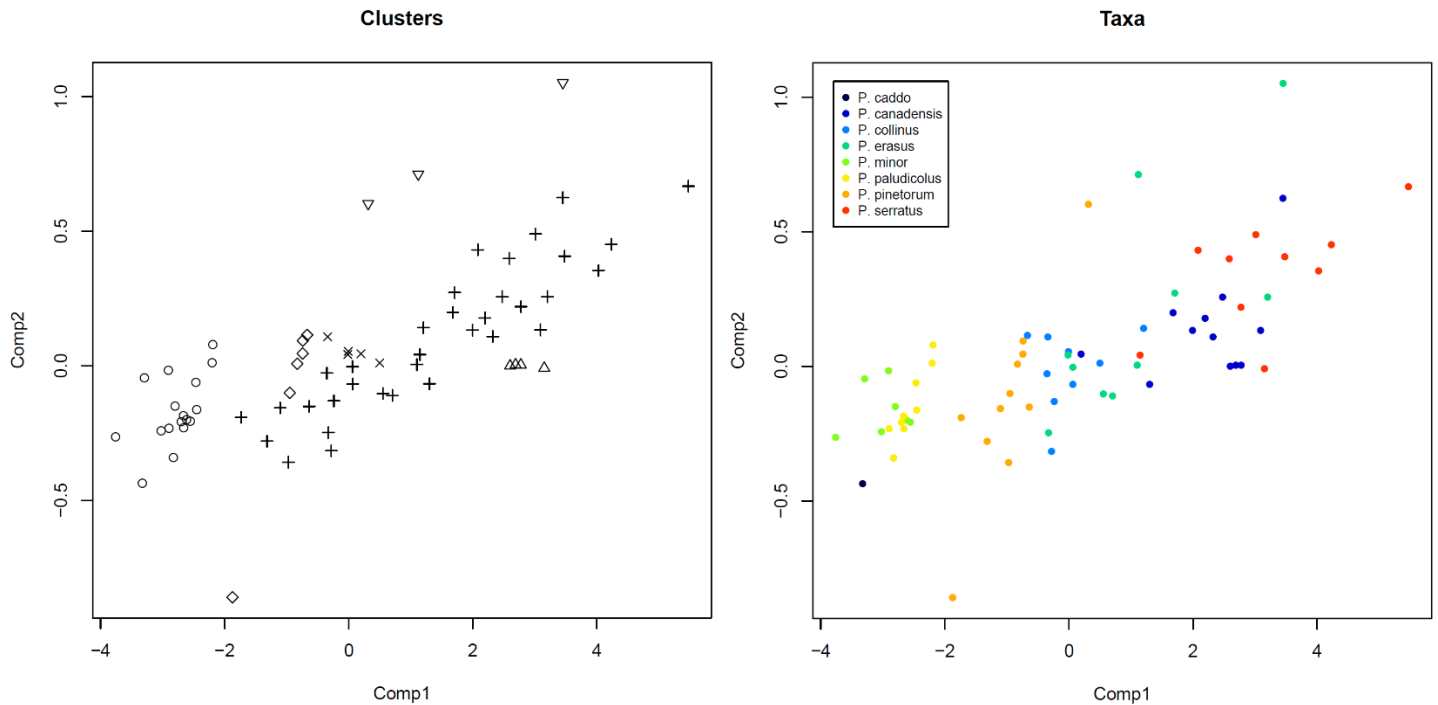


Figure 4.5. Multivariate clustering with PCA. Left panel: clusters identified by modified PCA. Right panel: *Pseudopolydesmus* specimens colored by species.

The species tree inferred by the *BEAST analysis is shown in Figure 4.6. The relationships in the *BEAST phylogeny differ from those in the RAxML-inferred phylogeny. In the *BEAST tree, *P. caddo* and *P. minor* are indicated as sister species with high support, and form a clade with *P. pinetorum*, but this clade is sister to all the other *Pseudopolydesmus* species. In this other clade, *P. paludicolus* is sister to the other *Pseudopolydesmus* species, and *P. erasus* is sister to the other three species. The species *P. collinus* is sister to a clade of *P. canadensis* and *P. serratus*, a relationship not found in the RAxML phylogeny. This may be a result of low support values in the *BEAST phylogeny, as ESS values for this phylogeny were very low, at 13. Within the phylogeny, only the relationship between *P. caddo* and *P. minor*, and *P. erasus* being sister to the (*P. collinus*, (*P. canadensis*, *P. serratus*)) clade are well-supported.

Due to the incongruence between the RAxML phylogeny and the *BEAST phylogeny, the relationships within the genus indicated by the RAxML phylogeny are followed in the Taxonomic Part of this study. This is due to the higher support values in the RAxML analysis, as well as its congruence with morphological characters.

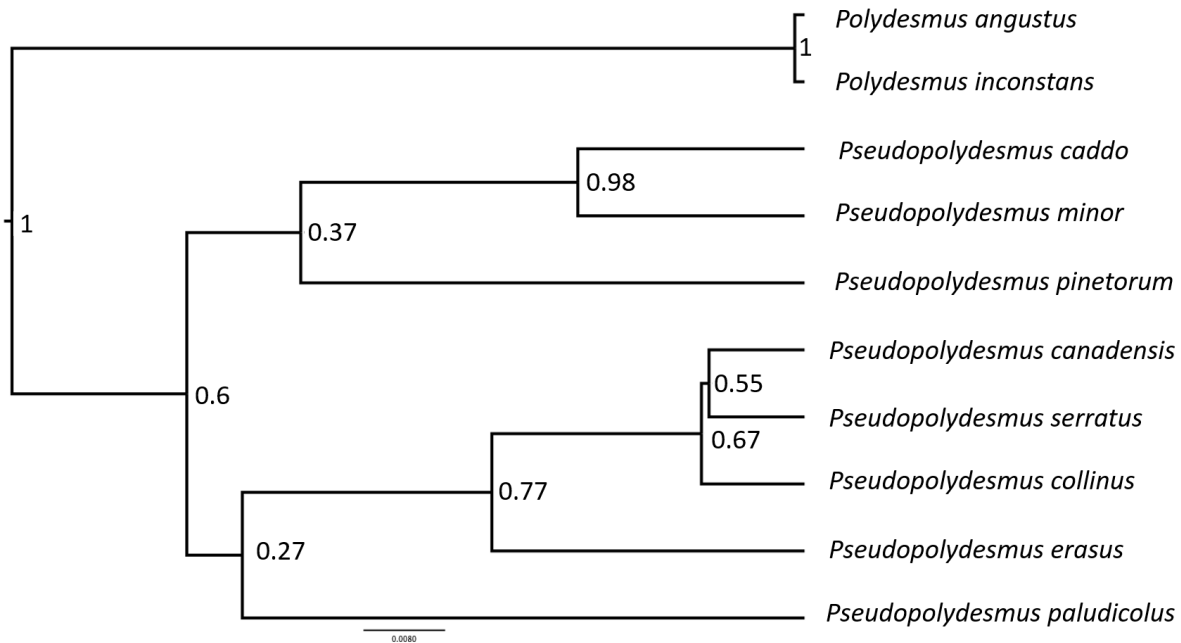


Figure 4.6. Species tree for *Pseudopolydesmus* inferred by *BEAST, with *Polydesmus* outgroups. Numbers at nodes indicate posterior probability support.

Taxonomic Part

Class Diplopoda de Blainville in Gervais, 1844

Order Polydesmida Pocock, 1887

Suborder Polydesmidea Leach, 1815

Family Polydesmidae Leach, 1815

Genus *Pseudopolydesmus* Attems, 1898

Type species: *Polydesmus canadensis* Newport, 1844.

Included species:

Pseudopolydesmus caddo Chamberlin, 1949
Pseudopolydesmus canadensis (Newport, 1844)
Pseudopolydesmus collinus Hoffman, 1974
Pseudopolydesmus erasus (Loomis, 1943)
Pseudopolydesmus minor (Bollman, 1888)
Pseudopolydesmus paludicolus Hoffman, 1950
Pseudopolydesmus pinetorum (Bollman, 1888)
Pseudopolydesmus serratus (Say, 1821)

Synonymies, morphological descriptions, gonopod images, and a key to the species of the genus can be found in Sierwald et al. (2019).

***Pseudopolydesmus caddo* Chamberlin, 1949**

Pseudopolydesmus bidens Loomis, 1959

Vernacular name: “The Caddo Marsh Flatback”

Figure 4.7, 8.

Diagnosis: Pulvillus pointed (Fig. 4.7). Lacking process *e1*, not present as in *P. erasus*; lacking process *e2*, not present as in all other *Pseudopolydesmus* species; process *e3* present and on a lamina, not absent as in *P. minor*; process *e4* absent, not present as in all other *Pseudopolydesmus* species; process *m1* fin-shaped, not triangular as in *P. minor*; process *m2* small, triangular, not large as in *P. minor*; process *m3* absent, not present as in *P. paludicolus*; process *m4* present, not absent as in *P. paludicolus*. Processes *m2* and *e3* connected by transverse ridge.



Figure 4.7. Left gonopod of *Pseudopolydesmus caddo* (MPE03746, St. Landry Parish, Louisiana), medial view (left) and ectal view (right).

Color: Color in life unknown, but preserved specimens dark maroon.

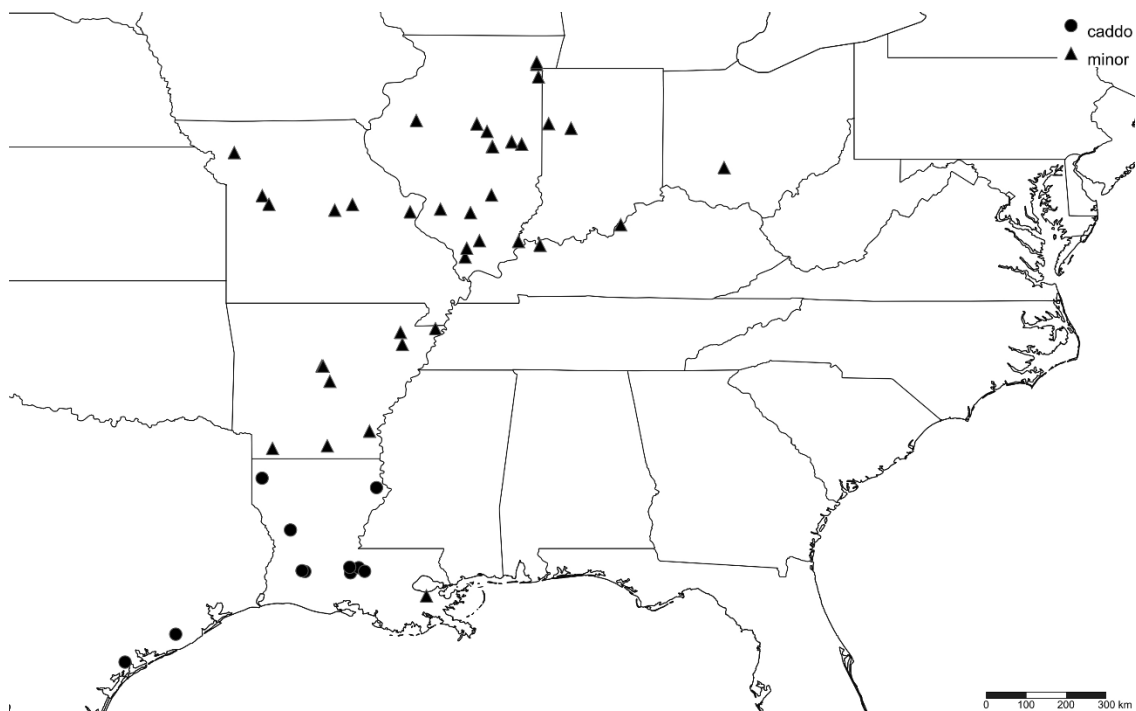


Figure 4.8. Distribution of *Pseudopolydesmus caddo* (dots) and *Pseudopolydesmus minor* (triangles) in the eastern United States.

Distribution: *Pseudopolydesmus caddo* is only known from Louisiana and eastern Texas (Fig. 4.8).

Ecology: *Pseudopolydesmus caddo* is one of the three species in the genus known from swamps and floodplains (the others being *P. minor* and *P. paludicolus*). Locality and habitat data indicate it is found in areas adjacent to streams, swamps, and rivers, and it has been collected on floating wooden debris in these habitats. This species is the most poorly-known in the genus, having only been collected from about a dozen localities. It has most often been collected during Spring and late Fall.

***Pseudopolydesmus canadensis* (Newport, 1844)**

Polydesmus glaucescens C. L. Koch, 1847
Polydesmus nitidus Bollman, 1887
Polydesmus branneri Bollman, 1887
Polydesmus echinogon Chamberlin, 1942
Polydesmus conlatus Chamberlin, 1943
Dixidesmus sylvicolens Chamberlin, 1943
Dixidesmus christianus Chamberlin, 1946
Dixidesmus catskillus Chamberlin, 1947
Dixidesmus phanus Chamberlin, 1951
Dixidesmus gausodicrorhachus Johnson, 1954

Vernacular name: “The Canadian Flatback”

Figures 4.2, 9-11.

Diagnosis: Pulvillus pointed (Fig. 4.9). Process *e1* present, abruptly bent basally, larger than in *P. collinus*; processes *e2* and *e3* present and connected by a basal stalk, *e2* not a single process as in *P. serratus*; process *e4* small, not absent as in *P. caddo*; processes *m1* and *m2* present, as in all *Pseudopolydesmus* species, quadrate and triangular, respectively; process *m3* present, triangular

and positioned near midline of acropodite, not absent as in *P. serratus*; process *m4* triangular, not absent as in *P. serratus*.



Figure 4.9. Left gonopod of *Pseudopolydesmus canadensis* (MPE00799, Craig County, Virginia), medial view (left) and ectal view (right).



Figure 4.10. *Pseudopolydesmus canadensis*, adult male from Ashe County, North Carolina (MPE04390).

Color: Specimens of *P. canadensis* most often have a broad longitudinal black dorsal stripe with brick-red paranota (Fig. 4.10), but the paranota color can sometimes be a lighter pink. Low-elevation populations in the Atlantic and Gulf Coastal Plains lack the black stripe and red paranota, and are instead light pink.

