

Distribution and diversity of semiaquatic Sparganophilidae earthworms in the Southern
Appalachian Piedmont, USA

by:

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(Under the Direction of Daniel Markewitz and Mac A. Callahan, Jr.)

ABSTRACT

There is general agreement among ecologists that earthworms are critical for ecosystem functioning. However, the ecology and diversity of the North American native family Sparganophilidae are still greatly understudied. These peculiar earthworms inhabit the interface between aquatic and terrestrial systems, occupying sediments along the margins of streams, lakes and wetlands. Three sizes of streams were compared between three US Forest Service Experimental Forests (Calhoun, Scull Shoals and Hitchiti) in the Southern Appalachian Piedmont region of North America. In each stream a transect was placed and systematically sampled during the spring, summer and fall of 2017. A total of ten species of sparganophilids were collected, all of them new to science, almost doubling the number of species within the family. None of the species were shared between sites, revealing high local endemism. In light of these results, this North American native family of earthworms is suggested to be an excellent candidate for further study as an eco-evolutionary model.

INDEX WORDS: Sparganophilidae, Species diversity, Endemism, New species, Sediment,
Community structure

DISTRIBUTION AND DIVERSITY OF SEMIAQUATIC SPARGANOPHILIDAE
EARTHWORMS IN THE SOUTHERN APPALACHIAN PIEDMONT, USA

by:

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DEDICATION

Through my short career in science, it has become obvious that my success is due to the sacrifices of three major groups of people, to whom I humbly dedicate my work. To some extent, it is also the result of their work.

Firstly, to all the first people of underrepresented minorities who are representing their own in any field of science. Thanks to the mostly unrecognized and unmeasurable sacrifices that you all have endured, today it is a little easier for us. Thanks for proving that science is not about race, ethnicities, genders, or languages.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Earthworms are generally classified as ecosystem engineers (Jouquet *et al.* 2006), defined as organisms that directly or indirectly regulate the availability of resources for other species by causing physical state changes in the ecosystem (Jones *et al.* 1994). Both ecologists and the general public recognize the importance of earthworms in ecosystems. Earthworms generate changes in ecosystems through their burrowing activities, promoting a generation of a seed bank by burying seeds (Decaëns *et al.* 2001, 2003; Eisenhauer *et al.* 2009b), accelerating decomposition of leaf litter and organic matter, generating aeration in soils, mixing soil horizons, changing nutrient content, and generating castings (Blanchart *et al.* 1999; Blouin *et al.* 2013; Lavelle *et al.* 2006). All of these together allow earthworms to directly and indirectly influence nutrient cycles (Bohlen *et al.* 2004a; b; Eisenhauer *et al.* 2007), diversity of microbes (Dempsey *et al.* 2011, 2013; Drake & Horn 2007; Li *et al.* 2002), invertebrates (Burtis *et al.* 2014; González *et al.* 2003; Mueller *et al.* 2016; Schlaghamerský *et al.* 2014), vertebrates including birds (Loss & Blair 2011, 2014) and salamanders (Cáceres-Charneco & Ransom 2010; Maerz *et al.* 2009; Ransom 2012, 2017), and plants (Eisenhauer *et al.* 2009a, 2010; Forey *et al.* 2011). However, in spite of much work describing ecological effects of earthworms, studies on their basic taxonomy and diversity have lagged significantly behind. For example, the Surales landscape, in the Orinoco Llanos, are dominated by unique soil formations that can be up to 5 m in diameter and up to 2 m tall created exclusively by earthworms. However, the species of earthworms responsible for creating these

remarkable structures and others species that inhabit them are still lacking formal published scientific descriptions (Zangerlé *et al.* 2016). In North America, most efforts have focused on the study of the invasive European lumbricids or the Asian pheretimoids because of the negative effects on native habitats and because of their agricultural importance (Hendrix *et al.* 2008; Moore *et al.* 2018; Snyder *et al.* 2013). Native species have received little attention, and their diversity is barely known.

North America is home to ~170 recognized earthworm species (Reynolds & Wetzel 2012), of which, ~130 are native. The west coast native earthworm diversity is dominated by members of the family Megascolecidae, including the genera *Arctiostrotus* McKey-Fender, 1982, *Argilophilus* Eisen, 1893, *Chetcodrilus* Fender & McKey-Fender, 1990, *Drilocheira* Fender & McKey-Fender, 1990, *Driloleirus* Fender & McKey-Fender, 1990, *Kincaidodrilus* McKey-Fender, 1982, *Macnabodrilus* Fender & McKey-Fender, 1990, *Nephralaxis* Fender & McKey-Fender, 1990, and *Toutellus* Fender & McKey-Fender, 1990, all with a total of 41 known species (Fender & McKey-Fender 1990; James 1994; McKey-Fender *et al.* 1994) and reportedly more than 50 undescribed species (Fender & McKey-Fender 1990). East of the Rockies, the native earthworm diversity of North America is currently understood to be dominated by the genus *Diplocardia* Garman, 1888, (family Acanthodrilidae) which contains ~50 known species (James 1990), and the genera *Bimastos* Moore, 1893, and *Eisenoides* Gates, 1969, (family Lumbricidae) with 12 and two species respectively (Csuzdi *et al.* 2017). The recognized eastern North American earthworm diversity is rounded out by the monotypic families Lutodrilidae and Komarekionidae, and the semiaquatic earthworms of the Sparganophilidae.

In comparison with terrestrial earthworms, semiaquatic species have received much less attention (James 1995). For example, the single member of the family Lutodrilidae, *Lutodrilus*

multivesiculatus, also known as the Louisiana mud worm, was not described until 1976, in spite of its large size (reaching up to 40 cm long), and unusual coloration with different tones of blue, green and purple (McMahan 1976). Similarly, another semi-aquatic family, the Sparganophilidae, has only 11-13 poorly known species and subspecies in a single genus: *Sparganophilus*. *Sparganophilus tamesis* Benham, 1892, was the first described species of the genus. It was collected from the Thames River in England, from which it takes its name. However, Benham did not consider it native to the river or even to the old world, as the novel genus and species most closely resembled the neotropical Rhinodrilidae (including Glossoscolecidae) to which he believed it belonged (Benham 1982). Over the course of more than a century, *Sparganophilus* has been assigned to different families including the Glossoscolecidae, Lumbricidae and Criodrilidae before it was broadly recognized as a family of its own in 2002, after the first earthworm genetic phylogeny was published (Jamieson *et al.* 2002) and was supported again later on (Anderson *et al.* 2017; James & Davidson 2012). However, no major systematic revision of the genus has been performed.

In addition to the scarce taxonomic and systematic treatment of the group, little is known about the ecology of Sparganophilidae other than they exist primarily along the shores of fresh water systems. Only two ecological works have been published since the original description. Both publications are more than 50 years old, and these report very different phenological patterns and major morphological differences within a single nominal species (Hague 1923; Harman 1965). Because of the importance of earthworms in terrestrial ecosystems, it is possible that the role of the semiaquatic species, including the Sparganophilidae, may be greatly significant in different ecological processes including but not limited to nutrient cycles, organic matter decomposition, and drivers of biodiversity changes.

Moreover, invasive species of earthworms are known to displace natives when appropriate environmental conditions and propagules of invasive taxa are present (Callaham *et al.* 2006; Chang *et al.* 2016b; Hendrix *et al.* 2006; Winsome *et al.* 2006; see Appendix A). In addition, semiaquatic and facultative semiaquatic exotic and potentially invasive species have already been reported in North America, including, for example, the lumbricids *Octolasion tyrtaeum*, *Eiseniella tetraedra*, *Helodrilus oculatus*, the ocnero-drilids *Eukerria kukenthali*, and *Eu. saltensis*, the megascolecid *Amyntas hupiensis* (Callaham *et al.* 2016; Chang *et al.* 2016a; Reynolds & Wetzel 2012). These taxa, and others that have not yet been detected or introduced, all have the potential to competitively exclude the native sparganophilids, and this represents a threat to the ecological functions the native species perform, as well as a serious threat to the biodiversity of the group, which is mostly undocumented, and poorly known.

The objectives of this research were to (1) determine which relative stream size is preferred by the Sparganophilidae and (2) describe their diversity at three sites on the Southern Appalachian Piedmont. Chapter 2 documents a seasonal survey that was carried out at three of the US Forest Service's Experimental Forests (EF): Calhoun EF near Union, South Carolina; Scull Shoals EF and Hitchiti EF, located in northern and southern units of Oconee National Forest in central Georgia, respectively. These collections were carried out at each Experimental Forest during the spring, summer and fall of 2017, on streams of three relative sizes (small, medium and large, all within a connected tributary system). All oligochaete specimens were identified using morphological characters. In Chapter 3, ten new species of Sparganophilidae are defined using morphological characters and these are placed in three different genera, almost doubling the number of known species in the family.

This research provided the basis to perform future ecological and evolutionary studies, because (1) sparganophilids are now understood to be much more diverse than originally thought; (2) new reproductive phenological data is provided, contrasting with previous studies; and (3) sparganophilids are now believed to be highly endemic, with species range distributions limited to relatively small geographical regions. Taken together, this suggests that the group could potentially serve as a good evolutionary model for studies of species radiation, species packing, bio- and phylo-geographical patterns and sympatric speciation.

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CHAPTER 2

EARTHWORM COMMUNITIES IN STREAM-SIDE SEDIMENTS OF THREE RIVER
SYSTEMS ON THE SOUTHERN APPALACHIAN PIEDMONT¹

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Abstract

Earthworms play a critical role in ecosystems, yet earthworm ecology and diversity are still greatly understudied. This is especially true for the semi-aquatic earthworms of the family Sparganophilidae. These earthworms inhabit the interface of terrestrial and aquatic ecosystems, occurring exclusively in saturated soils or sediments, along the shores of lakes, rivers and wetlands. This endemic North American family comprises eleven species in one genus, and has been virtually ignored in terms of diversity, taxonomy or ecology. Our objective was to determine the habitat preferences, and diversity of sparganophilid earthworms. Three sites (Calhoun, Scull Shoals, and Hitchiti Experimental Forests – all in the Southern Appalachian Piedmont) were selected for this study. At each stream (one small, medium and large at each site), a 50 m transect was placed along the stream bank, and samples (cylindrical, 30 cm diameter and 15 cm depth) were randomly taken at 10m intervals. These sites were sampled during the spring, summer and fall of 2017. Sediment was collected from summer and fall for particle-size analyses. Ten species of Sparganophilidae were identified, belonging to three genera, all of which are new to science. Additionally, collection of the exotic, *Eukerria saltensis* (Ocnerodrilidae), was common, while *Octolasion tyrtaeum* (Lumbricidae) and at least one *Amyntas* species (Megascolecidae) were also collected, but rarely. At least two species of aquatic oligochaetes were collected as well, one Tubificidae sp. and *Branchiura sowerbyi* (Tubificidae) but in lesser abundance. The species rarefaction curve of sparganophilids did not plateau, suggesting that more species may yet be discovered at these sites. However, only 19% of the specimens were identifiable adults, with most collected only at the spring sampling and none during the fall. Based on these data, we hypothesize a strong phenology, where reproduction occurs during late winter and/or early spring. Each site had a unique sparganophilid community with no species being shared between sites, suggesting

high levels of endemism. Sparganophilids was only explained by time, site and relative stream size, and not to percent sediment particle size (i.e., sand, silt, or clay). With this study, the number of sparganophilid species is almost doubled. This demonstrates high diversity within a tiny fraction of the distribution of the Sparganophilidae, and emphasizes a critical and evident need for further biodiversity studies focusing on earthworms.

Key Words: Sparganophilidae, species richness, diversity, endemism

Introduction

Earthworms are widely recognized as ecosystems engineers (Jouquet *et al.* 2006), with known direct and indirect interactions with microbial (Dempsey *et al.* 2011, 2013; Drake & Horn 2007; Li *et al.* 2002), plant (Decaëns *et al.* 2003; Eisenhauer *et al.* 2009b; Forey *et al.* 2011; Hale *et al.* 2006; Nuzzo *et al.* 2009), invertebrate (Burtis *et al.* 2014; Eisenhauer *et al.* 2007; González *et al.* 2003; Mueller *et al.* 2016; Schlaghamerský *et al.* 2014) and even vertebrate (Loss & Blair 2011; Maerz *et al.* 2009; Ransom 2017; Ziemba *et al.* 2016) diversity. Additionally, earthworms are known to influence nutrient cycles (Bohlen *et al.* 2004a; b; Hale *et al.* 2008), organic matter decomposition (Dempsey *et al.* 2011; Heneghan *et al.* 2007; Holdsworth *et al.* 2008), mineral weathering in soil (Carpenter *et al.* 2007; Resner *et al.* 2011), and soil structure (Blanchart *et al.* 1999; Crumsey *et al.* 2014; Snyder *et al.* 2009; Zangerlé *et al.* 2016). However, much of these studies are based almost exclusively on European lumbricids and Asian pheretimoid species because of their importance in agricultural systems and because of their negative effects in the ecosystem when occurring in North America as invasive species (Hendrix *et al.* 2008). Because of this, the diversity and basic ecology and biology of North American native species are still unknown. This is especially true for the semiaquatic earthworms. This diverse group of earthworms inhabit the ecotone habitat of terrestrial and aquatic systems, at the shore lines of streams, rivers, lakes, swamps and other water bodies (see Chanabun *et al.* 2013).

One prominent example of these taxa is the family Almididae, containing about five to six genera and 72 species. With recent studies on Asian species of *Glyphidrilus* Horst, 1889, this genus now represents the best studied taxon in the group, and this has resulted in a doubling of the number of described species (Chanabun *et al.* 2013, 2017; Chanabun & Panha 2015; Jirapatrasilp *et al.* 2016). Commonly found in a wide range of soil textures from 0-88% sand, 1.6-10% clay, and 8-

90% silt (Chanabun *et al.* 2013) and in the top 15 cm of sediment (Chanabun *et al.* 2013; Chanabun & Panha 2015; Jirapatrasilp *et al.* 2016).