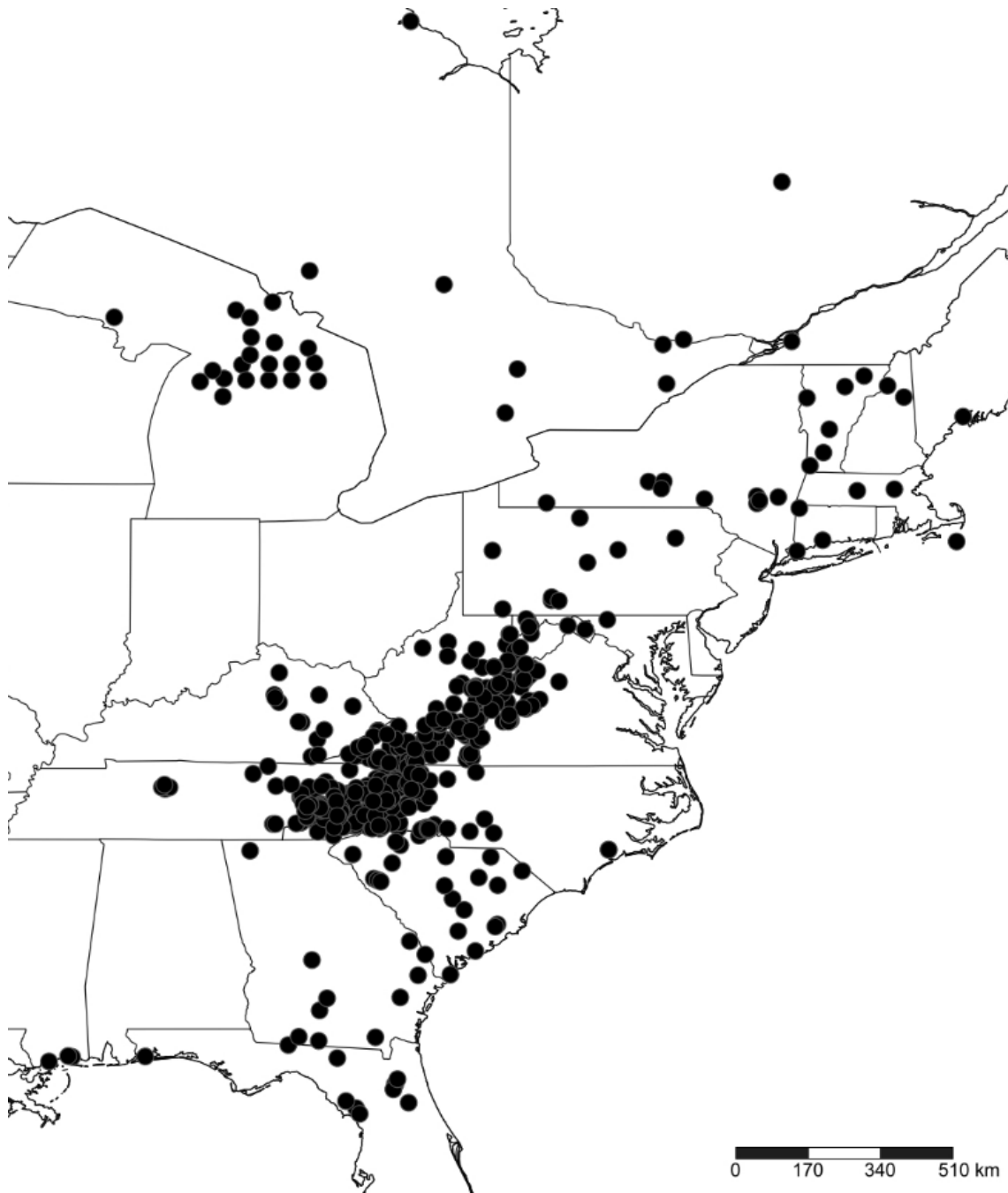


Figure 4.11. Distribution of *Pseudopolydesmus canadensis*.

Distribution: *Pseudopolydesmus canadensis* is one of the most widespread species in the genus, ranging from Québec and Ontario (Canada) south to Florida (Fig. 4.11). It is the most abundant *Pseudopolydesmus* species in Appalachia and the northern U.S. and Canada, but also ranges southward to the Atlantic and Gulf Coastal Plains. In this southern portion of its range, body size

is noticeably smaller and it lacks the bold coloration seen in the rest of its distribution.

Individuals in the Coastal Plain areas have light pink tergites and no contrastingly-colored paranota.

Ecology: This species has been found in a wide range of habitats, from generalized deciduous forests, to hemlock (*Tsuga* spp.) and rhododendron (*Rhododendron* spp.) cove forests, and sandy pine (*Pinus* spp.) forests. Label data indicate it has been collected in deciduous forests of oak (*Quercus* spp.), hickory (*Carya* spp.), maple (*Acer* spp.), and beech (*Fagus grandifolia*). It has been collected at near sea level in the Coastal Plain regions, all the way up to Mount Mitchell, North Carolina, the highest peak in the Appalachians.

***Pseudopolydesmus collinus* Hoffman, 1974**

Vernacular name: “The Rolling Hills Flatback”

Figures 4.12-14.

Diagnosis: Pulvillus pointed, slightly recurved (Fig. 4.12). Process *e1* small, straight, pointed, sometimes absent, not large as in *P. canadensis*; processes *e2* and *e3* connected by basal stalk, smaller than in *P. canadensis*, process *e2* not absent as in *P. serratus*; process *e4* triangular with a broad base, not absent as in *P. caddo*; process *m1* spade-shaped, not broadly quadrate as in *P. canadensis*; *m2* fin-shaped, not triangular as in *P. canadensis*; process *m3* absent, not present as in *P. canadensis*; process *m4* large, triangular, not absent as in *P. serratus*.



Figure 4.12. Left gonopod of *Pseudopolydesmus collinus* (MPE00842, Patrick County, Virginia), medial view (left) and ectal view (right).



Figure 4.13. *Pseudopolydesmus collinus*, dorsal view of adult male from Carroll County, Virginia (MPE03520).

Color: *Pseudopolydesmus collinus* has light pink to red paranota edges, with the rest of the tergites being a dark maroon to light pink color (Fig. 4.13).

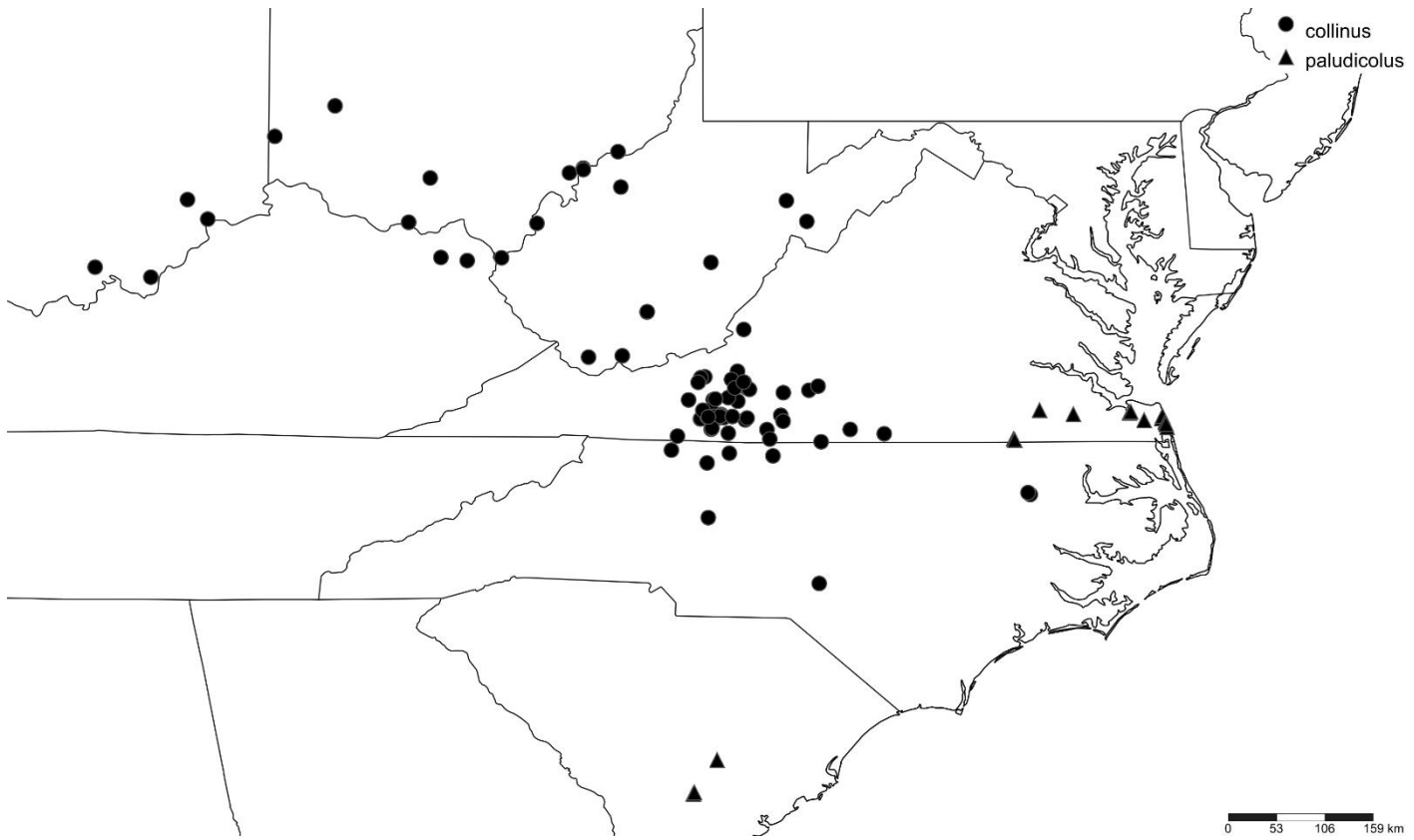


Figure 4.14. Distribution of *Pseudopolydesmus collinus* (dots) and *P. paludicolus* (triangles).

Distribution: *Pseudopolydesmus collinus* is known from six states: the southern portions of Indiana and Ohio and adjacent northern Kentucky, south through West Virginia, Virginia, and North Carolina (Fig. 4.14).

Ecology: This species is often found near rivers and streams at low to middle elevations, being recorded from elevations spanning from 169 m to 1109 m. Label data indicates it is most often found in deciduous forests of oak (*Quercus* spp.), maple (*Acer* spp.), tulip tree (*Liriodendron*

tulipifera), American beech (*Fagus grandifolia*), witch hazel (*Hamamelis virginiana*), and buckeye (*Aesculus* spp.). It has also been collected in hemlock (*Tsuga* spp.) and rhododendron (*Rhododendron* spp.) coves.

***Pseudopolydesmus erasus* (Loomis, 1943)**

Dixidesmus tallulanus Chamberlin, 1943

Dixidesmus penicillus Chamberlin, 1943

Dixidesmus humilidens Chamberlin, 1943

Vernacular name: “The Southern Flatback”

Figures 4.15-17.

Diagnosis: Pulvillus large, rounded distally (Fig. 4.15). Process *e1* pointed and straight, arising from transversely expanded area, not bent at base as in *P. canadensis*; process *e2* large, triangular, not connected to process *e3* by basal stalk as in *P. canadensis*; process *e3* small to medium sized and triangular, sometimes absent; process *e4* small, triangular, not absent as in *P. caddo*; process *m1* large, pointed to spade-shaped; process *m2* triangular, not fin-shaped as in *P. collinus*; process *m3* absent, not present as in *P. canadensis*; process *m4* triangular, with rounded apex, not absent as in *P. serratus*.

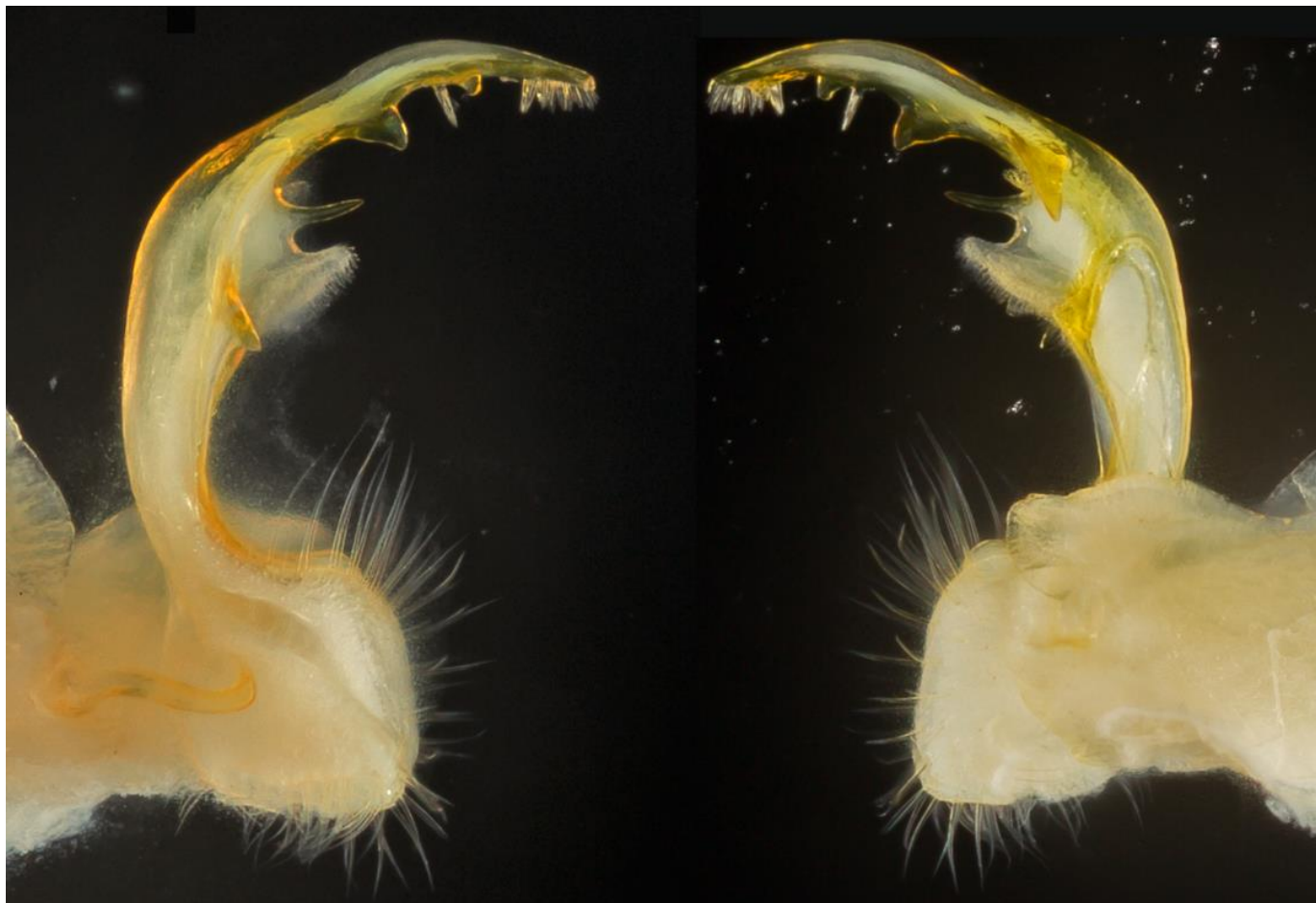


Figure 4.15. Left gonopod of *Pseudopolydesmus erasus* (MPE01326, Putnam County, Tennessee), medial view (left) and ectal view (right).



Figure 4.16. *Pseudopolydesmus erasus*, dorsal view of male from Van Buren County, Tennessee (MPE01229).

Color: *Pseudopolydesmus erasus* shows some color variability, but tends to have tergites mostly maroon-brown with paranota edges pink to light red, or sometimes light orange (Fig. 4.16).

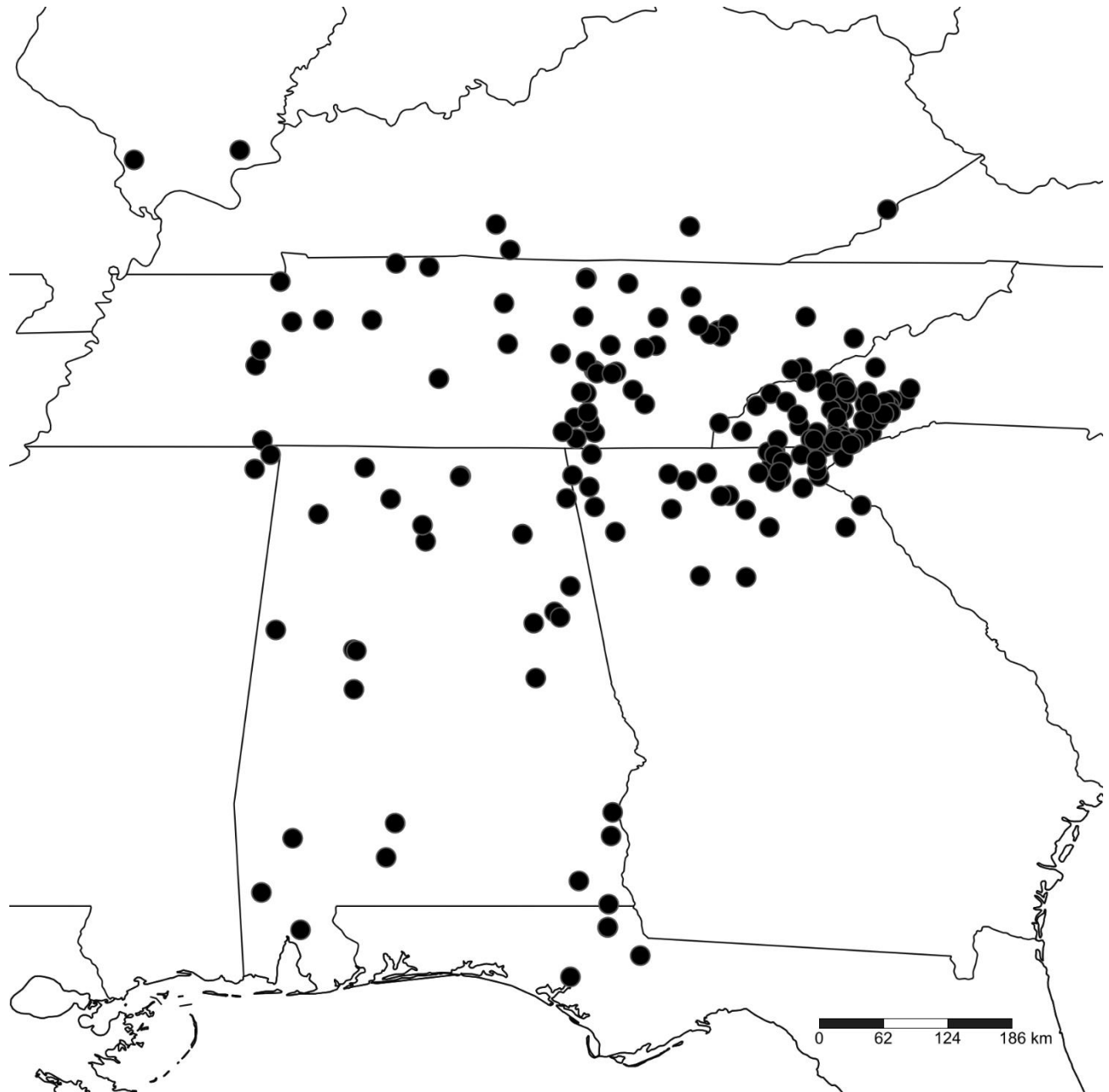


Figure 4.17. Distribution of *Pseudopolydesmus erasus*.

Distribution: *Pseudopolydesmus erasus* is found throughout the southern United States, ranging from the southern tip of Illinois south to the Florida panhandle (Fig. 4.17). It is the most common *Pseudopolydesmus* species throughout much of its range, but is sympatric with *P. canadensis*, *P. pinetorum*, and *P. serratus* in portions of its range.

Ecology: *Pseudopolydesmus erasus* is a general forest species, and can be found in mixed deciduous woods, pine woods, and hemlock-rhododendron coves. It occurs in a variety of habitats throughout its range, from low elevation plains to high elevation mountain forests. It is not particularly tied to streams or swamps, and can tolerate drier habitats.

***Pseudopolydesmus minor* (Bollman, 1888)**

Polydesmus neoterus Chamberlin, 1942

Polydesmus euthetus Chamberlin, 1942

Vernacular name: “The Small Swamp Flatback”

Figures 4.8, 18-19.

Diagnosis: Pulvillus pointed and elongate (Fig. 4.18). Process *e1* absent, not present as in *P. erasus*; process *e2* small, triangular, on ectal flange, not absent as in *P. caddo*; process *e3* absent, with the occurrence of an ectal flange in its place, not triangular as in *P. caddo*; process *e4* small, sharp, not absent as in *P. caddo*; process *m1* large, triangular, not fin-shaped as in *P. caddo*; process *m2* large, triangular, not small as in *P. caddo*; process *m3* absent, not present as in *P. paludicolus*; process *m4* triangular, not absent as in *P. paludicolus*.

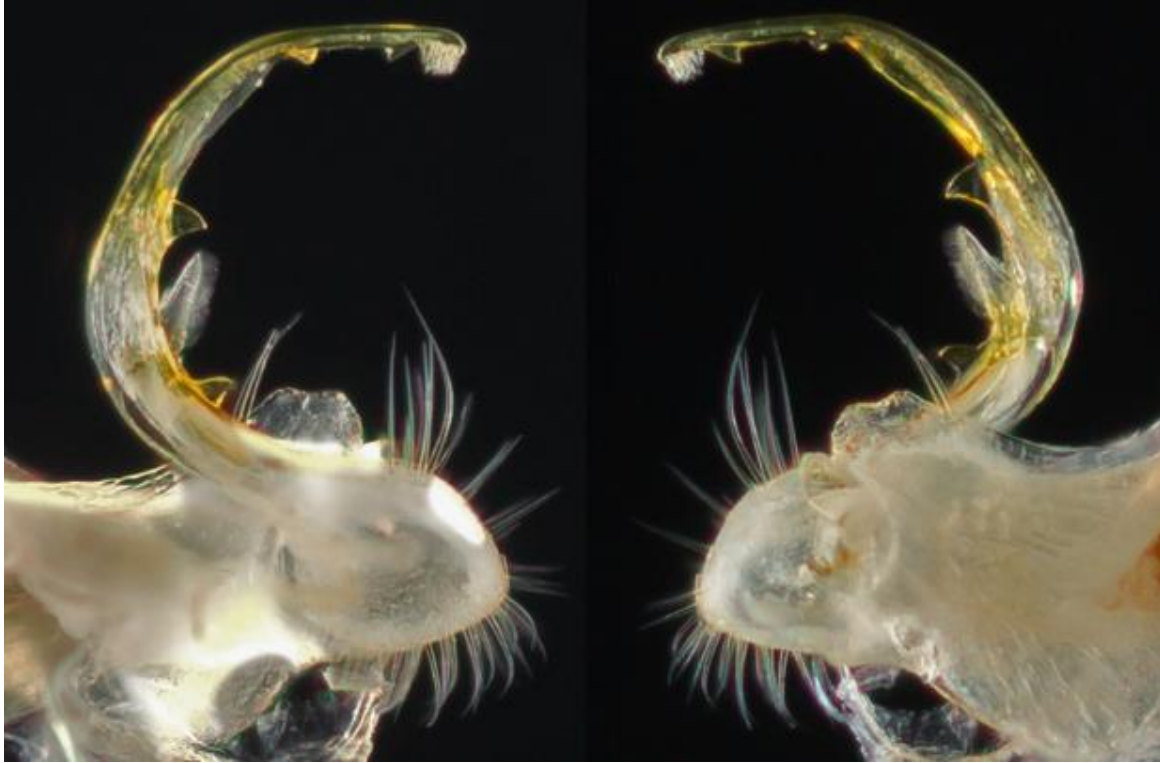


Figure 4.18. Left gonopod of *Pseudopolydesmus minor* (MPE03910, Champaign County, Illinois), medial view (left) and ectal view (right).



Figure 4.19. *Pseudopolydesmus minor*, dorsal view of adult female from Piatt County, Illinois (MPE03923).

Color: *Pseudopolydesmus minor* is a small species with dark brown tergites and paranota edges light tan-brown (Fig. 4.19).

Distribution: This species is the most widespread of all the wetland-adapted *Pseudopolydesmus* species (the others being *P. caddo* and *P. paludicolus*), and is found from Louisiana north to Indiana, mainly along the Mississippi River drainage (Fig. 4.8). Two records from far outside the core of the distribution are included, one from New Orleans, Louisiana, and another from Washington Court House, Ohio.

Ecology: *Pseudopolydesmus minor* is a swamp-adapted species found in floodplains and swamps associated with rivers and streams. Label data indicates it is associated with deciduous floodplain forests composed of oaks (*Quercus* spp.), maples (*Acer* spp., including *A. negundo* and *A. saccharinum*), hickory (*Carya* spp.), and American sycamore (*Platanus occidentalis*). Within these habitats, *P. minor* is often found associated with stumps in grassy areas, or under logs and other woody debris, particularly after recent flooding events.

***Pseudopolydesmus paludicolus* Hoffman, 1950**

Vernacular name: “The Virginia Swamp Flatback”

Figures 4.14, 20-21.

Diagnosis: Pulvillus small, pointed (Fig. 4.20). Process *e1* absent, not present as in *P. canadensis*; process *e2* small, pointed, not large as in *P. serratus*; process *e3* absent, not present as in *P. caddo*; process *e4* large, triangular, slightly curved distally, not small as in *P. serratus*; process *m1* small, spade-shaped, not large as in *P. erasus*; process *m2* triangular, connected to

process *m3* by lamina, both processes medium-sized, process *m2* not connected to *e2* by a transverse ridge as in *P. serratus*, process *m3* not absent as in *P. serratus*; process *m4* absent, not present as in *P. caddo*.

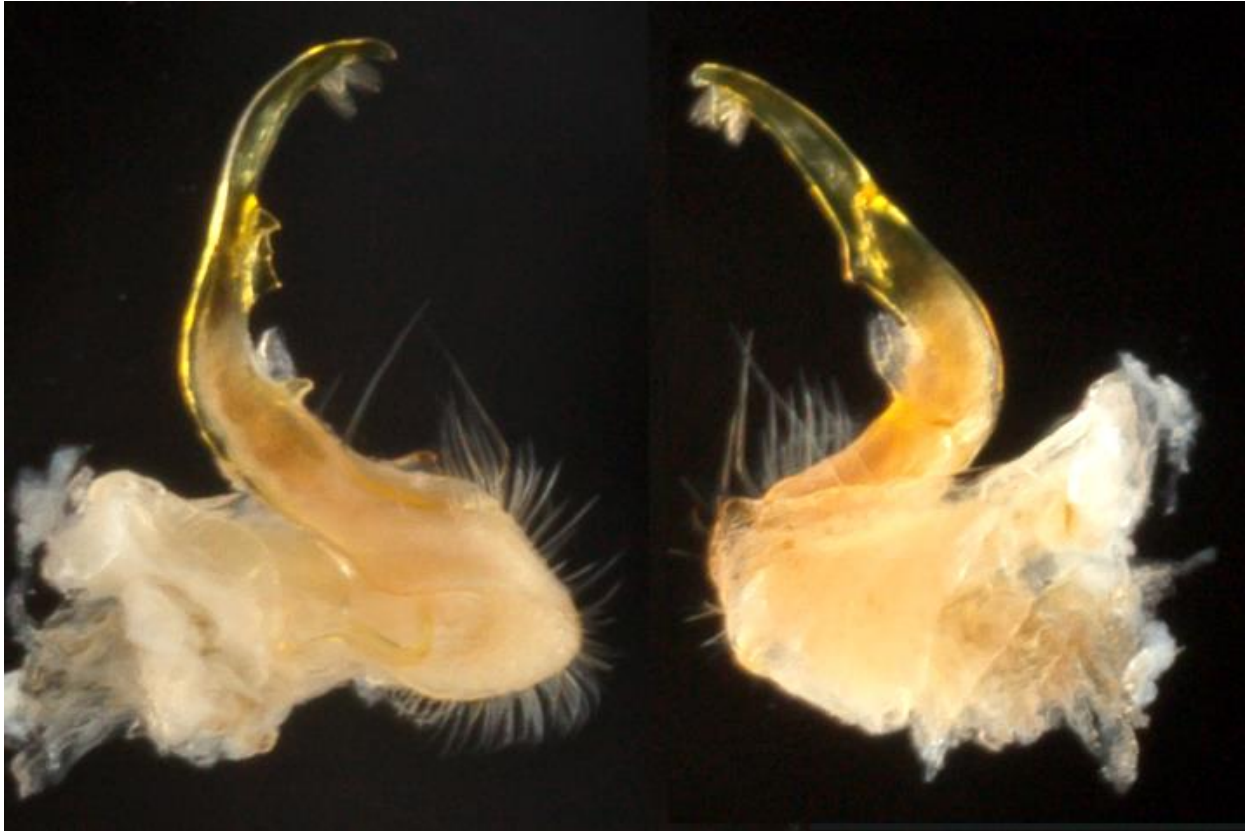


Figure 4.20. Left gonopod of *Pseudopolydesmus paludicolus* (MPE01169, Greensville County, Virginia), medial view (left) and ectal view (right).



Figure 4.21. *Pseudopolydesmus paludicolus*, dorsal view of adult female from Colleton County, South Carolina (MPE03525).

Color: Maroon-brown tergites with paranota the same color (Fig. 4.21).

Distribution: *Pseudopolydesmus paludicolus* is known from the Atlantic Coastal Plain in Virginia and South Carolina (Fig. 4.14). It has not yet been recorded from North Carolina, but with further collection in swampy habitats, will likely be found to occur in that state.

Ecology: A wetland-adapted species, *P. paludicolus* is found exclusively in swamps, often close to the water's edge. It has been recorded from swamp forests composed of bald cypress (*Taxodium distichum*), black gum (*Nyssa sylvatica*), live oak (*Quercus virginiana*), overcup oak (*Quercus lyrata*), loblolly pine (*Pinus taeda*), and sweet gum (*Liquidambar styraciflua*). It can be found in leaf litter or under logs and other woody debris, and has also been collected within sand dune habitats.

***Pseudopolydesmus pinetorum* (Bollman, 1888)**

Polydesmus americanus Carl, 1902

Polydesmus natchitoches Chamberlin, 1942

Polydesmus paroicus Chamberlin, 1942
Polydesmus hubrichti Chamberlin, 1943
Polydesmus modocus Chamberlin, 1943

Vernacular name: “The Pinelands Flatback”

Figures 4.22-24.