In North America, the semi-aquatic earthworm community is dominated by members of the family Sparganophilidae, with eleven currently recognized species and a single genus (*Sparganophilus*) within the family (but see Chapter 3). However, few studies have focused on these organisms, much less their ecological influence in ecosystems (*c.f.*, a total of two ecological studies identified in the literature [Hague 1923; Harman 1965], and a single taxonomic revision of the genus [Reynolds 1980]). The two ecological studies deal with one (purported) species: *S. eiseni* (currently *S. tamesis*). Hague (1923) studied the *Sparganophilus* communities of Douglas Lake in Michigan, and in Homer Park and Havana, both in Illinois, and reported that it was possible to collect specimens only in specific locations. Harman (1965) also described the soils where *Sparganophilus* were collected as sandy and sandy loam with a rich concentration of organic matter. Although both authors assigned all of their specimens to “*S. eiseni*,” both reported wide variation in some of the specimens’ characteristics. In light of this variation, it is possible that these authors were actually studying multiple species, but due to the primitive state of the taxonomy of the group, they referred to their specimens as *S. tamesis* for convenience. Support to this hypothesis may be found in the fact that both authors reported very different reproduction cycles in their respective studies: Hague (1923) reported cocoon production during late July to early September, whereas in Harman (1965), reproduction lasted from April to November.

Terrestrial earthworms are usually highly endemic to a restricted geographic area, with relatively few cosmopolitan and peregrine species found in various geographic areas due to human transportation (Hendrix *et al.* 2006, 2008). In tropical forests of French Guiana, earthworm diversity was high, and community species composition differed drastically over relatively short

distances (Decaëns *et al.* 2016). This general pattern of endemism is also true of semiaquatic earthworms *Glyphidrilus*, as many of these species appear to be restricted to a single river or stream with few present in more than one system (Chanabun *et al.* 2013). A similar pattern also appears to be the case of the sparganophilid earthworms, as many species have been only found within a single river, stream, or river basin (Ikeda *et al.* unpublished data), with a few exceptions including *S. tamesis*. However, many of these species are awaiting formal description (see Chapter 3).

The objectives of this project were to (1) study the distribution of sparganophilid communities in three river systems of the Southern Piedmont, with sampling along small, medium, and large streams in each system; (2) determine the distribution and location of *Sparganophilus* within shoreline habitats, and (3) determine relationships between visible surface castings and the community size and activity of sparganophilids at a particular location.

Methods

Study Sites: Three different rivers systems were used for this study: one at Scull Shoals Experimental Forest in northern parts of the Oconee National Forest (ONF), one at the Hitchiti Experimental Forest (a southern unit of ONF), both in Georgia, and a third at the Calhoun Experimental Forest (a unit of the Sumter National Forest) in South Carolina (Figure 2.1). In each of the three river systems, we selected sample sites along waterbodies of differing size ranging from < 3.0 m wide (small streams), 7-12 m wide (medium streams) to 40-115 m wide streams (large streams or rivers) [unnamed creeks to small creeks to rivers (Oconee, Ocmulgee, and Tyger rivers, respectively)] (Figure 2.1).

Sampling: At all sampling sites, we placed a 50 m transect along the waterline of the stream bank. We randomly selected a number between 1 and 10, and used this number to locate the first

sampling site within the first 10 m of the transect, and subsequent samples were collected at 10 m intervals along the remaining 40 m of the transect, for a total of 5 samples per transect. At each sampling site, we first counted all visible earthworm castings along 100 cm within 30 cm of the waterline. Earthworm castings, including those of the sparganophilids, are easily distinguishable from the soil surface as a cluster of aggregates, usually with obviously different texture and color of that of the soil surface (Figure 2.2). Earthworm sampling consisted of pushing a cylindrical (30 cm diameter) sampler to a depth of 15 cm into stream sediments, and hand-sorting the sediments inside the sampler for all oligochaetes. At one site (Ocmulgee River), it was necessary to establish a split transect consisting of a 20 m and a 30 m long segment, due to difficulty in placing a continuous 50 m transect that could be safely sampled. Each site was visited three times, in April-May, July, and October of 2017, with the exception of the Ocmulgee River, which was flooded during the April-May sample period. Earthworms and other oligochaetes were preserved in 95% ethanol in the field. All adult earthworms were identified to species or morphospecies, and juveniles were identified to the lowest possible taxonomic level possible.

Sediment Particle Size Analyses: The sediments collected from the summer and fall samples were air-dried and sieved through a 2.0 mm screen. Following Gee & Or (2002), the sediment was then divided into two homogeneous batches of 40.0 g each, one of these batches was dried at 105°C for 24 hrs. to calculate moisture corrected dry mass. The other was placed in a 500 ml bottle with 100 ml of 50 g/L sodium hexametaphosphate solution and 250 ml of DI water, then placed in a shaker at 300 rpm overnight. The sample was then transferred into a 1000 ml cylinder and filled with DI water up to 1000 ml. After suspending all particles into the water column with a plunger, a hydrometer was placed in the cylinder and readings were taken at 0.5, 1, 90, and 1440 minutes.

Statistical Analyses: To predict the abundance of adults, juveniles and all Sparganophilidae, *Eukerria saltensis* (an introduced earthworm species), species richness, all earthworms and all Annelids, we selected the best fitted Poisson model regressions based on a global model (Abundance ~ Stream Size + Site + Time + (Stream Size x Site) + (Site x Time) + (Stream Size x Site) + (Stream Size + Time) + (Stream Size x Site x Time) + Sand + Silt) to find the model that best explained the earthworm abundance. Similarly, we used a global model (Castings ~ Sparganophilidae + *Eukerria saltensis* + *Octolasion tyrtaeum* + *Amyntas* spp.) to determine which taxa of earthworms was most responsible of the production of castings. In both analyses, we identify the best fit and most parsimonious model (based on AICc approach) using the dredge function in R package MuMIn ver. 1.42.1 (Barton 2018), in Rstudio ver. 3.3.1 (R Core Team 2016). The best model or combination of models were considered to have a weight (w) (or cumulative w) ≥ 0.80 .

Species rarefaction curves, and their 95% confidence intervals were calculated using EstimateS ver. 9.1.0 (Colwell 2013) to estimate whether we were able to effectively sample the diversity of species within these systems.

Results

Species Composition: At least 15 species of oligochaetes were collected during the period of this study, 13 were earthworms. Ten of these were species of Sparganophilidae, putatively belonging to three different genera. All of these species are new to science and are described in Chapter 3, following the taxonomy of Carrera-Martínez et al. (unpublished data). In addition to the sparganophilids, the aquatic oligochaete *Branchiura sowerbyi* and another Tubificidae sp. and the invasive *Eukerria saltensis* (Ocnerodrilidae), *Amyntas* spp. (Megascolecidae) and *Octolasion*

tyrtaeum (Lumbricidae) were collected, but in lesser frequency and abundance. Only two specimens of *Amyntas* were adult *A. corticis*, the remaining four were juveniles unidentifiable to species (Table 2.1). The species accumulation curve for all earthworms, and rarefaction curve for the sparganophilid earthworms suggest that additional species may be discovered, as neither reached a horizontal asymptote (Figure 2.3).

Predicting Earthworm Presence and Abundance: Earthworm abundance of adult Sparganophilidae, juvenile Sparganophilidae, total Sparganophilidae, total earthworms, total annelids, and *E. saltensis* were all strongly explained by one or two models containing a combination of site, stream size and time with some interactions (cumulative $w > 0.800$, Table 2.2). This model consists of an interaction of site and stream size, with sample time as a block.

Adult sparganophilids were projected to decrease through time, being collected during the spring, with only a few during the summer and none during the fall (Figure 2.4). Juveniles sparganophilids, on the other hand, were collected throughout the year, but increasing in abundance through time (Figure 2.5). *Eukeria saltensis* followed a similar, but weaker trend as adult sparganophilids (Figure 2.6). Total earthworm abundance was relatively constant throughout the seasons (Figure 2.7), and richness slightly declined (Figure 2.8). No particular trend was detected in earthworm distribution with regards to stream size.

Casting Counts as a Proxy for Earthworm Abundance: Models were overall weak. The best fit model explained 33.8% of the data variation ($w = 0.338$) and was composed by the additive effect of Sparganophilidae and *E. saltensis*, the second most fitted model ($w = 0.184$, cumulative = 0.522) was composed only by Sparganophilidae, the third ($w = 0.143$, cumulative = 0.665) was composed by *E. saltensis*, Sparganophilidae and *O. tyrtaeum*, and the fourth ($w = 0.129$,

cumulative = 0.794) by *Amyntas* spp., *E. saltensis* and Sparganophilidae (Table 2.3). The data used in these models can be found in Appendix B.

Discussion

Earthworm Abundance and Distribution: Earthworm abundance on stream shores was strongly related to stream size, time and site for all taxa collected in this study (Table 2.1, Figure 2.4-2.8). Most specimens were collected in medium and, to some extent, in smaller streams. The semiaquatic earthworms live on the edge of different water bodies and oversaturated soils (Chanabun *et al.* 2013; Reynolds 1980). Bigger streams may represent a hostile habitat for these earthworms in different ways. First, rivers have a more constant water flow than smaller streams, which may prevent sediment and organic matter deposition and thus impede the maintenance of ideal habitat. Secondly, two of the rivers in this study, the Oconee and Ocmulgee, have a dam upstream of our sample sites, while the Tyger River did not. At least in the case of the Ocmulgee River, the release of water from the dam generated a highly variable hydroperiod which could have made conditions difficult for these earthworms to establish and survive. However, the dam on the Oconee River did not produce such drastic daily changes in the water table (data from <https://waterdata.usgs.gov/nwis/rt>), suggesting that the absence of earthworms in both of these rivers cannot be strictly attributed to the dams. On the other hand, smaller streams may be more exposed to full desiccation during dry seasons, preventing consistent ideal habitat conditions. During our study, only one juvenile sparganophilid was collected in the small stream in the Hitchiti site. Even though this stream did not fully dry during our study, the long drought of 2016 may have nearly extirpated the earthworm population of the stream, with only a few cocoons surviving

to the following year. It is also possible the stream was recolonized by individuals moving up from larger streams lower in the watershed.

The abundance of these taxa, however, was not strongly predicted by any particular model, and the best model differs between taxa (Table 2.1). Nonetheless, most of these models that explained reasonable portions of the variation had either a significant time difference, or site interactions. In general, adult Sparganophilidae were collected almost exclusively during the spring, with few specimens collected during the summer and none during the fall, whereas juveniles were collected throughout the year. Although our data were limited, they suggested a phenological pattern wherein individuals mature during winter and early spring, and reproduce during spring to early summer. Our observed pattern differs from those observed in Illinois (Hague 1923) and Louisiana (Harman 1965), where reproduction was observed during late July to early September, and from April to November, respectively. This inconsistency between reproductive periods may be the result of communities composed by different species or by differences in climate and habitats. Additionally, as noted above, the profound drought in the year preceding our study (2016), may have had some influence on the patterns we observed in our sampling.

Our data are currently insufficient to determine if differences in phonologies may be due to different community species compositions because adults accounted for only 18% of all sparganophilids collected, and our understanding of species barriers within the family is not yet well-established. Both Hague, (1923) and Harman (1965), identified all of their specimens as *Sparganophilus eiseni* (currently, a synonym of *S. tamesis*), even with a wide range of morphological variations. Although it is well known that *S. tamesis* is the most widespread species of the family (Rota *et al.* 2016; Ikeda *et al.* unpublished data), our understanding of Sparganophilidae species barriers is currently evolving. Ikeda *et al.* (unpublished data) found close

to 100 putative individual molecular taxonomic units, proposed to be unique species, after two years of sampling in the East and Central US. Thus, it is uncertain if Hague (1923) and Harman (1965) only collected *S. tamesis* or several species morphologically similar to *S. tamesis*, or even if *S. tamesis* was present in their collections.

On the other hand, climate may partially explain the observed phenological pattern. In Thailand, adults of the species *Glyphidrilus*, another semiaquatic earthworm taxa, were found more commonly during the dry and early wet season (March-July) in areas above 12°N, but south of this latitude, adults were found all year (Chanabun *et al.* 2017). It is possible that the sparganophilid species in Georgia and South Carolina are adapted to reproduction during spring and early summer because there is less probability of stress by desiccation, and a greater opportunity of dispersal due to a more consistent water flow and higher discharge. However, the biology of the family is basically unknown, and studies on their diversity, life cycle and stress evasion mechanisms must be performed.

The sparganophilids abundance was not determined by sediment texture, according to the best fitting models. However, most of the samples were collected from plots with a concentration of >85% sand, with little representation of higher percentages of finer textures. This may have prevented our models from detecting any pattern driven by sediment texture. Moreover, attention should also be given to having more replicates on a species level, as most specimens collected were juveniles unidentifiable to species. Reports of different species of *Glyphidrilus* have been made from sediments composed of 0-88% sand (Chanabun *et al.* 2013), but not all species were collected across the entire range. These authors reported the collection of seven new species, six of which were collected in sediment with 79.4-88.2 % sand, 8.7-14.0 % silt and 1.6-6.5 % clay, whereas *G. vesper* was collected from sediment composed of 90% silt and 10% clay.

Of the few previous studies on semiaquatic earthworms, most link the presence of these organisms with the presence of surface castings (Chanabun *et al.* 2013, 2017; Chanabun & Panha 2015; Jirapatrasilp *et al.* 2016). These studies have used castings to determine where to sample and collect the earthworms. However, no previous studies have taken into consideration the number of surface castings as a proxy for earthworm abundance. Our study confirms that castings can be used to estimate the population of earthworms, as they were correlated to the number of earthworms collected underneath them. However, caution should be employed as this approach could underestimate the abundance of *Eukerria saltensis*, as the correlation of this species abundance was weak and not significant. However, in systems where destructive sampling (e.g., digging) cannot be done, casting counts could be employed as a proxy for overall earthworm abundance or at the very least, as an index for their activity.