Diagnosis: Pulvillus large, rounded distally (Fig. 4.22). Process *e1* absent, not present as in *P. erasus*; process *e2* medium-sized, fin-shaped, lateral in position, not small and triangular as in *P. minor*; process *e3* large, fin-shaped, and recurved, not absent as in *P. serratus*; process *e4* small, triangular, not absent as in *P. caddo*; process *m1* small, quadrate, not large as in *P. erasus*; process *m2* medium-sized, triangular, not large as in *P. minor*; process *m3* absent, not present as in *P. canadensis*; process *m4* medium-sized, triangular, not absent as in *P. serratus*.



Figure 4.22. Left gonopod of *Pseudopolydesmus pinetorum* (MPE01006, Newton County, Arkansas), medial view (left) and ectal view (right).



Figure 4.23. *Pseudopolydesmus pinetorum*, dorsal view of adult male from St. Charles County, Missouri (MPE02753).

Color: Tergites maroon-brown, with paranota light pink to tan in color (Fig. 4.23). Quadrate paranota often times only slightly pinker than tergites.

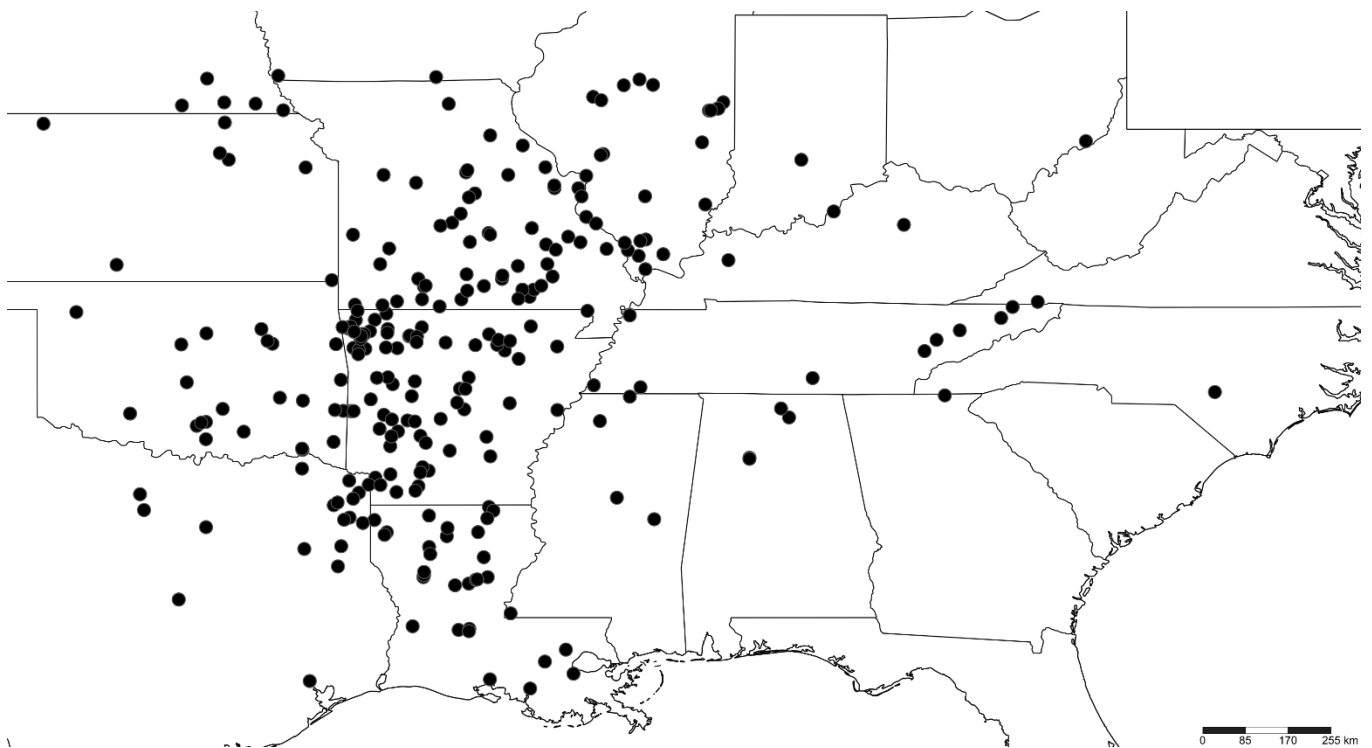


Figure 4.24. Distribution of *Pseudopolydesmus pinetorum*.

Distribution: *Pseudopolydesmus pinetorum* is the most common species west of the Mississippi River, but scattered records indicate its presence in farther eastern states (Fig. 4.24).

Ecology: This species can be found in generalized deciduous woods, as well as in pine forests throughout its range, where it occupies low to middle elevations up to 660 m.

***Pseudopolydesmus serratus* (Say, 1821)**

Polydesmus pennsylvanicus C. L. Koch, 1847

Polydesmus scopus Chamberlin, 1942

Polydesmus planicolens Chamberlin, 1942

Vernacular name: “The Common Pink Flatback”

Figures 4.25-27.

Diagnosis: Pulvillus large, slightly rounded distally (Fig. 4.25). Process *e1* absent, not present as in *P. canadensis*; process *e2* large, triangular, connected to process *m2* by transverse ridge, not connected to another process by a basal stalk as in *P. canadensis*; process *e3* absent, not present as in *P. erasus*; process *e4* small, triangular, not large as in *P. paludicolus*; process *m1* medium-sized, triangular, not large as in *P. minor*; process *m2* large, triangular, connected to process *e2* by transverse ridge, not connected to process *m3* as in *P. paludicolus*; process *m3* absent, not present as in *P. canadensis*; process *m4* absent, not present as in *P. canadensis*.

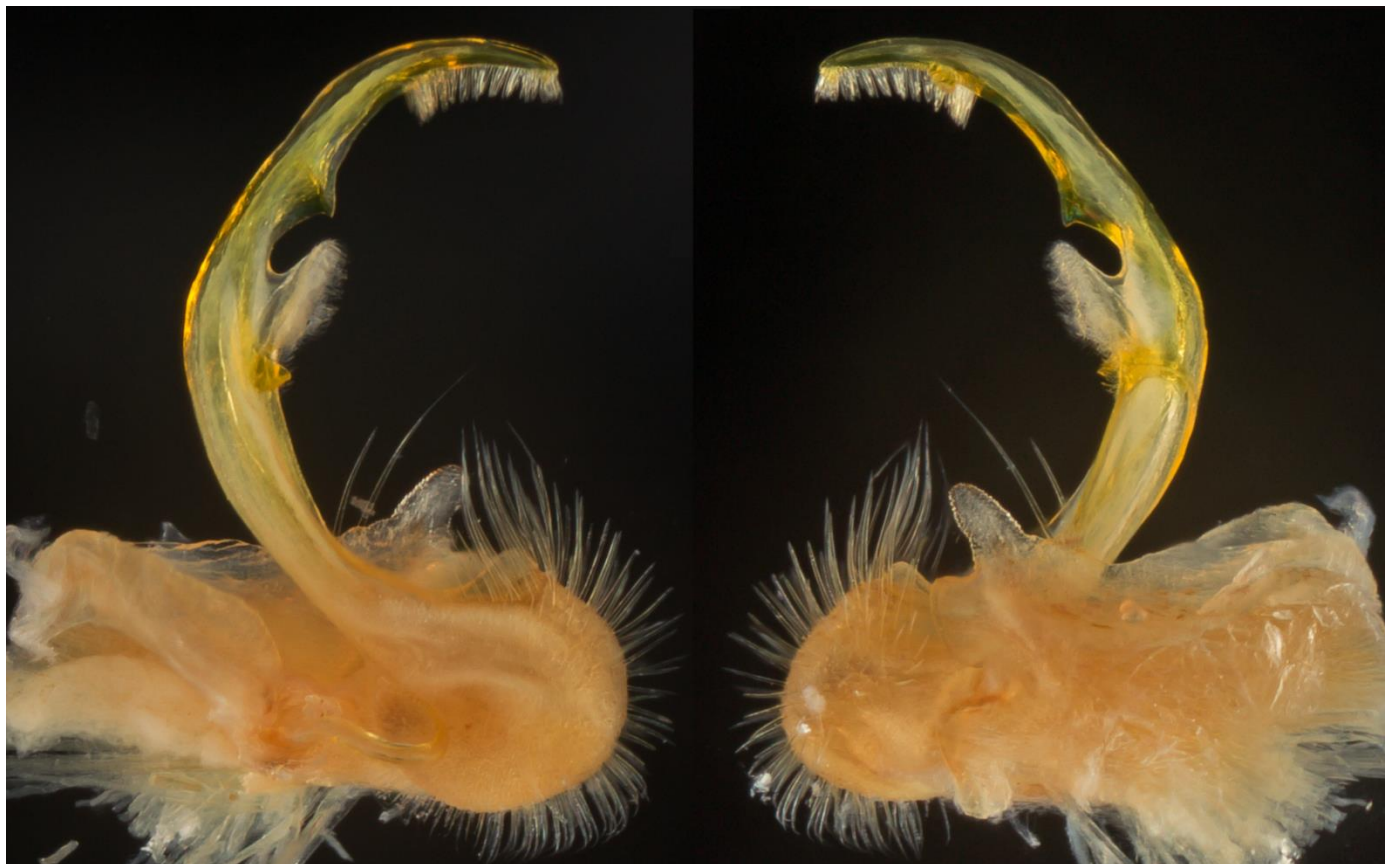


Figure 4.25. Left gonopod of *Pseudopolydesmus serratus* (MPE00803, Craig County, Virginia), medial view (left) and ectal view (right).



Figure 4.26. *Pseudopolydesmus serratus*, dorsal view of adult male from Powell County, Kentucky (MPE03105).

Color: Tergites dark brown to light pink, with pink to red paranota (Fig. 4.26).

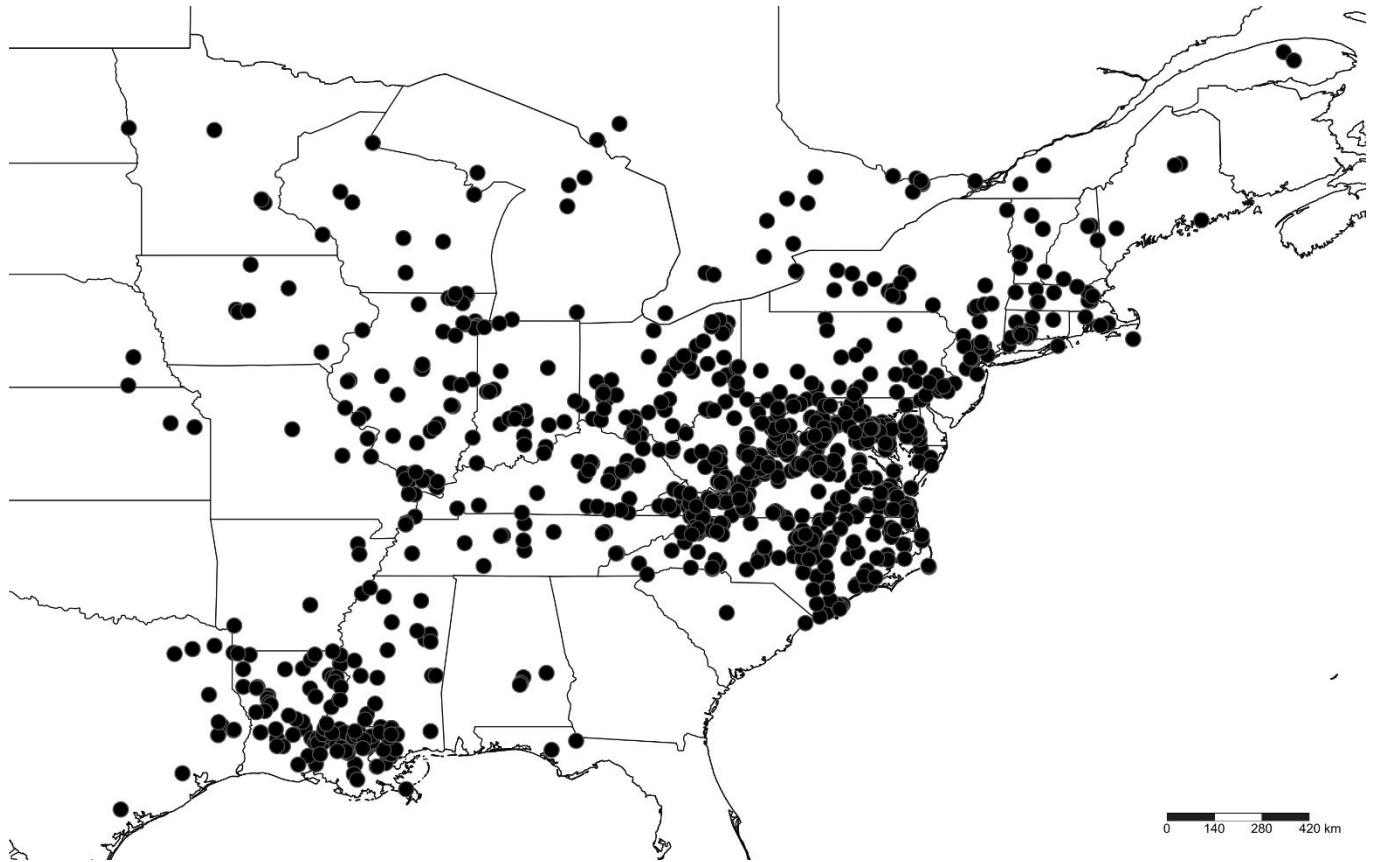


Figure 4.27. Distribution of *Pseudopolydesmus serratus*.

Distribution: The most widely-distributed species in the genus, *P. serratus* is known from Ontario and Québec south to Florida and Texas (Fig. 4.27). It is sympatric with every other species in various portions of its large range, but is not as common in the southeastern Coastal Plain and portions of its far western range.

Ecology: A generalist, *Pseudopolydesmus serratus* can be found in almost any suitable millipede habitat within its distribution, though it does not tend to occur at high elevations.

4.5 Discussion

Compared to the morphologically heterogeneous and ill-defined European and Asian polydesmid genera, *Pseudopolydesmus* has been generally assumed to be a monophyletic

assemblage, and this is supported by the genetic analyses presented here. The most closely-related genus appears to be *Epanerchodus* (Fig. 4.3); however, only one other of seven North American polydesmid genera was included, *Scytonotus*. The current concept of eight species in the genus advanced by Sierwald et al. (2019) was supported by the RAxML-inferred phylogeny.

Specimens of the potential new species identified in Hoffman's notes were collected from Russell County, Virginia. Hoffman used the designation "Adocetus" (from the Greek *adoketos*: unexpected) for specimens collected in Russell, Scott, and Washington Counties in southwest Virginia, that have the same gonopod processes as in *Pseudopolydesmus serratus*, but which have a greatly-curving acropodite, rather than the slightly-curving acropodite typically observed in *P. serratus*. These "Adocetus" specimens were included in the phylogenetic analysis to test if this feature was a characteristic of a cryptic species, as Hoffman surmised. The results from the RAxML phylogeny indicated that these "Adocetus" specimens are not representative of a cryptic species, as they fall well within the *Pseudopolydesmus serratus* clade and do not form their own separate clade. The "Adocetus" specimens are therefore just an example of standard morphological variation within *Pseudopolydesmus serratus*.