Semiaquatic Earthworm Diversity: At least 13 species of earthworms were collected during this study, ten of which were new species of Sparganophilidae, belonging to three novel genera (see Chapter 3). Five of these species were collected at the Hitchiti Experimental Forest (EF), three at Calhoun EF and two at Skull Shoals EF. None of the Sparganophilidae species were shared between sites. This high apparent endemism is consistent with recent observations on Sparganophilidae (Ikeda *et al.* unpublished data) and reports on the Asian *Glyphidrilus*, for which unique species are found within specific river basins, specific rivers, or unique sites along a river. Few species were found in more than one river basin (Chanabun *et al.* 2013, 2017; Chanabun & Panha 2015; Jirapatrasilp *et al.* 2016). Further, spatial separation within a given river course may be enough to promote speciation (Jirapatrasilp *et al.* 2015). Importantly, the landscape of the Southern Piedmont and its rivers are geologically quite old. The soils surrounding the Tyger River

are estimated to be on the order of 1.3 up to 3.1 million years old (Bacon *et al.* 2012). For semiaquatic earthworms, the ridges between river basins on the Piedmont represent dryer terrestrial barriers that they likely would not be able to cross during dispersal. Because of this, these geological barriers may have provided these earthworms enough isolation and time to diversify and speciate at a fairly fine level of resolution. Moreover, in the case of the Calhoun site, three species were collected but two were found exclusively on the Tyger River and the third in the medium and small streams only. Similar patterns of spatial relationship with diversification and speciation have been reported for tropical terrestrial, semiaquatic, and arboreal earthworms in French Guiana (Decaëns *et al.* 2016). The authors conducting the study in French Guiana increased the number of known species of earthworms by five times for the country, and also predicted that many more species are yet to be discovered.

The unexpected high diversity of sympatric species of sparganophilids, and potentially of their terrestrial relatives, may be explained by the “species packing” hypothesis. When resources are abundant, high diversity is supported by different uses of the same resource (MacArthur 1970; Pellissier *et al.* 2018; Werner 1977), by specializing into a particular narrow resource (i.e., niche). Traditionally, species packing is measured in terms of food as a resource; for example, the ability to consume a particular range of food sizes and functional morphological traits (Pellissier *et al.* 2018; Werner 1977). Currently, predation (Roughgarden 1975; Vandermeer *et al.* 2006) and spatial-temporal fluctuations (Kremer & Klausmeier 2017) are now being considered to influence species packing as well. By specializing in a particular niche (therefore limiting similarity between species), competition for resources is reduced and a stable coexistence is maintained as long as sufficient resources are present (Kremer & Klausmeier 2017; Pellissier *et al.* 2018).

For earthworms, food resources are present in two primary forms: vegetable material in the leaf litter, and organic matter associated with the mineral soil. Species generally consume some ratio of both resources (Bouché 1977; Chang *et al.* 2016b). Further, if soils are considered a three-dimensional habitable space that is used as a resource (it is both food and refuge), sympatric species of earthworm may be able to coexist by having different burrowing behaviors. Earthworm functional groups have long been defined by the combinations of how species occupy spatial resources, and consume organic matter resources, with epigeic species living in and feeding upon the leaf litter, endogeic species inhabiting and feeding mostly within the mineral soil, and anecic feeding from the leaf litter and constructing deep, vertical burrows in the mineral soil (Bouché 1977). However, such categories have been recently questioned (Chang *et al.* 2016b) and are not applicable to semiaquatic taxa, as shoreline sediments do not necessarily have stable or defined horization, and the feeding behavior of these worms is essentially unknown. However, sympatric earthworm species can behave differently when occurring together (Capowiez *et al.* 2001; Huang *et al.* 2016; Jégou *et al.* 2001; Lachnicht *et al.* 2002), and this may be true for the semiaquatic taxa as well, including the sparganophilids. For example, *Pontodrilus* is a peculiar genus of earthworms with some halophilic species, inhabiting coastlines. A recently described species, *P. longissimus*, was frequently found coexisting with the peregrine *P. litoralis*. However, these species differ in their burrowing behavior, *P. longissimus* digging much deeper and producing almost no surface castings compared to *P. litoralis* (Seesamut *et al.* 2018).

Although in the case of the sparganophilids more data will be needed before drawing conclusions, we consider it unlikely that an excess of resources is the only driver of their diversity in all three sites. This is because, even though these streams are indeed subject to large annual inputs of nutrients and organic matter, they also exhibit strong spatial-temporal fluctuation

patterns, including temperature changes, drastic changes in discharge and strongly seasonal inputs of nutrients through leaf litter. These patterns would suggest that species packing might be the driving force of their diversity (Kremer & Klausmeier 2017). However, this may not be true for all sites. In the case of the Calhoun Experimental Forest site, three species were found, of which one was only collected from the medium and small streams, whereas the other two (one of which is represented by a single specimen) were collected exclusively at the river, suggesting this community may have avoided competition by habitat preference rather than by species packing mechanisms. In the case of the Hitchiti Experimental Forest site, all species were collected together in the same plots, suggesting that their community may have achieved stability through species packing and niche partitioning. Scull Shoals Experimental Forest seems to also follow a species packing model, however only six identifiable specimens were collected belonging to two species. The consistently low number of earthworms in Scull Shoals suggests a rather low resource system for sparganophilid. If such low condition states are proven to be true in Scull Shoals, then only a small functional diversity can be sustained (Kremer & Klausmeier 2017; Pellissier *et al.* 2018).

The other three earthworm species collected in this study were all invasive, with *Eukerria saltensis* the most widespread, and *Octolasion tyrtaeum* and *Amyntas* spp. being relatively rare (Table 2.3). The presence of invasive or non-native species of earthworms is usually considered to be indicative of past disturbances (Hendrix *et al.* 2008). In the case of semiaquatic earthworms, invasiveness of exotic species has not been well studied, but a study in a highly disturbed wetland in Puerto Rico found four species inhabiting the riparian forest (Alfaro & Borges 1996). Out of these species only one was native, and the other three peregrine pantropical earthworm species. Additionally, invasive European terrestrial earthworms have been dispersed through rivers and other water bodies as many of them are sold as fishing baits (Costello *et al.* 2010; Keller *et al.*

2006). While the presence of exotic species in our study is likely, at least, an indirect result of anthropogenic disturbance and distribution, it is unclear to what extent these species presence and distribution is related to disturbances relative to the result of passive transportation and deposition of propagules in the water column. Further, it is unknown whether and to what extent these exotic species may be affecting the ecology of these streams, native earthworms, or other native organisms in this habitat.

Future Directions: Earthworms, in general, are considered ecosystem engineers (Jouquet *et al.* 2006), sustaining more interactions with all sorts of ecological processes and organisms than most other known living invertebrates. Nonetheless, with the exception of three publications looking at the effects of semiaquatic earthworms on the growth of rice plantations on paddy systems (Choosai *et al.* 2010; Jouquet *et al.* 2008; Owa *et al.* 2003), virtually no research has been conducted to understand the ecology of semiaquatic species. Since all three studies have shown an increase of plant production with the presence of semiaquatic earthworms (Choosai *et al.* 2010; Jouquet *et al.* 2008; Owa *et al.* 2003) and that their castings can represent pools with high concentrations of nutrients (Choosai *et al.* 2010), there is reason to expect that these organisms have important functions in the ecosystem. Further, the only studies of the life cycle of the Sparganophilidae in North America are more than 50 years old (Hague 1923; Harman 1965). More research is needed on the ecology of the Sparganophilidae in order to understand their role in aquatic systems of the Southern Piedmont, and wherever they occur (including virtually all of eastern North America). Finally, our study in combination with other studies (Chanabun *et al.* 2013, 2017; Chanabun & Panha 2015; Jirapatrasilp *et al.* 2016; Ikeda *et al.* unpublished data) have

shown that the diversity of these organisms is still not well understood, with dozens if not hundreds of species awaiting discovery and description.

The invasive species coexisting alongside the native sparganophilids, although rare in our sampling, is concerning. Similar to the native semiaquatic taxa, semiaquatic exotic earthworms have received limited to no attention and their effects in native communities are unknown. However, their terrestrial counterparts are known to be capable of reducing native earthworm abundance, resulting in a simplified community and net diversity loss (Burtis *et al.* 2014; Hale *et al.* 2006; Lobe *et al.* 2014; Maerz *et al.* 2009; Nuzzo *et al.* 2009). In the case of the semiaquatic taxa, the risk of losing native diversity may be even greater as some of the semiaquatic invasives are rather facultative, being capable of living in fully terrestrial ecosystems. For example, *O. tyrtaeum* is commonly associated with wetlands and saturated soils in its native range (Sims & Gerard 1999) and is well known to survive in anaerobic, saturated soils (Beylich & Graefe 2002; Keplin & Broll 2002; Plum & Filser 2005), but in North America it can be found in almost any terrestrial system that has been influenced by humans. Thus, this ability to exploit multiple habitats may result in a faster and wider dispersal of the invasives as they encounter fewer barriers to their spread.

Finally, because of the recently discovered diversity, relatively easy collection protocols, and being present throughout the year, the sparganophilids could be used as a study model to understand eco-evolutionary implications of diversity and biogeography with almost unlimited possibilities. For example, by understanding the mechanisms by which their diversity is driven and maintained, the sparganophilids may be used as bioindicators of a healthy, functional system. Further, it may expand our understanding of evolutionary and ecological forces (in particular competition or its avoidance) that promotes high diversity within the same habitat, particularly in

soils. Understanding these eco-evolutionary aspects are increasingly important, because it will allow predictions of how the sparganophilids could respond to the threats presented by invasive species, habitat loss, and climate change. However, before we are able to address these questions, studies on their basic biology and ecology, as well as a major systematic revision of the family are needed.

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Table 2.1. Species presence in each site, denoted by a 1. Taxonomy of the Sparganophilidae follows Chapter 3.

Family and species	Hitchiti			Skull Shoals			Calhoun		
	Ocmulgee	Falling Creek	Unnamed	Oconee	Sandy Creek	Moore's Mill Creek	Tyger River	Holcombe Branch	"Old Ray Tributary"
Sparganophilidae									
<i>GenusA sp3</i>								1	1
<i>GenusA sp7</i>		1							
<i>GenusA sp8</i>		1							
<i>GenusA sp2</i>							1		
<i>GenusA sp4</i>					1	1			
<i>GenusA sp5</i>					1	1			
<i>GenusA sp6</i>		1							
<i>GenusA sp1</i>							1		
<i>GenusB sp9</i>		1							
<i>GenusC sp10</i>		1							
Sparganophilidae juveniles	1	1	1	1	1	1	1	1	1
Lumbricidae									
<i>Octolasion tyrtaeum</i>						1			
Megascolecidae									
<i>Amyntas corticis</i>								1	
<i>Amyntas juveniles</i>					1	1		1	
Ocnerodrilidae									
<i>Eukerria saltensis</i>		1		1	1	1	1	1	
Tubificidae									
<i>Branchiura sowerbyi</i>							1		
Tubificidae sp	1				1	1	1		

Table 2.2. Best fitted model(s) predicting adult and juvenile sparganophilids, *Eukerria saltensis* and all earthworm species abundance and species richness.

Response	Model rank (<i>w</i>)	Parameters
Adult Sparganophilidae	1 (0.739)	Stream size + Site + Time + (Stream size x Site)
	2 (0.152)	Stream size + Site + Time + (Stream size x Site) + (Site x Time)
Juvenile Sparganophilidae	1 (0.843)	Stream size + Site + Time + (Stream size x Site) + (Stream size x Time) + (Site x Time)
All Sparganophilidae	1 (0.841)	Stream size + Site + Time + (Stream size x Site) + (Stream size x Time) + (Site x Time)
<i>Eukerria saltensis</i>	1 (0.697)	Stream size + Site + Time + (Stream size x Site) + (Stream size x Time)
	2 (0.236)	Stream size + Site + Time + (Stream size x Site) + (Stream size x Time) + (Site x Time)
Total number of earthworms	1 (0.477)	Stream size + Site + Time + (Stream size x Site) + (Stream size x Time) + (Site x Time) + (Stream size x Site x Time)
	2 (0.353)	Stream size + Site + Time + (Stream size x Site) + (Stream size x Time) + (Site x Time)
Earthworm species richness	1 (0.765)	Stream size + Site + Time + (Stream size x Site) + (Stream size x Time) + (Site x Time) + (Stream size x Site x Time)
	2 (0.119)	Stream size + Site + Time + (Stream size x Site) + (Stream size x Time)

Table 2.3. Top four models detected by the AICc approach, explaining the number of earthworm castings per 1 m long x 0.30 m wide section of shoreline at sample point.

Model rank	Parameters	Estimate (+/- SE)	<i>t</i> -value	P-value
1	Intercept	4.180 (1.797)	2.326	0.0216
	<i>E. saltensis</i>	2.377 (1.308)	1.818	0.0715
	Sparganophilidae	1.384 (0.321)	4.311	<0.001
2	Intercept	5.1677 (1.728)	2.99	0.003
	Sparganophilidae	1.389 (0.324)	4.29	<0.001
3	Intercept	4.168 (1.801)	2.314	0.022
	<i>E. saltensis</i>	2.679 (1.388)	1.930	0.056
	Sparganophilidae	1.375 (0.322)	4.272	<0.001
	<i>O. tyrtaeum</i>	-4.2984 (6.503)	-0.661	0.510
4	Intercept	4.264 (1.811)	2.354	0.020
	<i>E. saltensis</i>	2.487 (1.331)	1.868	0.064
	Sparganophilidae	1.385 (0.322)	4.301	<0.001
	<i>Amyntas</i> spp.	-2.879 (5.975)	-0.482	0.631

Figure 2.1. Map showing sites and plot locations in the Southern Appalachia Piedmont. Site 1 is Calhoun Experimental Forest (EF); 2, Scull Shoals EF; and 3, Hitchiti EF.

Figure 2.2. Casting produced by a Sparganophilidae specimen, observed in Sandy Creek, Scull Shoals Experimental Forest (33.7238N, 83.2864W) on February 7, 2017

Figure 2.3. Species rarefaction curves for all earthworm taxa (Top) and only sparganophils (Bottom). Green shade represents 95% confidence intervals.

Figure 2.4. Projected abundance of adult sparganophilid based on the best fitting model (Table 2.2), on all sites and streams sizes. Error bars are 95% confidence intervals.