The gonopod morphology of *P. erasus* and *P. tallulanus* (Chamberlin, 1943) is similar, and *P. tallulanus* has traditionally been separated by the presence of an additional process, *e3*, on the ectal side of the gonopod, but specimens conforming to the *P. tallulanus* morphotype nested within the *P. erasus* clade, supporting the synonymy of *P. tallulanus* within *P. erasus*. The gonopod processes of each species of each species are the same, except for the presence or absence of *e3*. Specimens of the *P. tallulanus* morphotype tended to be found at higher elevations in the southern Appalachian Mountains in North Carolina and Georgia, while specimens conforming to the *P. erasus* morphotype tended to be found at lower elevations from

Illinois south through Alabama and Georgia, though this did not hold true for every specimen. Specimens from throughout the distribution of *P. erasus* and that were represented in the molecular phylogeny were examined for the presence of process *e3*, and though many lacked this process entirely, there was variation. A specimen from Marshall County, Tennessee (MPE01288) was observed to have a very small process *e3*, as did a specimen from Towns County, Georgia (MPE03315), while specimens from Walker County, Georgia (MPE01957) and Tishomingo County, Mississippi (MPE02698) lacked this process entirely. Another specimen from Towns County, Georgia (MPE04338) instead had a very large process *e3*. By examining the morphological variation of these specimens within a molecular phylogenetic framework, none of this variation was found to be restricted to separate clades within *Pseudopolydesmus erasus*, supporting the conclusion that the *P. tallulanus* morphotype specimens are within the standard morphological variation of *P. erasus*.

Aside from this difference, the traditionally-supported species of *Pseudopolydesmus*, based solely on gonopod morphology, agree with the genetic analysis. This finding offers great hope for future systematic work based on genetics within the Polydesmidae (and possibly the Polydesmidea as well), offering a more objective basis to base species hypotheses on, instead of relying mainly on small gonopod morphological differences to delimit species and subspecies.

The multivariate morphological analyses were not as clarifying for species delimitation in the genus, however. Body length clearly drove the results of the analysis, with 96% of the variation coming from this component, while the second component accounted for about 2% of the variation. The PCA plot contains six clusters (Fig. 4.5), and the variables are highly positively correlated, indicating the extent to which they are driven by body size (length). Due to the comparatively uniform somatic morphology in the genus, the modified PCA was not as

useful as it might be in a group of taxa that shows more morphological and/or ecological variation. In taxa like *Pseudopolydesmus*, species delimitation based on the genital morphology and genetics, rather than somatic characters, is likely to be a more practical approach.

The previous hypothesis about species relationships within *Pseudopolydesmus* (Withrow 1988) posited two groups: a *canadensis* group (five species) and a *serratus* group (four species), based on the gonopod morphology. This hypothesis was not supported by the species phylogeny inferred by RAxML (Fig. 4.3) or by the *BEAST species tree (Fig. 4.6). The phylogenetic analyses each show two clades within *Pseudopolydesmus*, but with varying species compositions. The RAxML tree shows a clade with *P. paludicolus* and *P. serratus* as sister species, with the rest of the species in their own clade (see Results section). In the *BEAST tree, the two major clades within *Pseudopolydesmus* consist of *P. caddo* and *P. minor* as highly-supported sister species, and allied with *P. pinetorum*, while the rest of the species make up the other clade. The RAxML phylogeny has better support values than the *BEAST tree, lending it more credence, but nonetheless, both phylogenies do not support the previous morphological-based hypotheses.

These results show biogeographical and ecological patterns within the genus, particularly with regards to adaptation and convergent evolution in wetland habitats. *Pseudopolydesmus paludicolus* was not found to be closely related to *P. minor* and *P. caddo*, the two other wetland-adapted species. This suggests that small body size and adaptation to wetland habitats may have evolved independently twice within the genus. Body length in the three wetland-inhabiting species ranged from 12 – 17 mm, compared to 14 – 36 mm in the rest of the species (Appendix B). All the other species in the genus are general forest species, with some living at higher elevations. Some specimens within *Pseudopolydesmus canadensis* are clustered in a small clade,

and all of these specimens are from the southeastern Coastal Plain. However, while they are smaller in body size and have different coloration, they do not appear to be a separate species from *P. canadensis*, though Coastal Plain *P. canadensis* have been thought to warrant subspecific status by previous workers (Hoffman, 1999).

As a result of this study, *Pseudopolydesmus* is now known from 37 U.S. states and two Canadian provinces in eastern North America. It is the most widespread polydesmid genus in North America, and the best-studied polydesmid in North America, with summarized information on its morphology (Sierwald et al., 2019; Zahnle et al., 2020) and genetics available. As demonstrated here, future studies on polydesmids will be well-served by incorporating a genetic component for species delimitation.

4.6 Acknowledgements

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Chapter 5 – Conclusions

5.1 Summary & Conclusions

The Polydesmida remains a group rich with opportunities for study. In North America, the Xystodesmidae is one of the most well-known families taxonomically, but still has intriguing ecological and other biological questions awaiting answers. The family presents a superb opportunity as an introductory group for non-millipede specialists, due to its large number of species, beautiful color patterns, and interesting chemical ecology.

The results presented in this work highlight the evolution of color pattern and anti-predator defense in the family. The area of the reaction chamber of the chemical defense gland was found to have increased relative to the ancestral state in the eastern North American clade of the Xystodesmidae, indicating increased toxicity of the millipedes. The largest glands were observed in the tribe Apheloriini, which have some of the most striking color patterns in the family. Within the Apheloriini, differential directions of gland size change were found, however. In areas with sympatric *Apheloria* and *Brachoria* species, the *Brachoria* species that mimic *Apheloria* color patterns were found to have smaller reservoir chambers in their chemical defense glands, indicating reduced toxicity, while *Apheloria* had larger reservoir chambers. In other areas where species of *Brachoria*, *Dixioria*, and *Sigmoria*, occur without a sympatric *Apheloria* species, the reaction chambers of those species had larger areas, indicating higher toxicity. Therefore, it appears that co-occurring mimic apheloriines, such as *Brachoria* species, possess reduced toxicity as a result of “release” from predation conferred by protection by *Apheloria*. The mimicry ring still serves its protective function through Müllerian mimicry, but some mimic species of the community may “cheat,” rather than all involved species being equally toxic to predators. Further study is warranted with mimicry rings involving other species

and genera throughout the range of Xystodesmidae in the Appalachian Region, to determine whether this pattern is constant throughout the family.

While a large portion of North American Xystodesmidae has received taxonomic attention, the tribe Nannariini has not. It is comprised of two genera (including *Mimuloria* Chamberlin, 1928), the monotypic *Oenomaea* Hoffman, 1964 and *Nannaria* Chamberlin, 1918, with 23 described species. Large numbers of undescribed species have been known to exist in museum collections and through recent fieldwork for decades, but little progress has been made on describing them, until this study. We collected 374 *Nannaria* specimens from the *wilsoni* species group across seven states in Appalachia. Through molecular phylogenetic analysis of six genes and examination of morphological characters of the gonopods, I described 18 new species. This quadrupled the known diversity of the *wilsoni* species group from six species to 24. By examining 1,835 specimen records from museum collections, we provided distributional data and ecological data for each species, and found that the *wilsoni* species group is only found in the Appalachian Mountains of the United States, with a gap in their distribution in eastern Tennessee and western North Carolina. Additional new species are expected to be found in the southern Appalachian Mountains, due to a lack of specimen records in the region. Phylogenetic and morphological differences separate this group from the *minor* species group of *Nannaria*, allowing for easy identification of these two species groups. Many species of *Nannaria* were found to have small distributions, endemic to only a few counties, and thus are susceptible to habitat loss and other threats.

Another polydesmidan family in North America, the Polydesmidae, was investigated. Mostly Holarctic in distribution, the family has a number of poorly-defined genera and a confusing array of subgenera and subspecies. North America has only seven native genera, only

two of which are in eastern North America, making the taxonomic situation much simpler than in the rest of its range. The largest genus in eastern North America, *Pseudopolydesmus*, was recently reviewed based on morphology, but has not received any phylogenetic investigation to clarify species boundaries and intrageneric relationships. As a result, an integrated taxonomic revision of the genus was undertaken, to assess species boundaries and assess distributional limits of each species in North America. This was done with field collections over five years, resulting in the collection of 1,148 specimens, which were used for population genetic analyses. These records were combined with specimen records from museum specimens and literature records to generate a database of 3,462 specimen records. This database was then used to map each species' distribution. Recently collected specimens were used to infer a phylogeny based on five genes, resulting in intrageneric relationships that conflicted with previous morphology-based hypotheses about species relationships in the genus. A dataset of 15 morphological measurements was not successful in delimiting species boundaries in a modified PCA analysis, but demonstrated that body size accounted for most of the variation between species in *Pseudopolydesmus*, emphasizing the usefulness of the genitalia in identifying species in the family. A total of eight species were supported by the genetic analyses, supporting previous morphological analyses of the genus; however, intrageneric phylogenetic relationships were clarified. This study indicated the usefulness of phylogenetic data in more objectively defining species in the family, and provides a way forward for analyses of other polydesmid genera, which often have multiple gonopod processes of unknown homology, making generic diagnosis difficult with only one line of evidence.

Millipede taxonomy stands to benefit greatly from increased use of phylogenetics for taxonomy and evolutionary analysis. The tools developed in the past few decades are useful for a

more objective and repeatable analysis of hypotheses, particularly those pertaining to species delimitation. The field has a great opportunity to grow and inspire general interest among other biologists and the general public. With inexpensive, yet high-quality photography accessible to anyone, beautiful images of millipedes have never been easier to produce. Their striking colors and interesting morphological features instantly garner attention, but a lack of accessible identification resources for millipedes and other myriapods limits the accessibility to amateurs and professional scientists. It is my hope that these barriers to access will be lowered in the coming years, for the betterment of the field and the creatures we study.

Appendix A. *Nannaria wilsoni* group morphological scoring matrix and character key

Species	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18
<i>Nannaria austriicola</i>	2	1	1	1	1	1	7	3	2	2	2	1	1	2	1	2	2	3
<i>Nannaria ericacea</i>	1	1	1	1	1	1	5	1	1	2	2	1	1	2	1	2	1	2
<i>Nannaria morrisoni</i>	2	1	1	1	1	1	1	1	1	2	2	2	1	3	1	2	2	2
<i>Nannaria scutellaria</i>	2	1	1	1	1	1	7	3	3	6	1	1	1	2	1	2	4	1
<i>Nannaria shenandoa</i>	2	1	1	1	1	1	5	3	3	4	2	3	1	3	1	2	1	3
<i>Nannaria wilsoni</i>	2	1	1	1	1	1	4	2	1	3	2	1	1	3	1	2	3	1
<i>Nannaria</i> n. sp. “Aenigma”	2	1	1	1	1	1	1	1	2	2	2	1	1	3	1	2	3	1
<i>Nannaria</i> n. sp. “Amicalola”	2	1	1	1	1	1	2	5	1	2	2	2	1	2	1	2	4	1
<i>Nannaria</i> n. sp. “Augusta”	2	1	1	1	1	1	3	1	1	3	2	1	1	5	1	2	2	1
<i>Nannaria</i> n. sp. “BigStonyCreek”	2	1	1	1	1	1	4	2	1	3	2	1	1	3	1	2	2	1
<i>Nannaria</i> n. sp. “Brandywine”	1	1	1	1	1	1	7	1	2	2	2	1	1	2	1	2	1	4
<i>Nannaria</i> n. sp. “Coweeta”	1	1	1	1	1	1	7	3	3	3	2	1	1	2	1	2	4	4
<i>Nannaria</i> n. sp. “Crataegae”	1	1	1	1	1	1	1	1	1	2	2	1	1	2	1	2	2	1
<i>Nannaria</i> n. sp. “Dell”	2	1	1	1	1	1	2	2	1	2	2	1	1	2	1	2	2	2
<i>Nannaria</i> n. sp. “HarrisCove”	1	1	1	1	1	1	7	3	3	6	2	1	1	2	1	2	2	3
<i>Nannaria</i> n. sp. “Juglandis”	1	1	1	1	1	6	2	1	1	3	2	1	2	5	1	2	1	4
<i>Nannaria</i> n. sp. “LongSpringRun”	2	1	1	1	1	1	1	1	1	2	2	1	1	3	1	2	1	3
<i>Nannaria</i> n. sp. “Lutra”	2	1	1	1	1	1	5	2	1	3	2	1	1	3	1	2	2	1
<i>Nannaria</i> n. sp. “Nubiterrae”	2	1	1	1	1	1	4	3	3	6	2	2	1	3	1	2	2	4

<i>Nannaria</i> n. sp. “Pinnacles”	2	1	1	1	1	1	5	2	1	3	2	1	1	3	1	2	2	1
<i>Nannaria</i> n. sp. “PottsMountain”	2	1	1	1	1	1	4	1	2	2	2	1	1	5	1	2	2	1
<i>Nannaria</i> n. sp. “PucPuggy”	2	1	1	1	1	1	7	1	1	2	2	1	1	2	1	2	2	1
<i>Nannaria</i> n. sp. “Sanctimontis”	2	1	1	1	1	1	4	3	1	6	2	1	1	2	1	2	2	4
<i>Nannaria</i> n. sp. “Trimble”	1	1	1	1	1	1	5	3	1	6	2	1	1	2	1	2	2	2

Species	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31	C32	C33	C34
<i>Nannaria austriicola</i>	1	1	2	1	2	2	1	1	3	2	1	2	2	2	2	1
<i>Nannaria ericacea</i>	2	6	2	1	2	2	1	1	3	2	1	2	2	2	2	1
<i>Nannaria morrisoni</i>	1	2	2	2	2	3	2	2	3	2	1	2	2	2	2	1
<i>Nannaria scutellaria</i>	2	6	2	1	2	3	2	1	3	2	1	2	2	2	2	1
<i>Nannaria shenandoa</i>	1	2	2	1	2	4	1	1	3	2	1	2	2	2	2	1
<i>Nannaria wilsoni</i>	5	6	2	1	2	4	2	5	3	2	1	2	1	1	2	1
<i>Nannaria</i> n. sp. “Aenigma”	1	2	2	1	2	2	1	2	3	2	1	2	1	1	2	1
<i>Nannaria</i> n. sp. “Amicalola”	1	6	2	1	2	4	2	3	3	2	1	2	2	2	2	1
<i>Nannaria</i> n. sp. “Augusta”	5	1	3	1	2	3	2	1	3	2	1	2	2	2	2	1
<i>Nannaria</i> n. sp. “BigStonyCreek”	3	6	2	2	2	2	1	1	3	2	1	2	2	2	2	1
<i>Nannaria</i> n. sp. “Brandywine”	1	1	2	1	2	2	1	1	3	2	1	2	2	2	2	1
<i>Nannaria</i> n. sp. “Coweeta”	3	6	2	2	2	3	1	1	3	2	1	2	2	2	2	1
<i>Nannaria</i> n. sp. “Crataegae”	1	1	2	1	2	3	2	1	3	2	1	2	1	1	2	1
<i>Nannaria</i> n. sp. “Dell”	3	1	2	1	?	?	?	?	?	2	1	2	1	1	2	1
<i>Nannaria</i> n. sp. “HarrisCove”	3	2	2	1	2	3	1	3	3	2	1	2	2	2	2	1
<i>Nannaria</i> n. sp. “Juglandis”	4	2	2	2	2	2	1	3	3	2	1	2	2	2	2	1
<i>Nannaria</i> n. sp. “LongSpringRun”	2	2	2	2	?	?	?	?	?	2	1	2	2	2	2	1