Figure 2.5. Projected abundance of juvenile sparganophilid based on the best fitting model (Table 2.2), on all sites and streams sizes. Error bars are 95% confidence intervals.

Figure 2.6. Projected abundance of *Eukerria saltensis* based on the best fitting model (Table 2.2), on all sites and streams sizes. Error bars are 95% confidence intervals.

Figure 2.7. Projected abundance of all earthworm based on the best fitting model (Table 2.2), on all sites and streams sizes. Error bars are 95% confidence intervals.

Figure 2.8. Projected earthworm species richness based on the best fitting model (Table 2.2), on all sites and streams sizes. Error bars are 95% confidence intervals.

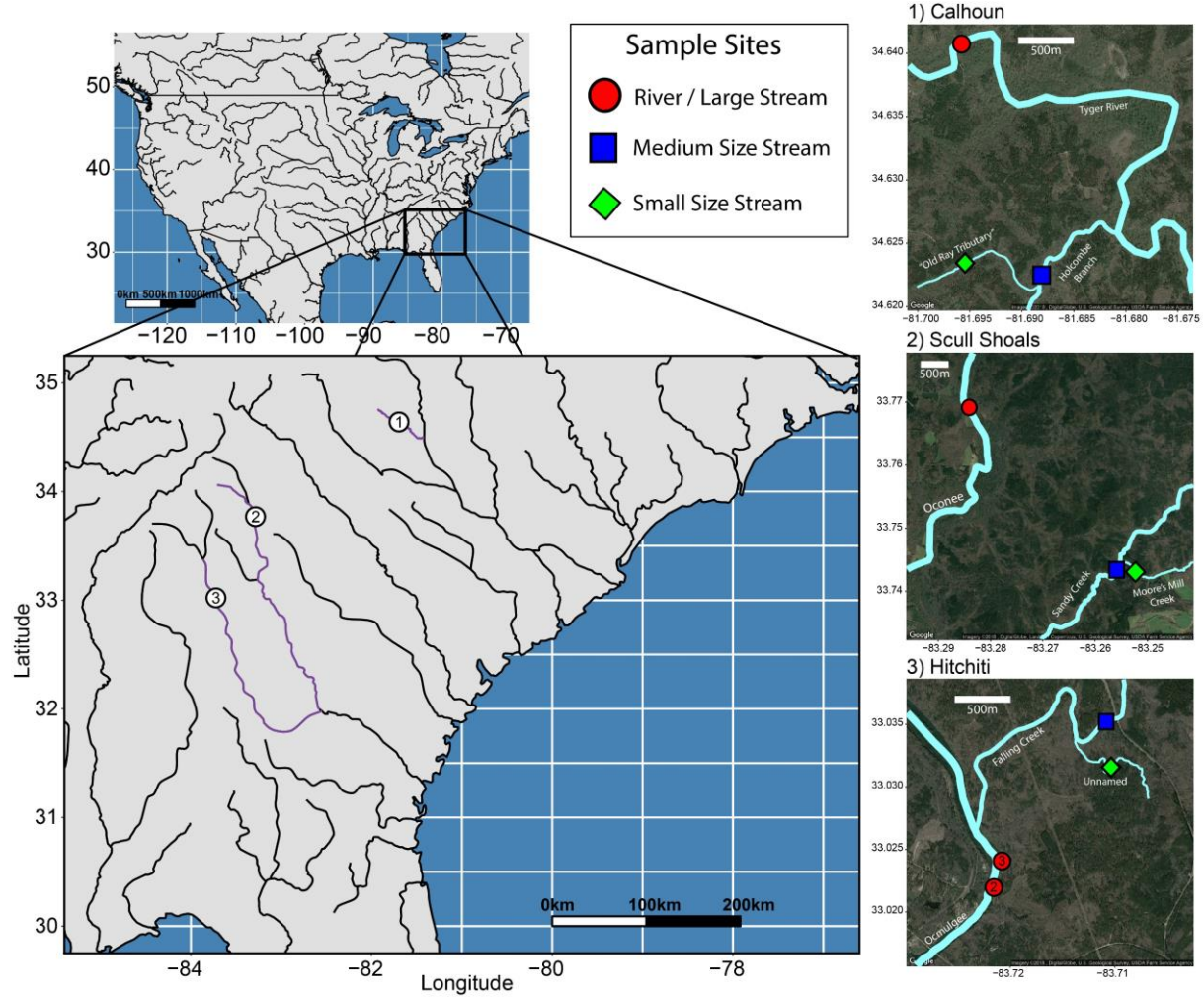


Figure 1.



Figure 2.

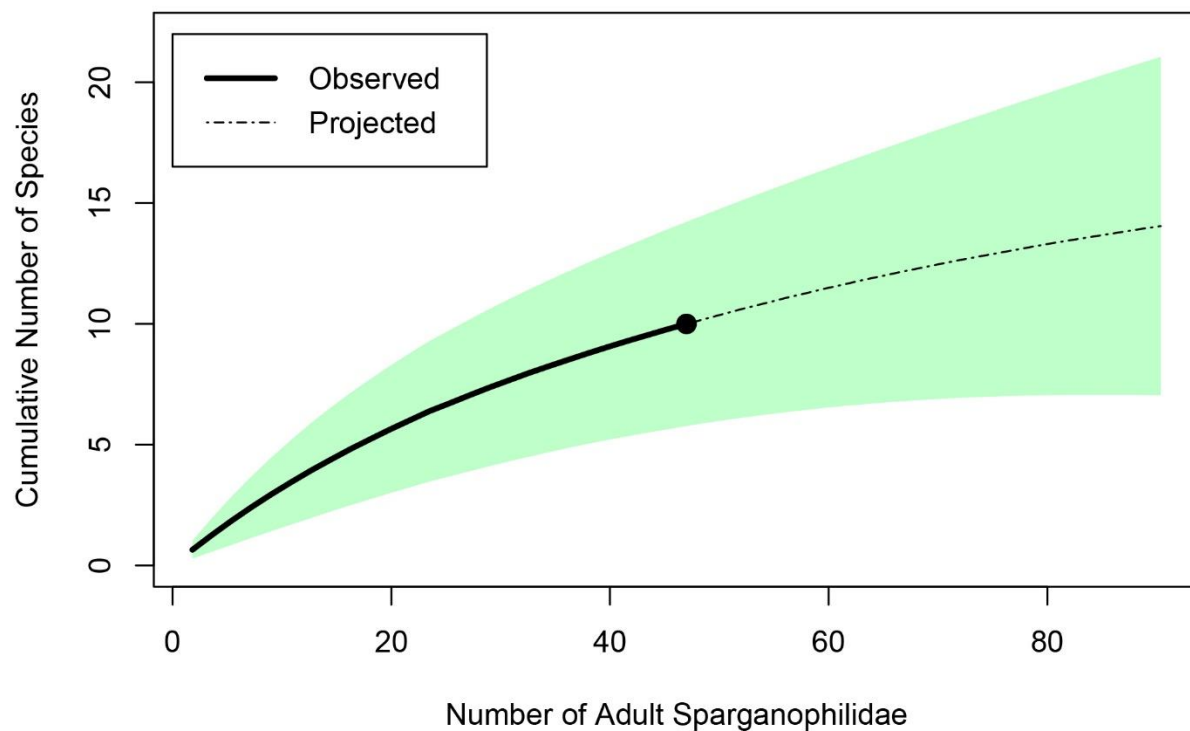
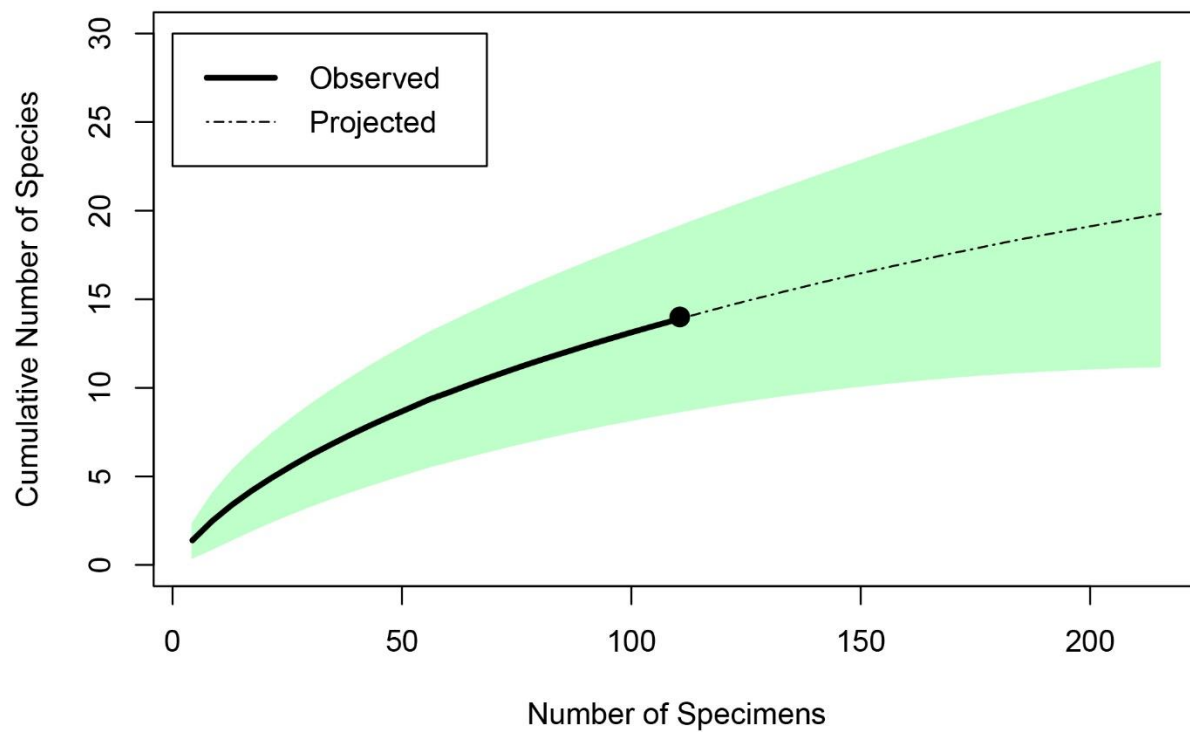


Figure 3.

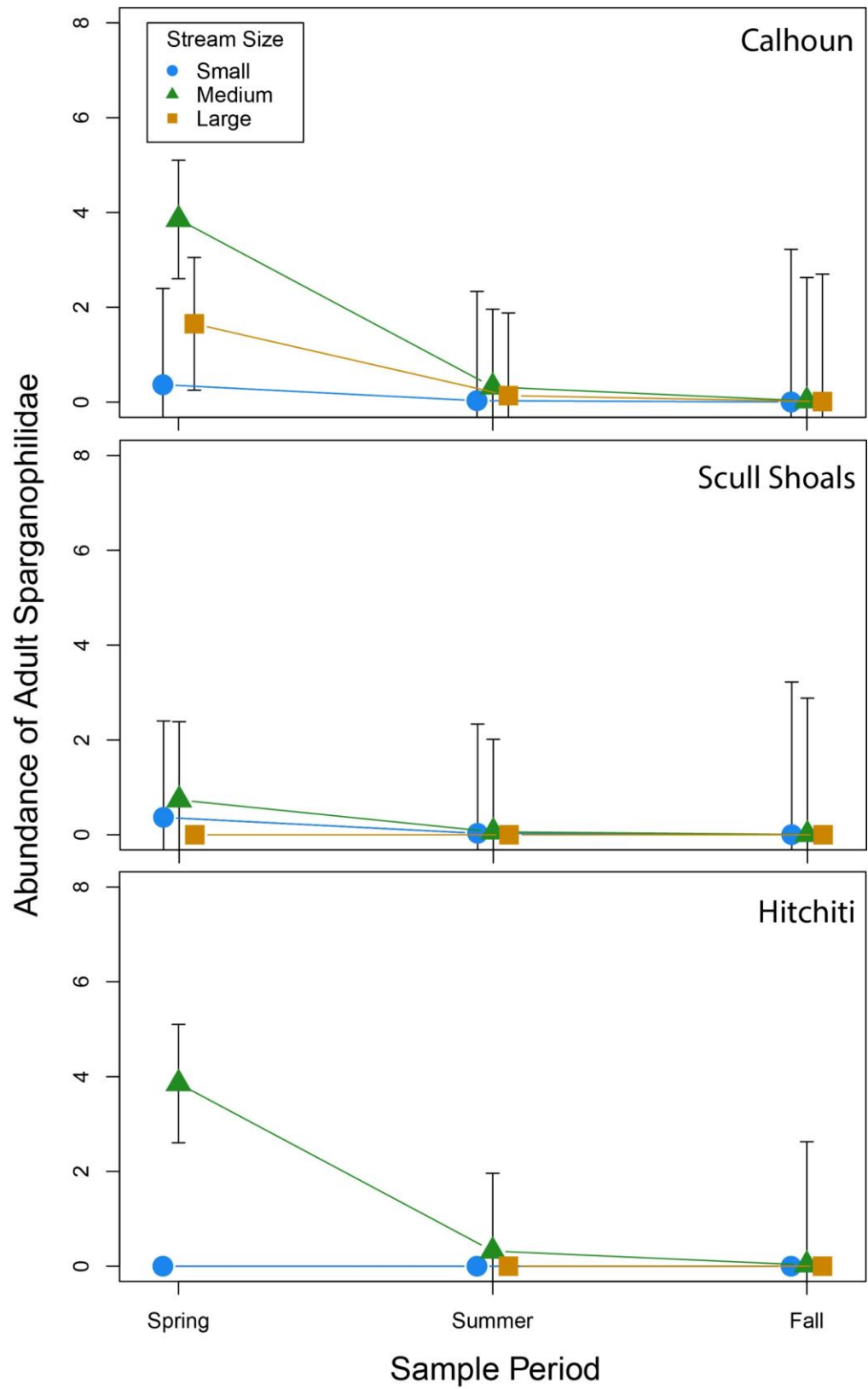


Figure 4.