<i>Nannaria</i> n. sp. “Lutra”	3	2	2	2	2	3	1	1	3	2	1	2	2	2	2	1
<i>Nannaria</i> n. sp. “Nubiterrae”	1	2	2	1	2	4	1	5	3	2	1	2	1	1	2	1
<i>Nannaria</i> n. sp. “Pinnacles”	3	6	2	2	2	2	1	1	3	2	1	2	1	1	2	1
<i>Nannaria</i> n. sp. “PottsMountain”	3	2	2	1	2	2	1	1	3	2	1	2	1	1	2	1
<i>Nannaria</i> n. sp. “PucPuggy”	1	2	2	2	2	2	2	3	3	2	1	2	1	1	2	1
<i>Nannaria</i> n. sp. “Sanctimontis”	1	2	2	1	2	4	1	3	3	2	1	2	1	1	2	1
<i>Nannaria</i> n. sp. “Trimble”	1	1	2	1	2	4	1	3	3	2	1	2	1	1	2	1

Species	C35	C36	C37	C38	C39	C40	C41	C42	C43	C44	C45	C46	C47	C48	C49	C50	C51
<i>Nannaria austriicola</i>	2	1	2	1	1	1	1	2	1	2	1	2	1	2	3	2	1
<i>Nannaria ericacea</i>	2	1	2	1	1	1	1	2	1	2	1	2	1	2	3	2	1
<i>Nannaria morrisoni</i>	2	1	2	1	1	1	1	2	1	2	1	2	1	2	5	5	1
<i>Nannaria scutellaria</i>	2	1	2	1	1	1	1	2	1	2	1	2	1	2	5	6	1
<i>Nannaria shenandoa</i>	2	1	2	1	1	1	1	2	1	2	1	2	1	2	6	7	1
<i>Nannaria wilsoni</i>	2	1	2	1	1	1	1	2	1	2	1	2	1	2	6	7	1
<i>Nannaria</i> n. sp. “Aenigma”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	4	2	1
<i>Nannaria</i> n. sp. “Amicalola”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	5	7	1
<i>Nannaria</i> n. sp. “Augusta”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	6	7	1
<i>Nannaria</i> n. sp. “BigStonyCreek”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	3	2	1
<i>Nannaria</i> n. sp. “Brandywine”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	3	8	2
<i>Nannaria</i> n. sp. “Coweeta”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	5	4	1
<i>Nannaria</i> n. sp. “Crataegae”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	5	9	2
<i>Nannaria</i> n. sp. “Dell”	2	1	2	1	1	1	1	2	1	2	1	2	1	?	?	?	1

<i>Nannaria</i> n. sp. “HarrisCove”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	6	4	1
<i>Nannaria</i> n. sp. “Juglandis”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	4	3	1
<i>Nannaria</i> n. sp. “LongSpringRun”	2	1	2	1	1	1	1	2	1	2	1	2	1	?	?	?	1
<i>Nannaria</i> n. sp. “Lutra”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	5	2	1
<i>Nannaria</i> n. sp. “Nubiterrae”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	6	7	1
<i>Nannaria</i> n. sp. “Pinnacles”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	3	2	1
<i>Nannaria</i> n. sp. “PottsMountain”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	4	7	1
<i>Nannaria</i> n. sp. “PucPuggy”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	3	2	1
<i>Nannaria</i> n. sp. “Sanctimontis”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	5	2	2
<i>Nannaria</i> n. sp. “Trimble”	2	1	2	1	1	1	1	2	1	2	1	2	1	2	6	2	1

Species	C52	C53	C54
<i>Nannaria austriicola</i>	3	1	2
<i>Nannaria ericacea</i>	2	1	1
<i>Nannaria morrisoni</i>	1	2	1
<i>Nannaria scutellaria</i>	3	1	1
<i>Nannaria shenandoa</i>	1	2	1
<i>Nannaria wilsoni</i>	2	1	1
<i>Nannaria</i> n. sp. “Aenigma”	2	1	1
<i>Nannaria</i> n. sp. “Amicalola”	3	1	2
<i>Nannaria</i> n. sp. “Augusta”	1	2	1
<i>Nannaria</i> n. sp. “BigStonyCreek”	2	1	1
<i>Nannaria</i> n. sp. “Brandywine”	2	1	1
<i>Nannaria</i> n. sp. “Coweeta”	3	2	1

<i>Nannaria</i> n. sp. “Cratagae”	3	1	1
<i>Nannaria</i> n. sp. “Dell”	3	2	1
<i>Nannaria</i> n. sp. “HarrisCove”	2	2	2
<i>Nannaria</i> n. sp. “Juglandis”	2	1	1
<i>Nannaria</i> n. sp. “LongSpringRun”	2	1	1
<i>Nannaria</i> n. sp. “Lutra”	2	1	1
<i>Nannaria</i> n. sp. “Nubiterrae”	2	2	1
<i>Nannaria</i> n. sp. “Pinnacles”	2	1	1
<i>Nannaria</i> n. sp. “PottsMountain”	2	1	1
<i>Nannaria</i> n. sp. “PucPuggy”	3	1	2
<i>Nannaria</i> n. sp. “Sanctimontis”	3	2	1
<i>Nannaria</i> n. sp. “Trimble”	2	1	1

Scoring key for *Nannaria wilsoni* group morphological characters. 1-22, 51-53, male gonopodal characters; 23-27, 48-50, female cyphopodal characters; 28-47, 54 somatic characters. Character system adapted from Marek and Bond, 2006.

- #1. gonopods
 - 1. do not cross in situ
 - 2. cross in situ
- #2. prefemoral basal spine <presence>
 - 1. absent
 - 2. present
- #3. prefemoral basal spine
 - 1. absent
 - 2. separate from prefemoral process for some part of length
 - 3. entire length fused with prefemoral process
- #4. prefemoral basal spine <size>
 - 1. absent
 - 2. large, pronounced
 - 3. small, reduced
- #5. prefemoral basal spine <shape>
 - 1. absent
 - 2. pointed, sharp
 - 3. pointed, blunt
 - 4. rectangular, shelf-like
 - 5. reduced to rounded bulge
- #6. prefemoral process arising from
 - 1. base of acropodite
 - 2. top of prefemoral spine
 - 3. prefemoral spine, dorsomedially
 - 4. prefemoral spine, ventromedially
 - 5. absent
 - 6. midway up the acropodite
- #7. prefemoral process shape
 - 1. acicular
 - 2. laminate
 - 3. stout
 - 4. sinuous
 - 5. simple, curving
 - 6. absent
 - 7. sinuous taper
- #8. prefemoral process curve, when viewed anteriorly
 - 1. straight
 - 2. medially
 - 3. laterally
 - 4. absent
 - 5. ventrally
- #9. prefemoral process crosses acropodite, when viewed anteriorly

1. does not cross
2. crosses dorsolaterally
3. crosses ventrolaterally
- #10. prefemoral process tip directed
 1. ventrally
 2. cephalically
 3. medially
 4. dorsally
 5. absent
 6. laterally
- #11. gonopodal basal zone lateral bulge <presence>
 1. absent
 2. present
- #12. basal zone width, when viewed anteriorly
 1. wider than space between acropodite and prefemoral process at greatest divide
 2. subequal to space between acropodite and prefemoral process at greatest divide
 3. thinner than space between acropodite and prefemoral process at greatest divide
- #13. basal zone height, when viewed anteriorly
 1. < half length of acropodite
 2. subequal to half length of acropodite
 3. > half length of acropodite
- #14. acropodite arc
 1. straight
 2. straight, with abrupt bend at tip
 3. gradual curve
 4. sinuous
 5. straight, with bend at midpoint
- #15. acropodite cingulum <presence>
 1. absent
 2. present
- #16. acropodite <swelling>
 1. swollen before apex
 2. not swollen before apex
- #17. acropodite medial flange <shape>
 1. absent
 2. lobed
 3. laminate
 4. tooth-like
- #18. acropodite tip medial flange <shape>
 1. absent
 2. triangular
 3. lobed
 4. laminate
- #19. acropodite tip lateral flange <shape>
 1. absent
 2. triangular

- 3. lobed
- 4. hooked
- 5. laminate
- #20. acropodite tip directed, when viewed anteriorly
 - 1. medially
 - 2. caudally
 - 3. laterally
 - 4. cephalically
 - 5. dorsally
 - 6. ventrally
- #21. gonopodal distal zone <length>
 - 1. absent
 - 2. short, less than 0.4x length of acropodite
 - 3. long, greater than or equal to 0.5x length of acropodite
- #22. gonopodal tip <shape>
 - 1. sharp
 - 2. blunt
- #23. cyphopodal receptacle <presence>
 - 1. absent
 - 2. present
- #24. cyphopodal receptacle <size at widest part>
 - 1. absent
 - 2. shorter than prefemur length
 - 3. subequal to prefemur length
 - 4. wider than prefemur length
- #25. cyphopodal valves <symmetry>
 - 1. symmetric
 - 2. asymmetric
- #26. cyphopodal valves <orientation>
 - 1. ventrally
 - 2. anteroventrally
 - 3. posteroventrally
 - 4. twisted posterior
 - 5. laterally
- #27. cyphopodal receptacle cuticle <surface>
 - 1. absent
 - 2. smooth
 - 3. sculptured
- #28. gnathochilarium lateral emargination <presence>
 - 1. absent
 - 2. present
- #29. antennomere one distal cuticle <conformation>
 - 1. cylindrical, not wrapped around cones
 - 2. wrapped around cones
- #30. collum ridges <presence>
 - 1. absent

- 2. present
- #31. caudolateral corners, paranota I-X <shape>
 - 1. acute, projecting caudally
 - 2. rounded
- #32. caudolateral corners, paranota I-XIX <shape>
 - 1. acute, projecting caudally on all segments
 - 2. rounded on segments I-X only
 - 3. rounded on segments I-XIX
- #33. metatergal pores, metatergites IX+X <presence>
 - 1. absent
 - 2. present
- #34. metatergal linear bump-pores <presence>
 - 1. absent
 - 2. present
- #35. lateral wrinkles, tergites IX+X
 - 1. tightly wrinkled
 - 2. loosely wrinkled
- #36. repugnatorial pores, paranota IX+X <orientation>
 - 1. laterally
 - 2. dorsally
- #37. metatergal dorsal slits <presence>
 - 1. absent
 - 2. present
- #38. metatergal dorsal microsculpture <mesh shape>
 - 1. isodiametric
 - 2. anisodiametric
- #39. paranota-dorsum, segments IX+X <angle>
 - 1. 130 degrees
 - 2. 180 degrees
- #40. paranotal segments IX+X <width>
 - 1. thin
 - 2. thick
- #41. gonapophyses <shape>
 - 1. cylinder-shaped
 - 2. goblet-shaped
- #42. sternal knobs, 4th leg pair <presence>
 - 1. absent
 - 2. present
- #43. pleural process, segments IX+X <presence>
 - 1. absent
 - 2. present
- #44. sternal triangular spines, segments IX+X <presence>
 - 1. absent
 - 2. present
- #45. sternal median bulge, segments IX+X <presence>
 - 1. absent

- 2. present
- #46. setae, sterna IX+X <presence>
 - 1. absent
 - 2. present
- #47. ventral excavation, sterna IX-X <presence>
 - 1. absent
 - 2. present
- #48. female leg 2 coxa <shape>
 - 1. normal, not expanded
 - 2. laterally expanded, covering cyphopod bursa
- #49. cyphopodal receptacle length <size compared to valves>
 - 1. absent
 - 2. < half as large as the valves
 - 3. half as large as the valves
 - 4. three-quarters as large as the valves
 - 5. subequal in size to the valves
 - 6. larger than valves
- #50. cyphopodal receptacle <shape>
 - 1. absent
 - 2. triangular
 - 3. capsule-shaped, with rounded tip
 - 4. finger-like projection
 - 5. laminate and leaf-like
 - 6. elongated club
 - 7. enlarged triangular hood, covering valves
 - 8. quadrate, recurved distally
 - 9. sinuous S-shaped, with blunted end
 - 10. dome-shaped, slightly tapered distally
- #51. gonopod acropodite basomedial process <presence>
 - 1. absent
 - 2. present
- #52. gonopod acropodite anterior bend twist <presence>
 - 1. absent
 - 2. shape of a smoothly-undulating helix
 - 3. acutely bent, appearing crimped
- #53. gonopod acropodite tip <shape>
 - 1. single, smooth, entire branch
 - 2. bifurcate
- #54. leg 10 prefemur spine <length>
 - 1. < than half as long as femur
 - 2. half as long as femur
 - 3. > half as long as femur
 - 4. subequal in size to femur

Appendix B. Morphological measurements of *Pseudopolydesmus* specimens. All measurements are in millimeters. Male measurements presented first, followed by females.

Species	Catalog Number	Body Length	Collum Width	Collum Length	Head Width	Left 3rd Antennomere Length	Gnathochilarium width	Gnathochilarium Length	Clypeus Width	Inter-antennal space width	Metazonite 6 Width
<i>caddo</i>	MPE03746	12.50	1.48	0.70	0.86	0.49	0.55	0.59	1.11	0.30	1.98
<i>canadensis</i>	MPE01650	29.00	2.79	1.06	1.56	1.18	1.10	1.10	2.13	0.62	4.71
<i>canadensis</i>	MPE01687	27.00	2.57	0.99	1.53	1.09	1.12	1.00	2.00	0.59	4.20
<i>canadensis</i>	MPE02102	27.30	2.85	1.03	1.58	1.15	1.09	1.10	2.13	0.60	4.47
<i>canadensis</i>	MPE03032	26.00	2.30	0.93	1.45	1.00	0.99	0.96	1.98	0.56	3.74
<i>canadensis</i>	MPE03068	31.00	2.67	1.06	1.61	1.05	1.10	1.04	2.13	0.62	4.28
<i>canadensis</i>	MPE03223	27.00	2.51	0.93	1.44	1.02	1.02	1.04	1.96	0.59	4.13
<i>canadensis</i>	MPE03294	26.00	2.54	1.00	1.53	1.00	1.04	1.02	2.03	0.59	4.05
<i>canadensis</i>	MPE03657	22.00	2.15	0.84	1.24	0.85	0.86	0.88	1.62	0.48	3.24
<i>canadensis</i>	MPE04137	28.00	2.41	0.98	1.55	1.08	1.03	1.00	2.00	0.60	4.29
<i>canadensis</i>	MPE04528	24.50	2.33	0.93	1.35	0.99	0.98	1.00	1.88	0.48	3.92
<i>canadensis</i>	MPE04575	27.31	2.70	1.10	1.65	1.10	1.13	1.05	2.13	0.63	4.63
<i>canadensis</i>	MPE04576	27.10	2.82	1.05	1.65	1.10	1.18	1.15	2.23	0.65	4.70
<i>collinus</i>	MPE00844	19.56	2.15	0.82	1.23	0.89	0.86	0.90	1.68	0.41	3.48
<i>collinus</i>	MPE01725	22.00	1.98	0.83	1.20	0.84	0.88	0.88	1.66	0.44	3.32
<i>collinus</i>	MPE01737	21.50	1.87	0.80	1.22	0.76	0.85	0.85	1.60	0.45	3.20
<i>collinus</i>	MPE01751	20.50	1.92	0.78	1.20	0.85	0.84	0.84	1.64	0.50	3.20
<i>collinus</i>	MPE01776	21.00	1.86	0.76	1.16	0.83	0.81	0.73	1.54	0.48	2.91
<i>collinus</i>	MPE03162	21.00	1.86	0.74	1.18	0.76	0.82	0.84	1.60	0.44	3.04
<i>collinus</i>	PSE00577	24.85	2.15	0.92	1.34	1.08	0.96	0.96	1.80	0.48	3.52
<i>collinus</i>	PSE00596	22.92	2.08	0.83	1.40	0.90	0.86	0.86	1.78	0.49	3.40
<i>collinus</i>	PSE00840	20.00	1.69	0.69	1.14	0.80	0.78	0.76	1.54	0.46	2.79
<i>erasus</i>	MPE01288	27.00	2.02	0.78	1.15	0.78	0.83	0.85	1.60	0.50	2.85
<i>erasus</i>	MPE01311	20.00	2.14	0.81	1.18	0.78	0.86	0.80	1.68	0.46	3.16
<i>erasus</i>	MPE01326	26.00	2.40	0.88	1.33	0.92	0.95	0.88	1.94	0.54	4.00

Species	Catalog Number	Meta-zonite 6 Length	Left Leg 6 Tarsus Length	Epiproct Width	Epiproct Length	Gonopod Aperture Width
<i>caddo</i>	MPE03746	0.51	0.44	0.59	0.43	0.91
<i>canadensis</i>	MPE01650	1.46	1.43	0.96	0.74	1.90
<i>canadensis</i>	MPE01687	1.36	1.34	0.99	0.75	1.80
<i>canadensis</i>	MPE02102	1.38	1.30	0.98	0.70	1.92
<i>canadensis</i>	MPE03032	1.18	1.15	0.93	0.71	1.67
<i>canadensis</i>	MPE03068	1.37	1.36	1.22	0.94	1.90
<i>canadensis</i>	MPE03223	1.39	1.38	0.94	0.74	1.68
<i>canadensis</i>	MPE03294	1.25	1.20	0.91	0.83	1.85
<i>canadensis</i>	MPE03657	0.98	0.93	0.83	0.69	1.44
<i>canadensis</i>	MPE04137	1.23	1.31	0.96	0.74	1.88
<i>canadensis</i>	MPE04528	1.24	1.17	0.88	0.60	1.69
<i>canadensis</i>	MPE04575	1.50	1.30	1.00	0.73	1.95
<i>canadensis</i>	MPE04576	1.50	1.32	1.02	0.84	1.92
<i>collinus</i>	MPE00844	0.90	1.02	0.86	0.66	1.46
<i>collinus</i>	MPE01725	0.83	0.98	0.77	0.48	1.43
<i>collinus</i>	MPE01737	1.04	1.02	0.86	0.69	1.38
<i>collinus</i>	MPE01751	1.10	0.95	0.80	0.55	1.36
<i>collinus</i>	MPE01776	0.93	0.90	0.78	0.70	1.26
<i>collinus</i>	MPE03162	0.83	0.92	0.75	0.50	1.35
<i>collinus</i>	PSE00577	1.12	1.08	0.96	0.76	1.56
<i>collinus</i>	PSE00596	1.08	0.94	0.75	0.59	1.50
<i>collinus</i>	PSE00840	0.91	0.82	0.83	0.75	1.22
<i>erasus</i>	MPE01288	0.96	0.98	0.80	0.68	1.48
<i>erasus</i>	MPE01311	0.92	1.08	0.70	0.51	1.53
<i>erasus</i>	MPE01326	1.18	1.20	0.90	0.78	1.72

Species	Catalog Number	Body Length	Collum Width	Collum Length	Head Width	Left 3rd Antennomere Length	Gnathochilarium width	Gnathochilarium Length	Clypeus Width	Inter-antennal space width	Metazonite 6 Width
<i>erasus</i>	MPE01366	24.00	2.26	0.85	1.33	0.94	0.96	0.96	1.86	0.51	3.70
<i>erasus</i>	MPE01526	30.00	2.74	1.13	1.55	1.12	1.11	1.25	2.15	0.56	4.50
<i>erasus</i>	MPE02696	23.00	2.48	0.88	1.36	0.88	0.88	0.93	1.75	0.48	3.65
<i>erasus</i>	MPE02698	22.00	2.03	0.78	1.15	0.78	0.84	0.74	1.60	0.45	3.16
<i>erasus</i>	MPE02809	23.00	2.25	0.88	1.28	0.90	0.90	0.88	1.83	0.53	3.44
<i>erasus</i>	MPE04374	34.00	2.55	0.90	1.30	0.88	1.00	0.96	1.90	0.50	4.08
<i>erasus</i>	PSE00669	21.45	1.95	0.80	1.25	0.90	0.90	0.88	1.68	0.51	3.20
<i>minor</i>	MPE03911	12.00	1.13	0.53	0.80	0.33	0.48	0.53	1.04	0.32	1.68
<i>minor</i>	MPE03953	14.00	1.15	0.53	0.75	0.41	0.48	0.53	1.06	0.28	1.73
<i>minor</i>	PSE00546	14.82	1.28	0.54	0.70	0.48	0.55	0.61	1.10	0.32	1.80
<i>minor</i>	PSE00646	14.40	1.39	0.62	0.91	0.44	0.63	0.63	1.15	0.31	1.96
<i>minor</i>	PSE00646	15.00	1.49	0.64	0.91	0.54	0.63	0.64	1.23	0.37	2.13
<i>minor</i>	PSE00646	14.68	1.46	0.64	0.94	0.47	0.62	0.64	1.25	0.32	2.13
<i>minor</i>	PSE00650	13.75	1.33	0.61	0.90	0.49	0.57	0.62	1.13	0.32	1.95
<i>paludicolus</i>	MPE01169	15.50	1.32	0.73	0.93	0.57	0.64	0.64	1.28	0.37	2.11
<i>paludicolus</i>	MPE03444	14.30	1.30	0.63	0.92	0.50	0.64	0.66	1.26	0.45	2.10
<i>paludicolus</i>	MPE03445	13.74	1.34	0.62	0.98	0.53	0.67	0.67	1.30	0.35	2.10
<i>paludicolus</i>	MPE03446	14.80	1.30	0.61	0.96	0.48	0.64	0.62	1.25	0.34	2.03
<i>paludicolus</i>	MPE03644	17.00	1.33	0.59	0.90	0.50	0.59	0.65	1.21	0.37	2.14
<i>paludicolus</i>	PSE00052	14.45	1.22	0.59	0.90	0.52	0.58	0.62	1.20	0.37	1.86
<i>paludicolus</i>	PSE00054	14.70	1.33	0.64	0.98	0.53	0.61	0.65	1.22	0.40	1.96
<i>paludicolus</i>	PSE00569	15.70	1.34	0.63	0.93	0.53	0.60	0.60	1.26	0.38	2.09
<i>paludicolus</i>	PSE00588	16.40	1.44	0.67	0.99	0.53	0.61	0.69	1.30	0.37	2.21
<i>pinetorum</i>	MPE01002	19.80	1.96	0.77	1.17	0.89	0.79	0.90	1.58	0.40	2.73
<i>pinetorum</i>	MPE01070	18.75	1.90	0.60	1.25	0.80	0.80	0.82	1.60	0.40	2.91

Species	Catalog Number	Meta-zonite 6 Length	Left Leg 6 Tarsus Length	Epiproct Width	Epiproct Length	Gonopod Aperture Width
<i>erasus</i>	MPE01366	1.13	1.25	0.86	0.68	1.65
<i>erasus</i>	MPE01526	1.36	1.31	1.06	0.74	1.78
<i>erasus</i>	MPE02696	1.05	1.06	0.77	0.54	1.55
<i>erasus</i>	MPE02698	0.98	0.96	0.70	0.51	1.44
<i>erasus</i>	MPE02809	1.02	1.09	0.64	0.46	1.50
<i>erasus</i>	MPE04374	1.10	1.24	0.88	0.73	1.68
<i>erasus</i>	PSE00669	0.94	0.98	0.82	0.70	1.42
<i>minor</i>	MPE03911	0.53	0.35	0.53	0.38	0.90
<i>minor</i>	MPE03953	0.50	0.38	0.51	0.51	0.88
<i>minor</i>	PSE00546	0.58	0.43	0.63	0.51	1.00
<i>minor</i>	PSE00646	0.63	0.48	0.65	0.58	1.02
<i>minor</i>	PSE00646	0.65	0.50	0.64	0.45	1.04
<i>minor</i>	PSE00646	0.64	0.53	0.68	0.54	1.07
<i>minor</i>	PSE00650	0.60	0.48	0.61	0.50	0.96
<i>paludicolus</i>	MPE01169	0.58	0.58	0.63	0.53	0.94
<i>paludicolus</i>	MPE03444	0.66	0.63	0.69	0.47	1.01
<i>paludicolus</i>	MPE03445	0.64	0.56	0.66	0.51	0.96
<i>paludicolus</i>	MPE03446	0.60	0.58	0.59	0.46	0.99
<i>paludicolus</i>	MPE03644	0.65	0.56	0.64	0.50	0.90
<i>paludicolus</i>	PSE00052	0.62	0.56	0.56	0.38	0.91
<i>paludicolus</i>	PSE00054	0.64	0.59	0.59	0.54	0.96
<i>paludicolus</i>	PSE00569	0.63	0.53	0.59	0.54	0.93
<i>paludicolus</i>	PSE00588	0.69	0.37	0.64	0.60	0.96
<i>pinetorum</i>	MPE01002	0.84	0.93	0.74	0.64	1.28
<i>pinetorum</i>	MPE01070	0.96	0.93	0.64	0.35	1.20

Species	Catalog Number	Body Length	Collum Width	Collum Length	Head Width	Left 3rd Antennomere Length	Gnathochilium width	Gnathochilium Length	Clypeus Width	Inter-antennal space width	Metazonite 6 Width
<i>pinetorum</i>	MPE01163	25.00	1.90	0.72	1.08	0.71	0.75	0.83	1.46	0.41	2.70
<i>pinetorum</i>	MPE01283	17.00	1.71	0.74	1.05	0.59	0.70	0.70	1.38	0.35	2.43
<i>pinetorum</i>	MPE02718	19.50	1.85	0.73	1.15	0.70	0.78	0.75	1.63	0.43	2.52
<i>pinetorum</i>	MPE02752	14.08	2.20	0.84	1.24	0.84	0.78	0.78	1.61	0.47	2.91
<i>pinetorum</i>	MPE02762	17.35	1.92	0.72	1.16	0.78	0.77	0.82	1.49	0.42	2.70
<i>pinetorum</i>	MPE03921	20.00	1.80	0.78	1.12	0.77	0.82	0.82	1.50	0.45	2.48
<i>pinetorum</i>	MPE04339	18.26	1.93	0.78	1.14	0.71	0.78	0.82	1.52	0.41	2.60
<i>pinetorum</i>	MPE04598	20.00	1.85	0.80	1.14	0.77	0.77	0.77	1.53	0.41	2.60
<i>pinetorum</i>	PSE00545	20.23	1.82	0.78	1.12	0.83	0.78	0.82	1.50	0.40	2.54
<i>serratus</i>	MPE01517	28.00	2.26	0.98	1.40	0.94	1.16	1.04	1.93	0.51	3.56
<i>serratus</i>	MPE01671	32.50	2.91	1.18	1.67	1.24	1.18	1.28	2.42	0.68	4.78
<i>serratus</i>	MPE01713	29.00	2.88	1.12	1.64	1.18	1.21	1.18	2.23	0.53	4.55
<i>serratus</i>	MPE01839	36.00	3.16	1.27	1.83	1.32	1.33	1.36	2.51	0.61	5.31
<i>serratus</i>	MPE01847	32.50	2.95	1.13	1.72	1.14	1.18	1.18	2.38	0.58	4.75
<i>serratus</i>	MPE02803	28.50	2.76	1.02	1.55	1.04	1.06	1.12	2.05	0.53	3.80
<i>serratus</i>	MPE03040	31.00	2.70	1.15	1.58	1.16	1.16	1.16	2.18	0.58	4.50
<i>serratus</i>	MPE03050	30.00	2.57	1.14	1.58	1.01	1.04	1.13	2.17	0.59	4.25
<i>serratus</i>	MPE03308	28.00	3.00	1.10	1.54	1.06	1.13	1.08	2.10	0.55	4.55
<i>serratus</i>	MPE04278	24.00	2.45	0.92	1.44	0.90	0.98	1.04	1.90	0.53	3.64

Species	Catalog Number	Meta-zonite 6 Length	Left Leg 6 Tarsus Length	Epiproct Width	Epiproct Length	Gonopod Aperture Width
<i>pinetorum</i>	MPE01163	0.78	0.81	0.80	0.62	1.18
<i>pinetorum</i>	MPE01283	0.78	0.75	0.64	0.56	1.13
<i>pinetorum</i>	MPE02718	0.80	0.80	0.66	0.42	1.28
<i>pinetorum</i>	MPE02752	0.81	0.84	0.67	0.42	1.20
<i>pinetorum</i>	MPE02762	0.82	0.81	0.75	0.63	1.18
<i>pinetorum</i>	MPE03921	0.83	0.77	0.64	0.59	1.18
<i>pinetorum</i>	MPE04339	0.85	0.84	0.74	0.67	1.20
<i>pinetorum</i>	MPE04598	0.88	0.76	0.74	0.69	1.23
<i>pinetorum</i>	PSE00545	0.80	0.76	0.74	0.77	1.12
<i>serratus</i>	MPE01517	1.16	1.20	0.91	0.84	1.58
<i>serratus</i>	MPE01671	1.45	1.58	1.03	0.99	1.92
<i>serratus</i>	MPE01713	1.34	1.58	0.96	0.76	1.95
<i>serratus</i>	MPE01839	1.60	1.55	1.24	1.14	2.20
<i>serratus</i>	MPE01847	1.46	1.48	1.04	0.68	1.88
<i>serratus</i>	MPE02803	1.21	1.28	1.14	0.94	1.63
<i>serratus</i>	MPE03040	1.36	1.33	0.99	0.88	1.88
<i>serratus</i>	MPE03050	1.30	1.14	0.94	0.88	1.74
<i>serratus</i>	MPE03308	1.34	1.20	1.06	0.95	1.75
<i>serratus</i>	MPE04278	1.10	1.06	0.91	0.74	1.58