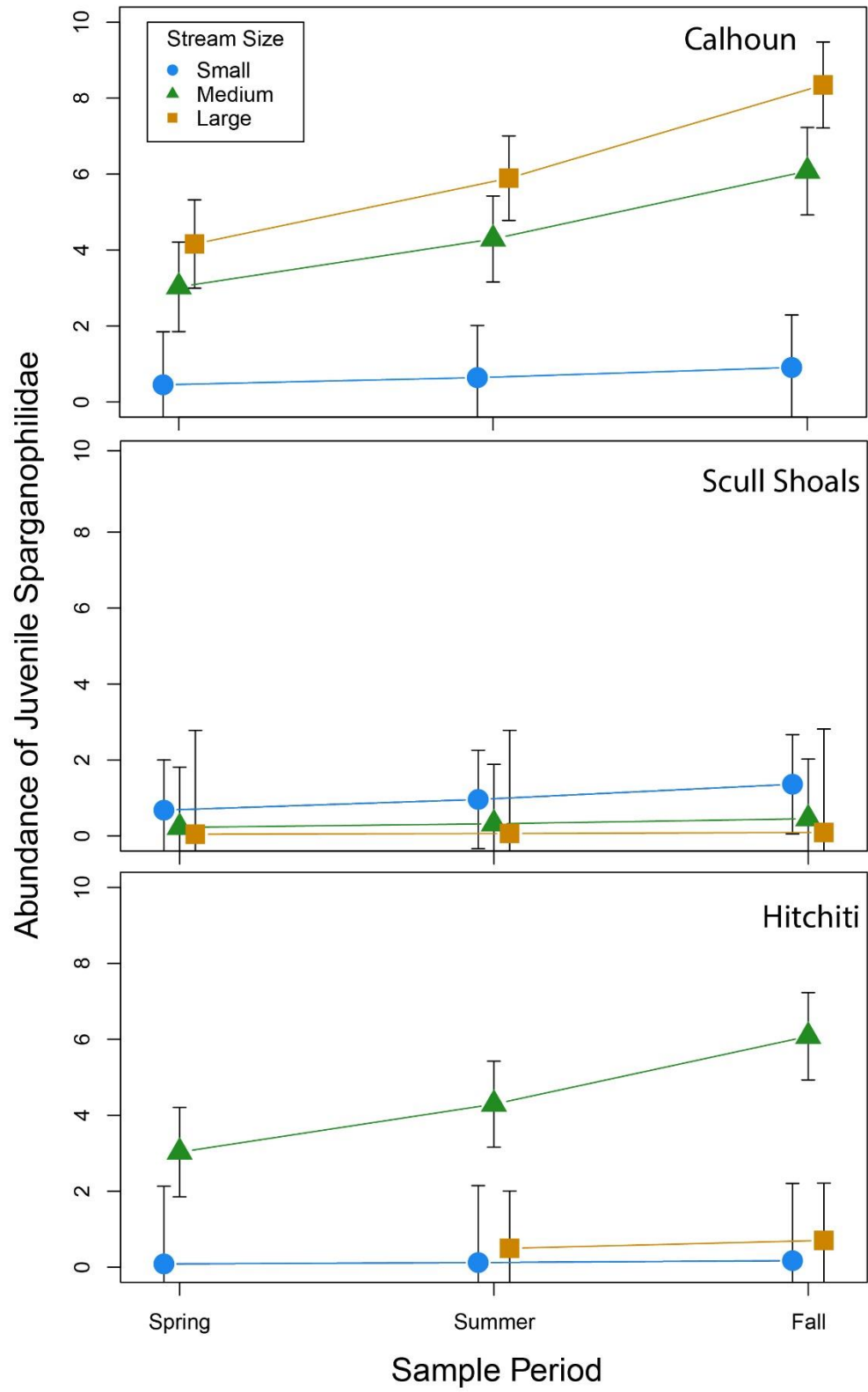


Figure 5.

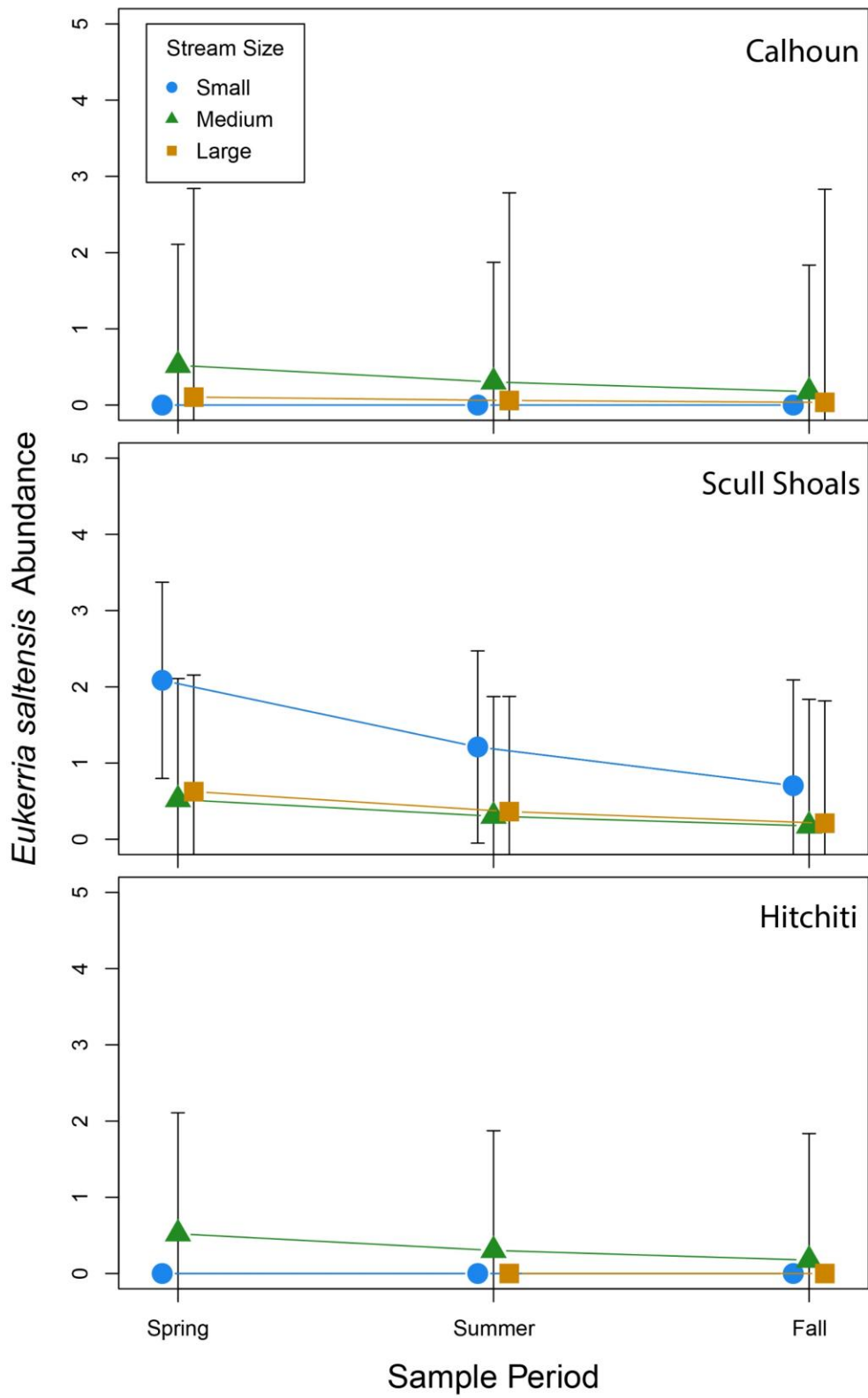


Figure 6

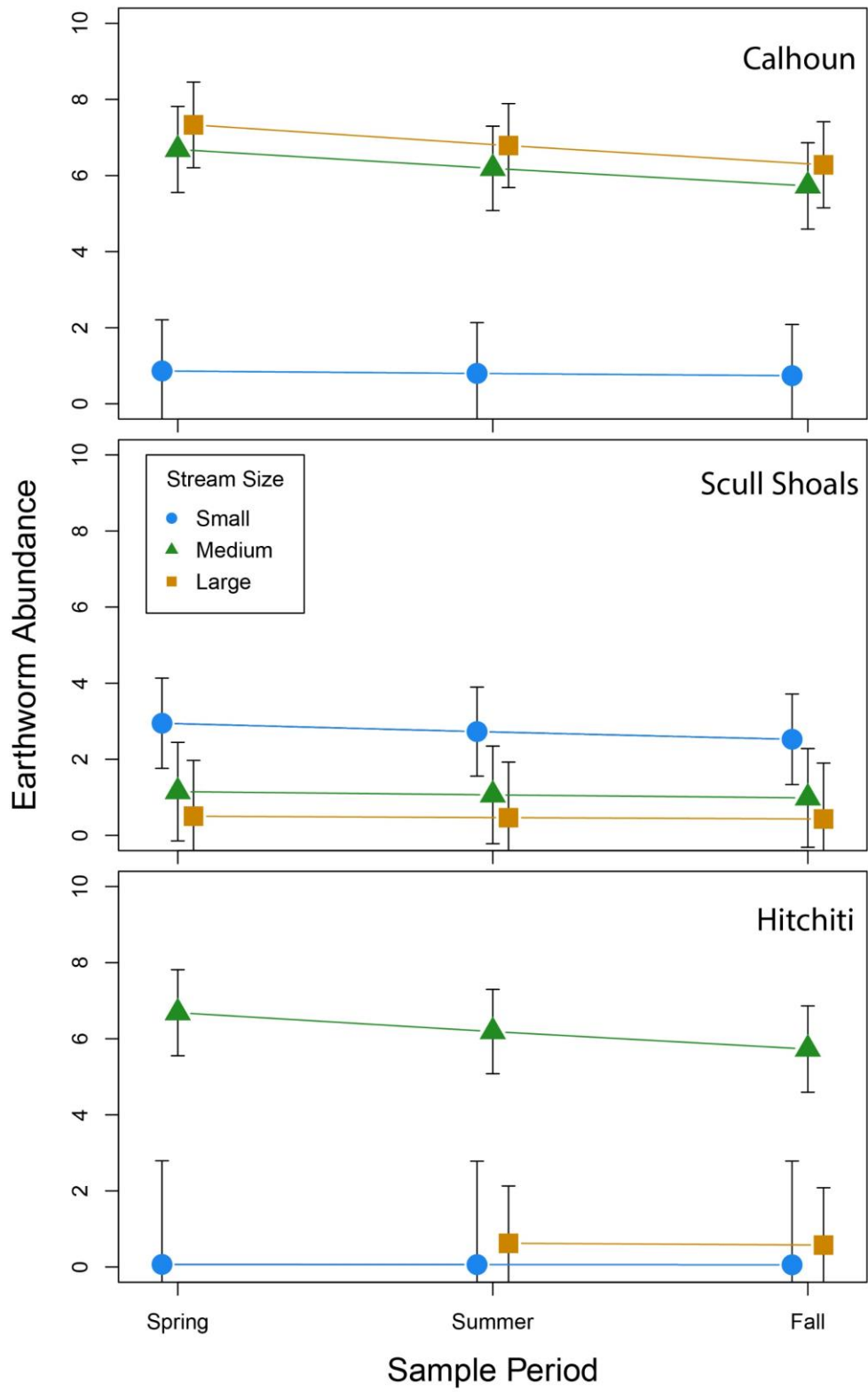


Figure 7

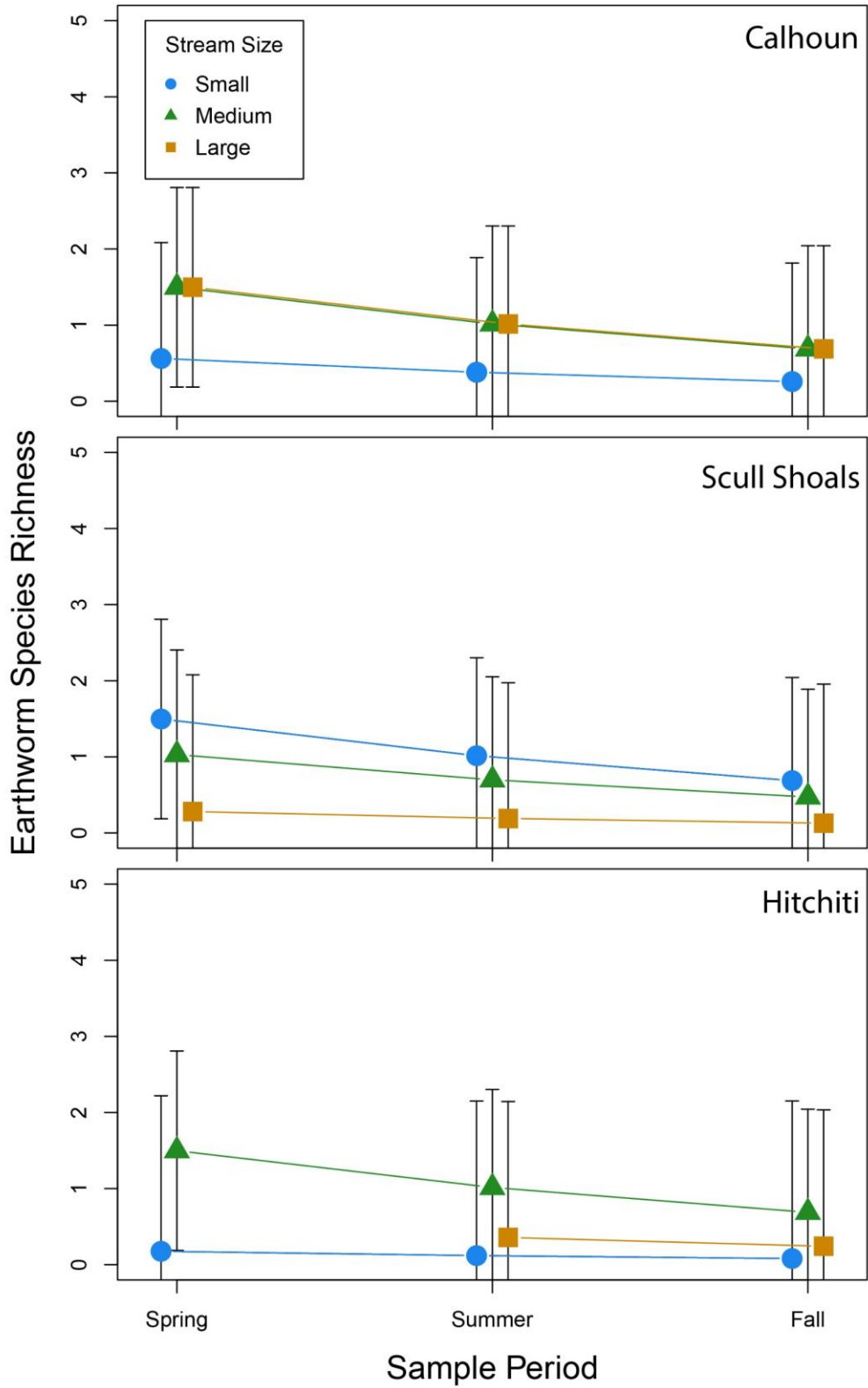


Figure 8

CHAPTER 3

A NEW GENUS AND TEN NEW SPECIES OF EARTHWORMS (OLIGOCHAETA:
SPARGANOPHILIDAE) COLLECTED FROM RIVER SEDIMENTS ON THE SOUTHERN
APPALACHIAN PIEDMONT, USA^{2,3}