Species	Catalog Number	Body Length	Collum Width	Collum Length	Head Width	Left 3rd Antennomere Length	Gnathochilarium width	Gnathochilarium Length	Clypeus Width	Inter-antennal space width	Metazonite 6 Width
<i>caddo</i>	MPE03748	12.16	1.34	0.64	0.88	0.42	0.56	0.59	1.15	0.31	1.78
<i>caddo</i>	PSE00520	10.70	1.09	0.50	0.75	0.35	0.47	0.52	1.00	0.30	1.33
<i>canadensis</i>	MPE01544	24.00	2.45	1.03	1.43	0.83	1.04	0.98	1.90	0.61	3.48
<i>canadensis</i>	MPE01649	23.00	2.79	1.17	1.67	1.00	1.13	1.10	2.18	0.66	4.13
<i>canadensis</i>	MPE01844	25.50	2.54	1.04	1.56	1.03	1.10	1.06	2.05	0.62	3.56
<i>canadensis</i>	MPE02006	29.00	2.67	1.08	1.63	1.08	1.12	1.10	2.13	0.65	3.90
<i>canadensis</i>	MPE03033	28.00	2.70	1.16	1.64	1.04	1.10	1.08	2.18	0.62	3.96
<i>canadensis</i>	MPE03086	26.00	2.76	1.16	1.67	1.05	1.22	1.18	2.23	0.63	3.80
<i>canadensis</i>	MPE03339	26.00	2.57	1.04	1.49	1.07	1.09	1.10	1.98	0.58	3.72
<i>canadensis</i>	MPE03658	19.00	1.94	0.79	1.18	0.63	0.83	0.83	1.55	0.48	2.70
<i>canadensis</i>	MPE04072	28.00	2.88	1.16	1.61	1.09	1.18	1.16	2.20	0.59	4.50
<i>canadensis</i>	MPE04529	20.72	2.45	1.02	1.46	0.93	1.08	1.02	1.92	0.54	3.44
<i>collinus</i>	MPE00097	18.88	2.23	0.93	1.43	0.78	0.96	0.92	1.82	0.60	3.12
<i>collinus</i>	MPE01759	23.50	2.35	0.90	1.40	0.99	0.98	0.98	1.94	0.52	3.44
<i>collinus</i>	MPE01780	22.00	2.11	0.85	1.20	0.85	0.90	0.90	1.75	0.53	2.92
<i>collinus</i>	MPE03163	21.50	2.13	0.83	1.32	0.81	0.88	0.90	1.75	0.54	3.03
<i>collinus</i>	MPE03441	17.70	2.10	0.65	1.27	0.78	0.80	0.96	1.72	0.48	2.85
<i>collinus</i>	MPE03538	24.00	2.40	0.95	1.48	0.93	1.03	1.02	1.95	0.59	3.60
<i>collinus</i>	MPE03539	24.00	2.43	0.95	1.50	0.86	1.00	1.00	1.95	0.55	3.45
<i>collinus</i>	PSE00574	21.58	2.23	0.90	1.38	0.84	0.91	0.98	1.83	0.55	3.02
<i>collinus</i>	PSE00840	20.65	2.15	0.88	1.30	0.91	0.93	0.91	1.73	0.46	3.10
<i>erasus</i>	MPE01330	23.00	2.43	0.95	1.38	0.81	0.92	0.92	1.84	0.53	3.12
<i>erasus</i>	MPE01365	27.00	2.72	1.05	1.58	0.98	1.18	1.10	2.13	0.58	3.93
<i>erasus</i>	MPE01390	20.50	2.17	0.87	1.26	0.80	0.86	0.83	1.70	0.43	2.95
<i>erasus</i>	MPE01401	26.00	2.56	0.96	1.54	0.92	0.95	1.05	1.98	0.55	3.56

<i>Species</i>	Catalog Number	Metazonite 6 Length	Left Leg 6 Tarsus Length	Epiproct Width	Epiproct Length	Cyphopod Aperture Width
<i>caddo</i>	MPE03748	0.42	0.34	0.59	0.42	0.74
<i>caddo</i>	PSE00520	0.41	0.27	0.49	0.38	0.53
<i>canadensis</i>	MPE01544	1.16	0.80	0.89	0.70	1.09
<i>canadensis</i>	MPE01649	1.27	0.91	1.00	0.72	1.39
<i>canadensis</i>	MPE01844	1.10	0.84	1.02	0.76	1.23
<i>canadensis</i>	MPE02006	1.09	0.94	1.04	0.67	1.32
<i>canadensis</i>	MPE03033	1.30	0.86	1.00	0.78	1.17
<i>canadensis</i>	MPE03086	1.30	0.90	1.05	0.76	1.26
<i>canadensis</i>	MPE03339	1.23	0.94	1.08	0.69	0.98
<i>canadensis</i>	MPE03658	0.86	0.64	0.74	0.59	0.93
<i>canadensis</i>	MPE04072	1.20	1.00	1.09	0.80	1.20
<i>canadensis</i>	MPE04529	1.14	0.80	0.96	0.69	1.10
<i>collinus</i>	MPE00097	0.88	0.76	0.90	0.65	1.16
<i>collinus</i>	MPE01759	1.12	0.78	0.96	0.68	1.04
<i>collinus</i>	MPE01780	0.88	0.75	0.80	0.55	0.98
<i>collinus</i>	MPE03163	0.87	0.77	0.86	0.69	1.02
<i>collinus</i>	MPE03441	0.90	0.68	0.74	0.52	0.88
<i>collinus</i>	MPE03538	1.08	0.88	0.88	0.68	1.08
<i>collinus</i>	MPE03539	0.95	0.84	0.90	0.68	1.02
<i>collinus</i>	PSE00574	0.93	0.71	0.74	0.61	1.01
<i>collinus</i>	PSE00840	0.94	0.77	0.86	0.66	1.05
<i>erasus</i>	MPE01330	1.04	0.78	0.76	0.80	1.17
<i>erasus</i>	MPE01365	1.09	0.86	0.96	0.68	1.50
<i>erasus</i>	MPE01390	0.90	0.74	0.83	0.51	1.55
<i>erasus</i>	MPE01401	1.17	0.83	0.99	0.72	1.18

Species	Catalog Number	Body Length	Collum Width	Collum Length	Head Width	Left 3rd Antennomere Length	Gnathochilarium width	Gnathochilarium Length	Clypeus Width	Inter-antennal space width	Metazonite 6 Width
<i>erasus</i>	MPE01528	26.00	2.88	1.09	1.61	0.96	1.08	1.04	2.17	0.63	4.20
<i>erasus</i>	MPE02693	24.00	2.56	1.04	1.40	0.92	0.96	0.96	1.78	0.50	3.44
<i>erasus</i>	MPE02699	22.00	2.36	1.16	1.36	0.80	0.93	0.90	1.72	0.48	3.08
<i>erasus</i>	MPE02810	22.50	2.28	0.96	1.28	0.84	0.90	1.02	1.80	0.44	3.13
<i>erasus</i>	MPE04375	21.70	2.85	1.05	1.35	0.83	1.00	0.96	2.00	0.55	3.92
<i>minor</i>	MPE03936	13.00	1.15	0.58	0.83	0.35	0.53	0.50	1.03	0.33	1.53
<i>minor</i>	MPE03938	12.50	1.16	0.56	0.83	0.35	0.56	0.59	1.10	0.32	1.36
<i>minor</i>	MPE03950	12.50	1.15	1.18	0.80	0.35	0.56	0.56	1.04	0.29	1.40
<i>minor</i>	MPE03952	13.00	1.20	0.56	0.80	0.34	0.53	0.56	1.04	0.30	1.08
<i>minor</i>	PSE00646	14.31	1.46	0.67	0.93	0.53	0.63	0.65	1.22	0.34	1.86
<i>minor</i>	PSE00650	16.25	1.46	0.70	0.94	0.49	0.62	0.64	1.23	0.33	1.93
<i>paludicolus</i>	MPE03442	18.40	1.62	0.84	1.20	0.86	0.83	0.86	1.62	0.50	1.07
<i>paludicolus</i>	MPE03530	13.45	1.33	0.59	0.98	0.44	0.63	0.65	1.26	0.37	1.86
<i>paludicolus</i>	PSE00051	14.10	1.23	0.61	0.89	0.41	0.56	0.62	1.15	0.32	1.64
<i>paludicolus</i>	PSE00569	12.10	1.39	0.64	0.95	0.55	0.62	0.67	1.23	0.37	1.87
<i>paludicolus</i>	PSE00588	13.79	1.36	0.65	0.98	0.46	0.60	0.70	1.25	0.38	1.88
<i>pinetorum</i>	MPE01003	22.86	2.41	0.98	1.35	0.94	0.88	0.94	1.76	0.40	3.28
<i>pinetorum</i>	MPE01069	16.64	1.83	0.81	1.29	0.62	0.80	0.80	1.53	0.43	2.44
<i>pinetorum</i>	MPE01162	27.00	2.45	0.90	1.33	0.84	0.93	0.93	1.77	0.50	3.25
<i>pinetorum</i>	MPE02637	18.00	1.78	0.75	1.14	0.64	0.74	0.74	1.47	0.42	2.36
<i>pinetorum</i>	MPE02720	20.00	1.90	0.80	1.25	0.63	0.85	0.80	1.63	0.48	2.68
<i>pinetorum</i>	MPE02740	23.00	2.53	0.94	1.42	0.88	0.99	1.02	1.86	0.54	3.36
<i>pinetorum</i>	MPE04599	19.00	1.95	0.68	1.21	0.65	0.81	0.78	1.58	0.47	2.79

Species	Catalog Number	Metazonite 6 Length	Left Leg 6 Tarsus Length	Epiproct Width	Epiproct Length	Cyphopod Aperture Width
<i>erasus</i>	MPE01528	1.30	0.88	1.00	0.75	1.30
<i>erasus</i>	MPE02693	1.12	0.75	1.05	0.88	1.20
<i>erasus</i>	MPE02699	0.96	0.70	0.93	0.68	1.15
<i>erasus</i>	MPE02810	0.94	0.76	0.70	0.46	1.20
<i>erasus</i>	MPE04375	1.13	0.85	0.92	0.72	1.20
<i>minor</i>	MPE03936	0.50	0.33	0.55	0.40	0.60
<i>minor</i>	MPE03938	0.50	0.31	0.59	0.37	0.74
<i>minor</i>	MPE03950	0.50	0.33	0.54	0.40	0.48
<i>minor</i>	MPE03952	0.40	0.32	0.56	0.43	0.60
<i>minor</i>	PSE00646	0.59	0.42	0.70	0.61	0.71
<i>minor</i>	PSE00650	0.59	0.38	0.70	0.58	0.76
<i>paludicolus</i>	MPE03442	0.76	0.66	0.70	0.46	0.90
<i>paludicolus</i>	MPE03530	0.60	0.41	0.63	0.43	0.51
<i>paludicolus</i>	PSE00051	0.49	0.42	0.58	0.45	0.61
<i>paludicolus</i>	PSE00569	0.54	0.39	0.60	0.53	0.64
<i>paludicolus</i>	PSE00588	0.54	0.39	0.65	0.61	0.60
<i>pinetorum</i>	MPE01003	0.98	0.86	0.85	0.48	1.00
<i>pinetorum</i>	MPE01069	0.80	0.58	0.68	0.44	0.90
<i>pinetorum</i>	MPE01162	0.90	0.78	0.80	0.58	1.06
<i>pinetorum</i>	MPE02637	0.65	0.58	0.60	0.48	0.89
<i>pinetorum</i>	MPE02720	0.80	0.62	0.67	0.62	0.96
<i>pinetorum</i>	MPE02740	0.99	0.89	0.93	0.73	1.10
<i>pinetorum</i>	MPE04599	0.74	0.64	0.93	0.74	1.00

Species	Catalog Number	Body Length	Collum Width	Collum Length	Head Width	Left 3rd Antennomere Length	Gnathochilium width	Gnathochilium Length	Clypeus Width	Inter-antennal space width	Metazonite 6 Width
<i>pinetorum</i>	PSE00545	19.96	1.80	0.78	1.10	0.62	0.75	0.86	1.49	0.41	2.35
<i>pinetorum</i>	PSE00552	18.50	1.98	0.83	1.23	0.74	0.80	0.88	1.58	0.41	2.57
<i>serratus</i>	MPE01522	23.00	2.28	0.98	1.38	0.93	0.92	0.98	1.88	0.54	3.08
<i>serratus</i>	MPE01670	31.00	3.04	1.19	1.80	1.22	1.24	1.33	2.32	0.59	4.65
<i>serratus</i>	MPE01712	31.00	3.32	1.28	1.80	1.16	1.33	1.27	2.42	0.66	4.80
<i>serratus</i>	MPE01838	32.00	2.96	1.16	1.77	1.13	1.13	1.25	2.35	0.64	4.60
<i>serratus</i>	MPE01848	32.50	3.25	1.33	1.92	1.15	1.21	1.24	2.39	0.64	4.85
<i>serratus</i>	MPE02804	28.00	2.91	1.15	1.55	1.05	1.15	1.05	2.15	0.56	4.15
<i>serratus</i>	MPE03037	28.00	2.91	1.22	1.72	1.09	1.13	1.23	2.30	0.66	4.45
<i>serratus</i>	MPE03051	27.00	2.60	1.15	1.64	0.92	1.06	1.08	2.12	0.56	3.76
<i>serratus</i>	MPE03414	29.00	3.32	1.27	1.71	1.04	1.21	1.15	2.33	0.67	4.80
<i>serratus</i>	MPE03913	24.00	2.24	1.05	1.43	0.86	1.02	1.02	1.95	0.54	3.48

Species	Catalog Number	Metazonite 6 Length	Left Leg 6 Tarsus Length	Epiproct Width	Epiproct Length	Cyphopod Aperture Width
<i>pinetorum</i>	PSE00545	0.79	0.64	0.84	0.82	0.68
<i>pinetorum</i>	PSE00552	0.84	0.66	0.84	1.23	0.90
<i>serratus</i>	MPE01522	0.96	0.78	0.86	0.64	1.00
<i>serratus</i>	MPE01670	1.40	1.14	1.10	0.94	1.24
<i>serratus</i>	MPE01712	1.43	1.08	1.21	0.96	1.38
<i>serratus</i>	MPE01838	1.43	0.96	1.10	1.03	1.27
<i>serratus</i>	MPE01848	1.43	1.03	1.03	0.90	1.48
<i>serratus</i>	MPE02804	1.30	0.93	1.13	0.93	1.18
<i>serratus</i>	MPE03037	1.35	0.94	1.10	0.91	1.28
<i>serratus</i>	MPE03051	1.15	0.84	0.98	0.80	1.25
<i>serratus</i>	MPE03414	1.35	0.88	1.15	0.98	1.20
<i>serratus</i>	MPE03913	1.13	0.78	0.98	0.70	1.05