²Based after unpublished work by Ikeda *et al.* and Carrera-Martínez *et al.*

³Carrera-Martínez, R.; Callaham Jr., M.A.; Snyder, B.A.; James, S.W; to be submitted to the
Journal of Natural History

Abstract

After one year of surveying semiaquatic earthworms (Oligochaeta, Sparganophilidae), *GenusA sp3 n. sp.*, *GenusA sp7 n. sp.*, *GenusA sp8 n. sp.*, *GenusA sp2 n. sp.*, *GenusA sp4 n. sp.*, *GenusA sp5 n. sp.*, *GenusA sp6 n. sp.*, *GenusA sp1 n. sp.* and *GenusB sp9 n. sp.* are described based on an extensive suite of morphological characters. The species of *GenusA* here described can be organized into species groups: (1) *GenusA sp3* and *GenusA sp4* with annular clitellum, (2) *GenusA sp8* with only a single pair of spermathecae, (3) *GenusA sp6*, *GenusA sp7*, *GenusA sp5*, *GenusA sp2*, and *GenusA sp1* having a saddle-shaped clitellum. *GenusB sp9 n. sp.* is characterized by having three discreet pairs of tubercula pubertatis and three pairs of spermathecae. A new genus, *GenusC n. gen.* is proposed to accommodate a single species, *GenusC sp10 n. sp.*, based on the extension of the tubercula pubertatis and nephridial structure.

Key words: New species, New genus, Piedmont, Semiaquatic, Saturated soil, Descriptions

Introduction

Earthworm biodiversity in North America is still largely unknown. With an estimate of more than 170 native and introduced species (Reynolds & Wetzel 2012), every year new records are encountered and new species are discovered (e.g. Callaham *et al.* 2016; Carrera-Martínez & Snyder 2016). One of the biggest limitations in the study of earthworm biodiversity in North America is the small number of experts based on the continent throughout history, with never more than three experts working simultaneously (personal observation, corroborated through literature study). Another complication is the fact that many of the existing formal species descriptions are incomplete, which impedes the accurate identification of specimens. Finally, most of the effort in studying these organisms focuses on invasive species of the families Lumbricidae and Megascolecidae, whereas native species have not received as much attention.

One example of a poorly known North American family is Sparganophilidae. *Sparganophilus* was first described from specimens collected from the Thames River in London, United Kingdom in 1892 (Benham 1892). Benham concluded that the type species, *S. tamesis* belonged to the family Rhinodrilidae, which then was composed of Central and South American species. However, subsequent authors disagreed about the appropriate family designation for *Sparganophilus*, and the genus was variously placed within the Lumbricidae, Geoscolecidae, Glossoscolecidae and a family of its own (see Reynolds 1980). It was not until 2002 when Sparganophilidae was unanimously accepted as an independent family as the result of the first molecular phylogenetic analyses of all earthworms (Jamieson *et al.* 2002). The family Sparganophilidae differs from all other families of earthworms by having the combination of 1) spermathecae and without diverticulum all anterior to the testes; 2) a simple digestive system with no gizzards, no calciferous glands (or lamellae), and no typhlosole; 3) meganephridia never

anterior to xiii; and 4) male pores within the clitellum and lacking true prostatic glands. Sparganophilids are also inhabitants of saturated soils and river sediments, rather than fully terrestrial habitats. Contrasting with its closest relative [based on genetic analysis (James & Davidson 2012; Jamieson et al. 2002)], the monotypic and elusive Komarekionidae inhabits terrestrial soils, has a strong gizzard in vi, and meganephridia present anterior to xiii. The North American families Sparganophilidae and Komarekionidae together with Kynotidae, from Madagascar, and Biwadrilidae, from Japan, form a clade that constitute the earliest extant earthworm branch (James & Davidson 2002).

Although the position of the Sparganophilidae in the annelid evolutionary tree has been clarified, its species diversity has not, and different authorities differ in the number of valid species recognized. Even worse, authorities differ on the definitions of species and intraspecific variation that are recognized. For example, Reynolds (1980, 2008) recognized the validity of *S. eiseni* Smith, 1895, *S. elongatus* Friend, 1921, and *S. langi* Bouché & Qui, 1998 as different species, while Jamieson (1971) and Rota *et al.* (2016) included these three nominal species as junior synonyms of *S. tamesis*. Reynolds (1980, 2008) justifies the validity of each of these species primarily on differences in the *cd* setae location (mid-ventral in *S. tamesis*, dorsal in the rest), number of prostate-like glands (absent in *S. elongatus*) and the origin of the intestines (reportedly, in vii in *S. langi* and in ix in the rest). Jamieson (1971) expanded the definition of *S. tamesis* to include these variations, as he did not recognize them to be species-specific. It is worth mentioning that both authors reported that they studied the type series of *S. tamesis*, but observed different morphologies between them, especially the location of the *cd* setal lines, and male and female pores in relation to setal lines. Additionally, Reynolds (1980) used a strict definition of *S. tamesis* while Jamieson (1971) amplified it to include a relatively wide range of variations on the location of the male

pores, number of prostate-like glands and auxiliary glands, extension of the clitellum and tubercula pubertatis, and presence or absence of genital markings. Even additional variations in the location of the first pairs of meganephridia was added to *S. eiseni* by Hague (1923), before Jamieson included it as a synonym of *S. tamesis*.

This wide definition by Jamieson (1971) is accepted by Rota *et al.* (2016), as they demonstrated that *S. tamesis* Cytochrome c Oxidase I (COI) sequences from 16 specimens were almost identical to those of specimens freshly collected close to the type localities of these three species and from the expanded distribution of *S. eiseni* and *S. tamesis* and 10 additional sequences obtained from the BOLD database. Furthermore, Rota *et al.* (2016) question the validity of some of the species described by Reynolds (1980), and concluded that the differences between the definitions of *S. tamesis* by Reynolds (1980) and Jamieson (1971) are due to mistakes originating with the former author. No mention of the setal formula or location is given in the original description by Benham (1892), but in a cross-section illustration, the setal lines *cd* are clearly mid-ventral, not dorsal. This is one of the characters that Smith (1895) used to define *S. eiseni*. Neither Jamieson (1971) nor Rota *et al.* (2016) indicated whether this was considered to be an inaccurate illustration of the setal lines by Benham or not. The inconsistency between authors in combination with the incompleteness of the original descriptions of most of the species makes the group taxonomically and ecologically challenging to study, as the most basic biological concept – that of what a species is – has not been well established for this family yet, even after 125 years since the description of the first species.

Currently, Sparganophilidae has only eleven to thirteen recognized species in a single genus, *Sparganophilus*, but unpublished records revealed four to eight undescribed genera and 90-100 new species (Ikeda *et al.*, unpublished data). Additionally, as a result of sampling performed

in three river systems in the Southeastern United States piedmont (see Chapter 2), a series of specimens collected were determined to be new to science, including one new genus not recorded in Ikeda *et al.* and Carrera-Martínez *et al.* (unpublished data). The goal of this study is to provide formal descriptions of these species.

Methods

Earthworm collection was performed in three different river systems, each including sample sites on a small, medium and large stream or river. Sites were located at Hitchiti Experimental Forest (Jones County, GA), Scull Shoals Experimental Forest (Greene County, GA), and the Calhoun Experimental Forest and Critical Zone Observatory (Union County, SC). All sparganophilid specimens were collected from randomly selected plots within a 50 m transect along the river shoreline and preserved in 95% ethanol, following procedures described in Chapter 2. After extracting a tissue sample for future molecular studies, the specimens were then fixed with formalin 10% for at least one week before further examination, after which were then preserved in 70% ethanol. Collections were performed during March, July and September 2017 at all sites.

Morphological description of the specimens was performed using a dissection microscope. All specimens were described externally, after which the internal anatomy was described by performing a dorsal dissection.

Descriptions

Family Sparganophilidae

Genus A

Diagnosis: Punctuated dark markings in preclitellar segments, in or close to the mid-lateral line between *bc*, equatorial or almost equatorial. Clitellum starting in xiv or xv. Tubercula pubertatis band-shaped starting in any of xv-xviii. Dorsal pores present or absent. Segmentation simple or secondary annulation present. Male pores in any of xviii-xx, within the tubercula pubertatis. One to four pairs of spermathecae, one pair per segment in vi-ix or vii-ix. Intestinal origin variable. Auxiliary prostate-like glands absent. Prostate-like glands present in the region of xxii-xxvii. Meganephridia vesicle U-shaped, bladder tubular and coiled laterally (Figure 3.1B-C).

GenusA spl n. sp.

Figure 3.2, A-C

Holotype: T-01 From sediments on the shore of the Tyger River, Calhoun Experimental Forest and Critical Zone Observatory, Sumter National Forest, Union, Union Co., South Carolina, USA (Coordinates: 33.76915N, 83.28407W). Col. R. Carrera-Martínez, M.K. Taylor, M.A. Callaham Jr. & G. Chapman. 18.MAY.2017.

Paratype: T-02, T-03, T08-10 together with Holotype. T06: One adult, same location as holotype. Col. R. Carrera-Martínez, M.K. Taylor, & G. Chapman. 19.JUL.2017.

Diagnosis:

Length 45-55mm. Dorsal pores absent. Clitellum saddle-shaped in xv-xxiii, xxiv, xxv, ventral limit in *b*. Tubercula pubertatis continuous in (1/n)xvii-xxii, band-shaped, ventral limit dorsal to *b*. Setae closely paired: $aa < bc$, $ab = cd$, aa or $bc < dd$. Spermathecal pores level with *cd*, in 6/7-8/9, very small. Prostatic pores in xxiii-xxvi or absent. Secondary annulation absent. Genital tumescences surrounding *ab* in xiv-xxiii, xxv all weakly developed. Intestinal origin in ix. One pair of spermathecae in each of vii-ix, spermathecae differentiated into ampulla and duct, duct tubular, twice as long or longer than the globular ampulla. Prostate-like glands and auxiliary prostate-like glands in xxiii-xxvi or absent. First nephridia in xiii.

External Characteristics (Figure 3.2A):

Length 45-55 mm. Diameter in x, 2.3-2.8 mm, in clitellum 3.3-3.8 mm. Number of segments, 150-180. Body ovate in cross-section anteriorly, dorsoventrally flattened at the clitellum, quadrangular postclitellarly, tail quadrangular and dorsoventrally flattened, anus dorsal. Preserved specimens with pale-whitish coloration, reddish-pink anteriorly, clitellum pinkish-white. Cuticle with strong green-blue iridescence. Prostomium zygotelic. Dorsal pores absent. Spermathecal pores in 6/7-8/9 level with *cd*, minute or inconspicuous. Female pores minute in xiv, anterior to *a*. Male pores lateral to *b* in xix, slightly presetal, within tubercula pubertatis. Clitellum in xv-xxiii, xxiv, xxv saddle-shaped, ventral limit in *b*. Tubercula pubertatis continuous (1/n)xvii-xxii, band-shaped, ventral limit dorsal to *b*. Prostate-like pores in xxiii-xxvi or absent. Nephridiopores anterior to *b*, starting in segment xiii. First setae in ii, lumbricine, closely paired. In x, $aa:ab:bc:cd:dd = 4.56:1.03:5.69:1.00:7.56$, in xxx, = $4.81:1.00:5.62:1.00:7.56$ (Figure 3.2C), *cd* and most *ab* absent in clitellum. Secondary annulation absent. Genital tumescences weak in xiv-xxiii, xxv surrounding *ab*.

Internal Characteristics:

Septa 7/8-8/9 thickened. Pharynx ends in segment vi, joining small esophagus. Intestine originates gradually in ix. Intestinal caeca, typhlosole, calciferous glands and gizzards absent.

One pair of spermathecae in each of vii-ix, without diverticulum. Spermathecal structure differentiated into ampulla and duct, all of similar size. Duct tubular, slender, two to three times as long as the ampulla. Ampulla globular, smooth (Figure 3.2B). Duct connects to the intersegmental furrows level with the setal line *cb*. Spermathecae located between weak muscular fiber originating anteriorly in the intersegmental section of the esophagus and connecting

posteriorly to the intersegmental-septum joint. Ovaries in xiii, free, string-like. Oviduct connects in segment xiv, anterior to *b*.

Testes free in x and xi. Epididymis in x and xi. Seminal vesicles, free in xi, and xii, large, filling most of the coelomic cavity, ramous. Vas deferens within muscular body wall. Tubular, prostate-like glands and auxiliary prostate-like glands absent or if present, greatly reduced in xxiii-xxv.

One pair of meganephridia per segment, starting at xiii. Meganephridia of xiv reduced and smaller than xiii. Those of xiii of same size as the ones in xv-xxvii, but smaller than those in xviii and afterwards. Meganephridia tubular, bladder composed of highly coiled tubules dorsally, ventrally with a u-shaped vesicle.

One pair of moniliform hearts per segment in vii-xi; all of approximately same size. Dorsal and ventral vessels present, and simple. Paired lateral vessels present, joining dorsal vessel in xiv, filiform.

Remarks: *GenusA sp1 n. sp.* differs from all other sparganophilids in its spermathecal structure and reduction or complete absence of the prostate-like glands.

GenusA sp2 n. sp.

Figure 3.3, A-C

Holotype: T-04 From sediments on the shore of the Tyger River, Calhoun Experimental Forest and Critical Zone Observatory, Sumter National Forest, Union, Union Co., South Carolina, USA (Coordinates: 33.76915N, 83.28407W). Col. R. Carrera-Martínez, M.K. Taylor, M.A. Callaham Jr. & G. Chapman. 18.MAY.2017.

Diagnosis:

Length 31 mm. Dorsal pores absent. Clitellum saddle-shaped in xv-xxiii, ventral limit in *b*. Tubercula pubertatis continuous in xviii-xix, xx, band-shaped, ventral limit dorsal to *b*. Setae closely paired: $aa < bc$, $ab = cd$, $aa < dd$, $bc < dd$. Spermathecal pores between *cd*, in 5/6-7/8, minute. Pores of prostate-like glands in xx-xxiii. Annulation simple. Genital tumescences surrounding *ab* weakly developed in xx-xxii. Intestinal origin in viii. One pair of spermathecae per segment in vi-viii, spermathecal structure moderately differentiated into ampulla and duct, smooth, duct tubular, three times as long as the globular ampulla. Prostate-like glands in xx-xxiii and auxiliary prostate-like glands absent. First nephridia in xiii, tubular bladder, wide u-shaped vesicle.

External Characteristics (Figure 3.3A):

Length 31 mm. Diameter in x 1.8 mm, in the clitellum, 2.8 mm. Number of segments, 104. Body ovoid in cross-section anteriorly, ventrally flattened at the clitellum, quadrangular in cross-section postclitellarly. Preserved specimens with a whitish-pale coloration, clitellum darker than

body. Cuticle with a strong purple iridescence. Prostomium zygalobitic. Dorsal pores absent. Spermathecal pores in intersegmental furrows 5/6-7/8 between *cd*, small. Female pores minute in xiv, anterior and ventral to *aa*, minute but visible. Male pores lateral to *b* in xix, equatorial, at the dorsal margin of tubercula pubertatis. Clitellum in xv-xxiii saddle-shaped, ventral limit in *b*. Tubercula pubertatis continuous xviii-xix at the right side, xviii-xx at the left, band-shaped, ventral limit dorsal to *b*. Prostate-like pores in xx-xxiii next to *b*, on small porophores. Nephridiopores anterior to *ab*, closer to *a*, starting in segment xiii, minute but visible. First setae in ii, lumbricine, setae closely paired. In *x*, *aa:ab:bc:cd:dd* = 4.5:1.0:6.5:1.0:7.5, in *xxx*, 5.0:1.0:6.5:1.5:7.5 (Figure 3.3C). Setae *cd* absent in clitellum. Annulation simple. Genital tumescences surrounding *ab* in xx-xxii, weakly developed. Postsetal dark spots visible just ventral to *c*, in a regular row in i-xiv. One dorsal pit in the equator of peristomium.

Internal Characteristics:

Septa 6/7-7/8 thickened. Pharynx ends in segment iv, joining a small esophagus. Intestine originates abruptly in viii. Intestinal caeca, typhlosole, calciferous glands and gizzards absent.

One pair of spermathecae per segment in vi-viii, without diverticulum. Spermathecal structure moderately differentiated into ampulla and duct, all of similar size. Duct about three times as long as the ampulla, tubular, wide. Ampulla small, globular, smooth, ovate (Figure 3.3B). Duct connects to the intersegmental furrows level with *cb*. Spermathecae located between a weak muscular fiber originating anteriorly in the intersegmental section of the esophagus and connecting posteriorly to the intersegmental-septum joint. Ovaries in xiii, free, string-like. Oviduct connects in segment xiv, anterior to *a*.

Testes free in x and xi. Epididymis not observed. Seminal vesicles, free in xi, and xii, small. Vas deferens within muscular body wall. Tubular, prostate-like glands in xx-xxiii, greatly reduced. Auxiliary prostate-like glands absent.

One pair of meganephridia per segment, starting at xiii. Meganephridia tubular, bladder composed of highly coiled tubules dorsally, ventrally with a narrow u-shaped vesicle, all of same size. Meganephridia of xiv joint to 13/14 septa. A vessel stoma is attached at the distal part of the vesicle.

One pair of moniliform hearts per segment in vii-xi, all of similar size. Dorsal and ventral vessel complete, and simple. Paired lateral vessel present, joining dorsal vessel in xiii, filiform.

Remarks: *GenusA sp2 n. sp.* differs from all sparganophilids in having prostate-like glands in xx-xxiii and reduced, having paired filiform lateral vessels joining dorsal and ventral trunks in xiii and in the position of its spermathecae.

GenusA sp3 n. sp.

Figure 3.4, A-C

Holotype: C-02 From sediments on the shore of Holcombe's Branch, Calhoun Experimental Forest and Critical Zone Observatory, Sumter National Forest, Union, Union Co., South Carolina, USA (Coordinates: 33.74339N, 83.25570W). Col. R. Carrera-Martínez, M.K. Taylor, M.A. Callaham Jr. & G. Chapman. 27.APR.2017.

Paratype: C-03-04, C-08-10 Adults. Same information as the Holotype. C07: one adult, same location as holotype, Col. R. Carrera-Martínez, M.K. Taylor, & G. Chapman. 20.JUL.2017. **C-01, 05**: two adults, From sediments on the shore of "Old Ray Tributary," (Coordinates: 34.62344N, 81.69545W). Col. R. Carrera-Martínez, M.K. Taylor, M.A. Callaham Jr. & G. Chapman. 27.APR.2017.

Diagnosis:

Length > 40 mm. Dorsal pores absent. Clitellum annular in xv-xxvii. Tubercula pubertatis continuous in xviii-xxii, band-shaped, ventral limit at *a*. Setae closely paired: $aa < bc$, $ab = cd$, $aa < dd$, $bc < dd$. Spermathecal pores between *cd*, in 6/7-8/9, inconspicuous. Pores of prostate-like glands in xxiii-xxvi. Annulation simple. Genital pad extending between *aa*, band-shaped in xxvii. Intestinal origin in ix. One pair of spermathecae per segment in vii-ix, spermathecal structure well differentiated into ampulla and duct, smooth, duct conical, as long as the globular, elongated

ampulla. Prostate-like glands in xxiii-xxvi, and auxiliary prostate-like glands absent. First nephridia in xiii, tubular bladder, wide u-shaped vesicle.

External Characteristics (Figure 3.4A):

Length >40 mm. Diameter in x, 2.5 mm, in clitellum, 3.4 mm. Number of segments, >147. Body ovoid in cross-section anteriorly, ventrally flattened at the clitellum, quadrangular in cross-section postclitellarly. Preserved specimens with whitish-pale coloration, clitellum darker. Cuticle with strong green-blue-purple iridescence. Prostomium zygotobic. Dorsal pores absent. Spermathecal pores in 6/7-8/9 between *cd*, inconspicuous. Female pores minute in xiv, anterior to *ab*, closer to *a*, minute but visible. Male pores lateral to *b* in xix, equatorial, within tubercula pubertatis. Clitellum in xv-xxvii annular, less developed between *bb*. Tubercula pubertatis continuous xviii-xxii, band-shaped including *b*, ventral limit at *a*. Prostate-like pores in xxiii-xxvi, next to *b*. Nephridiopores anterior to *ab*, closer to *a*, starting in xiii, minute but visible. First setae in ii, lumbricine, closely paired. In x $aa:ab:bc:cd:dd = 5.0:1.0:6.5:1.0:8.5$, in xxx $= 5.0:1.0:6.5:1.0:8.5$ (Figure 3.4C); *cd* absent in clitellum, some of *ab* absent in xviii-xxii. Annulation simple. Genital tumescences and genital markings, none observed, genital pad between *aa* in xxvii. Dark spots visible just at or slightly dorsal to mid-lateral line, in a regular row in i-xiv, and in some postclitellar segments. One dorsal pit in the equator of the peristomium.

Internal Characteristics:

Septa 6/7-8/9 slightly thickened, 6/7 thinnest. Pharynx ends in iv, joining a small esophagus. Intestine originates abruptly in ix. Intestinal caeca, typhlosole, calciferous glands and gizzards absent.

One pair of spermathecae in vii-ix, without diverticulum. Spermathecal structure well differentiated into ampulla and duct, all of similar size. Duct as long as or slightly longer than the ampulla, slightly conical, wide. Ampulla globular, smooth, elongated or ellipsoidal (Figure 3.4B). Duct connects to the intersegmental furrows level with *cb*. Spermathecae located between a weak muscular fiber originating anteriorly in the intersegmental section of the esophagus and connecting posteriorly to the intersegmental-septum joint. Ovaries in xiii, free, string-like. Oviduct connects in segment xiv, anterior to *a*.

Testes free in x and xi, with epididymis. Seminal vesicles, free in xi, and xii, large and each restricted to one segment. Vas deferens within muscular body wall. Tubular, prostate-like glands in xxiii-xxvi, long, coiled. Auxiliary prostate-like glands absent.

One pair of meganephridia per segment, starting at xiii. Meganephridia tubular, bladder composed of highly coiled tubules dorsally, ventrally with a narrow u-shaped vesicle, those of xiii-xxii slightly smaller than the rest. Meganephridia of xiv joined to 13/14 septa. A vessel stoma is attached at the basal part of the vesicle.

One pair of moniliform hearts per segment in vii-xi, size increasing posteriorly. Dorsal and ventral vessel complete, and simple. Paired lateral vessel present, joining dorsal vessel in xiii, filiform.

Remarks: *GenusA sp3 n. sp.* differs from other species in the genus by the location of the tubercula pubertatis in relation to its setal rows and having a genital pad in xxvii extending between *aa*.

GenusA sp4 n. sp.

Figure 3.5, A-C

Holotype: SS-04 From sand bars of Sandy Creek, near Scull Shoals Experimental Forest, Oconee National Forest, Greene Co., Georgia, USA. (Coordinates: 33.76915N, 83.28407W). Coll. R. Carrera-Martínez, M.K. Taylor, & M.A. Callaham Jr. 18.APR.2017.

Diagnosis:

Length 65 mm. Dorsal pores absent. Clitellum annular in xiv-xxv, ventrally less developed. Tubercula pubertatis continuous in (1/n)xvii-xxii, band-shaped, ventral limit in *a*. Setae closely paired: $aa > bc$, $ab = cd$, $aa < dd$, $bc < dd$. Spermathecal pores between *cd*, in 6/7-8/9, small. Prostatic pores inconspicuous in xxi-xxiii. Secondary annulation absent. Genital tumescences surrounding *ab* absent, genital pad extending between *b-b* in xxv-xxvi, xxvi. Intestinal origin in ix. One pair of spermathecae in vii-ix, spermathecal structure well differentiated into ampulla and duct, duct tubular, longer or as long as the globular, ellipsoidal and annulated ampulla. Prostate-like glands in xxi-xxiii and auxiliary prostate-like glands absent. First nephridia in xiii, tubular bladder, wide u-shaped vesicle.

External Characteristics (Figure 3.5A):

Length 65 mm. Diameter in x, 2.8 mm, in clitellum, 4.0 mm. Number of segments, 182. Body ovoid in cross-section anteriorly, ventrally flattened at the clitellum, quadrangular in cross-section postclitellarly. Anus as a dorsoventral slit. Preserved specimens with a whitish-pale

coloration, clitellum darker than body, pinkish. Cuticle with a strong purple iridescence. Prostomium zygalobitic. Dorsal pores absent. Spermathecal pores in 6/7-8/9 between *cd*, small. Female pores minute in xiv, anterior to *a*, conspicuous, ventral to nephridiopore. Male pores lateral to *b* in xix, equatorial or nearly so, within the tubercula pubertatis. Clitellum in xv-xxv annular, less developed ventrally between *bb*. Tubercula pubertatis continuous xvii-xxii, wide band-shaped, ventral limit at *a*. Prostate-like pores in xxi-xxiii next to *b*, inconspicuous. Nephridiopores in each segment anterior to *ab*, starting in segment xiii, very small. First setae in ii, lumbricine, setae closely paired. In x $aa:ab:bc:cd:dd = 4.00:1.00:3.33:1.00:8.33$, in xxx = $4.33:1.00:3.33:1.00:8.33$ (Figure 3.5C). Setae *cd* absent in the clitellar segments, *b* not visible in the tubercula pubertatis segments. Annulation simple. Genital tumescences and markings absent, genital pads present between *b-b* in xxv-xxvi. Postsetal dark spots visible, minute at the mid-lateral line, in a regular row in i-xiv. Dorsal pit in the equator of the peristomium.

Internal Characteristics:

Septa 7/8-8/9 only slightly thickened. Pharynx ends in iv, joining a small esophagus. Intestine originates abruptly in ix. Intestinal caeca, typhlosole, calciferous glands and gizzards absent.

One pair of spermathecae per segment in vii-ix, without diverticulum. Spermathecal structure well differentiated into ampulla and duct, all of similar size. Duct about as long as or longer than the ampulla, tubular. Ampulla globular, with lateral annulations, ellipsoidal or elongated, and with a digitiform tip (Figure 3.5B). Duct connects to the intersegmental furrows level with *cb*. Spermathecae located between a weak muscular fiber originating anteriorly in the

intersegmental section of the esophagus and connecting posteriorly to the intersegmental-septum joint. Ovaries in xiii, free, string-like. Oviduct connects in segment xiv, anterior to *a*.

Testes free in x and xi. Epididymis in x and xi, iridescent. Seminal vesicles, free in xi, and xii, large, and each restricted to a single segment. Vas deferens within muscular body wall. Tubular, prostate-like glands unpaired in xxi-xxiii, small, delicate. Auxiliary prostate-like glands absent.

One pair of meganephridia per segment, starting at xiii. Meganephridia of xiv joint to 13/14 septa. Those in xiii-xxiii smaller than the rest, xiv the smallest. Meganephridia tubular, bladder composed of highly coiled tubules dorsally, ventrally with a narrow u-shaped vesicle. A vessel stoma is attached at the ectal part of the vesicle.

One pair of moniliform hearts per segment in vii-xi, size increasing posteriorly. Dorsal and ventral vessel complete, and simple. Paired lateral vessel present, joining dorsal vessel in xiv, filiform.

Remarks: *GenusA sp4 n. sp.* resembles *GenusA sp3 n. sp.* in having the ventral limit of the tubercula pubertatis aligned to *a*. However, *GenusA sp4* differs from *GenusA sp3* by having the clitellum annular in xiv-xxv rather than xv-xvii, prostate-like glands in xxi-xxiii instead of xxiii-xxvi, and having the genital pad extending between *bb* in xxv-xxvi instead of being restricted to one segment in xxvii and between *aa*.

GenusA sp5 n. sp.

Figure 3.6, A-C

Holotype: SS-02 From sand bars of Sandy Creek, near Scull Shoals Experimental Forest, Oconee National Forest, Greene Co., Georgia, USA (Coordinates: 33.76915N, 83.28407W). Col. R. Carrera-Martínez, M.K. Taylor, & M.A. Callaham Jr. 18.APR.2017.

Paratype: SS-03 Same information as the holotype. SS-01 One adult, From sand bars of Moore's Mill Creek (Coordinates: 33.74306N, 83.25230W), Coll. R. Carrera-Martínez, M.K. Taylor, & M.A. Callaham Jr. 14.APR.2017. SS-06: One adult, Moore's Mill Creek, Coll. Carrera-Martínez, & M.K. Taylor, 06.JUL.2017.

Diagnosis:

Length >57, 75 mm. Dorsal pores absent or present pre- and post-clitellum, first in 2/3. Clitellum saddle-shaped in xv-xxv, xxvi. Tubercula pubertatis continuous in xvii-xxii, band-shaped, ventral limit in *b*. Setae closely paired: $aa < bc$, $ab = cd$, $aa < dd$, $bc = dd$. Spermathecal pores level with *c*, in 6/7-8/9. Prostatic pores inconspicuous in xxiii-xxvi. Secondary annulation absent. Genital tumescences surrounding *ab* weakly developed in xvii-xxii, genital pad extending between *b-b* in xxvii. Intestinal origin in ix. One pair of spermathecae per segment in vii-ix, spermathecal structure well differentiated into ampulla and duct, duct tubular, longer than or as long as the globular, ellipsoidal and undulated margin ampulla. Prostate-like glands in xxiii-xxvi

and auxiliary prostate-like glands absent. First nephridia in xiii, tubular bladder, wide u-shaped vesicle.

External Characteristics (Figure 3.6A):

Length >57, 75 mm. Diameter in x 2.5-2.8 mm, in clitellum, 3.5-4.0 mm. Number of segments, >182, 189. Body ovoid in cross-section anteriorly, ventrally flattened at the clitellum, quadrangular in cross-section postclitellarly. Anus as a dorsoventral slit. Preserved specimens with a whitish-pale coloration, clitellum darker than body, pinkish. Cuticle with a strong purple iridescence. Prostomium zygotobic. Dorsal pores absent or present pre- and postclitellarly starting in 2/3. Spermathecal pores in 6/7-8/9 between *cd*, small or inconspicuous. Female pores minute in xiv, anterior to *a*, conspicuous, ventral to nephridiopore. Male pores lateral to *b* in xix, equatorial or nearly so, within the tubercula pubertatis. Clitellum in xv-xxv, xxvi saddle-shaped. Tubercula pubertatis continuous xvii-xxii, ventral limit in *b*. Prostate-like pores in xxiii-xxvi next to *b*. Nephridiopores anterior to *ab*, starting in segment xiii, very small. First setae in ii, lumbricine, closely paired. In x $aa:ab:bc:cd:dd = 6.00:1.25:8.75:1.00:10.50$, in xxx = $6.25:1.00:8.75:1.00:10.25$ (Figure 3.6C). Setae *cd* absent in clitellum. Annulation simple. Genital tumescences surrounding *b* or *ab* in xvii-xxvi, markings absent, genital pads present between *b-b* in xxvii. Postsetal dark spots visible, minute at the mid-lateral line, in a regular row in i-xiv. Dorsal pit in the equator of the peristomium.

Internal Characteristics:

Septa 7/8-8/9 only slightly thickened. Pharynx ends in iv, joining a small esophagus. Intestine originates abruptly in ix. Intestinal caeca, typhlosole, calciferous glands and gizzards absent.

One pair of spermathecae per segment in vii-ix, without diverticulum. Spermathecal structure well differentiated into ampulla and duct, all of similar size. Duct about as long or longer than the ampulla, tubular. Ampulla globular, with lateral annulations, ellipsoidal or elongated, and with a digitiform tip (Figure 3.6B). Duct connects to the intersegmental furrows level with the setal line *cb*. Spermathecae located between a weak muscular fiber originating anteriorly in the intersegmental section of the esophagus and connecting posteriorly to the intersegmental-septum joint. Ovaries in xiii, free, string-like. Oviduct connects in segment xiv, anterior to *a*.

Testes free in x and xi. Epididymis in x and xi, iridescent. Seminal vesicles, free in xi, and xii, large and restricted to their segment. Vas deferens within muscular body wall. Tubular, prostate-like glands in xxiii-xxvi. Auxiliary prostate-like glands absent.

One pair of meganephridia per segment, starting at xiii. Meganephridia of xiv joint to 13/14 septa. Those in xiii-xxiii smaller than the rest, xiv the smallest. Meganephridia tubular, bladder composed of highly coiled tubules dorsally, ventrally with a narrow u-shaped vesicle. A vessel stoma is attached at the ectal part of the vesicle.

One pair of moniliform hearts per segment in vii-xi, size increasing posteriorly. Dorsal and ventral vessels complete, and simple. Paired lateral vessels present, joining dorsal vessel in xiv, filiform.

Remarks: *GenusA sp5 n. sp.* resembles *GenusA sp3 n. sp.* in having the clitellum in xv-xxv, xxvi and xv-xxvii (respectively), and prostate-like glands in xxiii-xxvi. *GenusA sp5* differs from *GenusA sp3* by having the ventral limit of the tubercula pubertatis level with *b*, the tubercula pubertatis in xvii-xxii and by having dorsal pores.

GenusA sp6 n. sp.

Figure 3.7, A-C

Holotype: **H-04** From sediments of Falling Creek, Hitchiti Experimental Forest, Oconee National Forest, Jones Co., GA, USA. Collected from silty sediments on the creek's shore, rich in organic matter, under a slope with some perennial herbs (Coordinates: 33.03521N, 83.71082W). Col. R. Carrera-Martínez, M.K. Taylor. 2.MAY.2017.

Paratype: H03-04, 06- 09, 12, 15, 14, 16-18. Same information as Holotype.

Diagnosis:

Length 45-80 mm. Dorsal pores absent, or if present postclitellarly, rudimentary. Clitellum saddle-shaped in xv-xxvi, xxvii, ventral limit in *b*. Tubercula pubertatis continuous in xvii-xxii, band-shaped, ventral limit in *b*. Setae closely paired: $aa < bc$, $ab \approx cd$, $aa < dd$, $bc < dd$. Spermathecal pores between *cd*, in 6/7-8/9, small. Prostatic pores in xxiii-xxv, rarely in xxvi. Annulation simple. Genital tumescences surrounding *ab* moderately developed in xxiii-xxv. Band-shaped genital pad in xxvii. Genital marking surrounding *ab* present in x, or usually absent. Intestinal origin in ix. Paired spermathecae in vii-ix, spermathecal structure moderately to well differentiated into ampulla and duct, smooth, duct tubular, three to four times as long as the globular ampulla. Prostate-like glands in xxiii-xxv, rarely in xxvi, and auxiliary prostate-like glands absent. First nephridia in xiii, tubular bladder, wide u-shaped vesicle.

External Characteristics (Figure 3.7A):

Length of unamputated specimens 45-80 mm. Diameter in x 2.0-2.5 mm, in clitellum, 3.0-3.5 mm. Number of segments of unamputated specimens, 129, 165-225. Body ovoid in cross-section anteriorly, ventrally flattened at the clitellum, quadrangular in cross-section postclitellarly. Anus as a dorsal slit. Preserved specimens with a yellow-pale coloration, clitellum darker than body. Cuticle with a strong blue-green-purple iridescence. Prostomium zygotobic. Dorsal pores absent. Spermathecal pores in intersegmental furrows 6/7-8/9 between *cd*, small. Female pores minute in xiv, anterior to *a*, minute but visible. Male pores lateral to *b* Postsetal in xix, within tubercula pubertatis. Clitellum in xv-xxvi, xxvii saddle-shaped, ventral limit in *b*. Tubercula pubertatis continuous xvii-xxii, band-shaped, ventral limit in *b*. Prostate-like pores in xxiii-xxv next to *b*, on small porophores. Nephridiopores to *ab*, closer to *a*, starting in segment xiii, very small. First setae in ii, each segment has eight setae closely paired. In x $aa:ab:bc:cd:dd = 4.95:1.05:6.60:1.00:9.07$, in xxx = $5.23:1.00:6.92:1.03:9.62$ (Figure 3.7C); *cd* absent in clitellum. Annulation simple. Genital tumescences surrounding *ab* in some or all of xv-xxvi, moderately developed. Genital pad band-shaped in xxvii, extending between *bb*. Genital markings surrounding *ab* in x present in two specimens. Postsetal dark spots visible just ventral to *c*, in a regular row in i-xiv. Dorsal pit on the peristomium not observed.

Internal Characteristics:

Septa 6/7-8/9 thickened. Pharynx ends in iv, joining a small esophagus. Intestine originates abruptly in ix. Intestinal caeca, typhlosole, calciferous glands and gizzards absent.

One pair of spermathecae per segment in vii-ix, without diverticulum. Spermathecal structure moderately to well differentiated into ampulla and duct, all of similar size. Duct three to

four times as long as the ampulla, conical. Ampulla globular, smooth, spherical to ovate (Figure 3.7B). Duct connects to the intersegmental furrows level with *cb*. Spermathecae located between a weak muscular fiber originating anteriorly in the intersegmental section of the esophagus and connecting posteriorly to the intersegmental-septum joint. Ovaries in xiii, free, string-like. Oviduct connects in segment xiv, anterior to *a*.

Testes free in x and xi. Epididymis in x and xi, iridescent. Seminal vesicles, free in xi, and xii, large, each restricted to a single segment or those in xii extending into xiii. Vas deferens within muscular body wall. Tubular, prostate-like glands in xxiii-xxv, large, long, highly coiled, rarely present in xxvi (2 specimens). Auxiliary prostate-like glands absent.

One pair of meganephridia per segment, starting at xiii. Meganephridia of xiv joint to 13/14 septa, all of similar size. Meganephridia tubular, bladder composed of highly coiled tubules dorsally, ventrally with a narrow u-shaped vesicle. A vessel stoma is attached at the basal part of the vesicle.

One pair of moniliform hearts per segment in vii-xi, all of similar size. Dorsal and ventral vessels complete, and simple. Paired lateral vessels present, joining dorsal vessel in xiv, filiform.

Remarks: *GenusA sp6 n. sp.* resembles *GenusA sp3 n. sp.*, and *GenusA sp5 n. sp.* in the location and extension of the clitellum and tubercula pubertatis. However, *GenusA sp6* has secondary annulation, lacks of dorsal pores, spermathecal duct 3-4 times as long as the ampulla, and has the prostate-like glands in xxiii-xxv.

GenusA sp7 n. sp.

Figure 3.8, A-C

Holotype: H-13 From sediments of Falling Creek, Hitchiti Experimental Forest, Oconee National Forest, Jones Co., GA, USA. Collected from silty sediments at the creek's shore, rich in organic matter, under a slope with some perennial herbs (Coordinates: 33.03521N, 83.71082W). Col. R. Carrera-Martínez, M.K. Taylor. 2.MAY.2017.

Paratype: H-19. Collected with holotype.

Diagnosis:

Length >55 mm. Dorsal pores absent. Clitellum saddle-shaped in xv-xxv, ventral limit dorsal to *b*. Tubercula pubertatis continuous in xviii-xxi, band-shaped, ventral limit dorsal to setal line *b*. Setae closely paired: $aa = bc$, $ab = cd$, $aa < dd$, $bc < dd$. Spermathecal pores between *cd*, in 6/7-8/9, inconspicuous. Prostatic pores in xxiii-xxv. Annulation simple. Genital tumescences surrounding *ab* weakly developed in xv-xxii. Intestinal origin in ix. One pair of spermathecae per segment in vii-ix, spermathecal structure well differentiated into ampulla and duct, smooth, duct conical, as long as the spherical ampulla. Prostate-like glands in xxiii-xxv and auxiliary prostate-like glands absent. First nephridia in xiii, tubular bladder, wide u-shaped vesicle.

External Characteristics (Figure 3.8A):

Length >55 mm (>13mm on paratype). Diameter in x 2.0-2.5 mm, in clitellum, 2.8-3.5 mm. Number of segments, >110 (>30). Body ovoid in cross-section anteriorly, ventrally flattened at the clitellum, quadrangular in cross-section postclitellarly. Preserved specimens with a yellow-pale coloration, clitellum pink. Cuticle with a strong blue-green iridescence. Prostomium zygotobitic. Dorsal pores absent. Spermathecal pores in intersegmental furrows 6/7-8/9 between *cd*, inconspicuous. Female pores minute in xiv, anterior to *a*, minute but visible. Male pores lateral to *b* equatorial, at the dorsal margin of the tubercula pubertatis in xxi. Clitellum in xv-xxv saddle-shaped, ventral limit dorsal to *b*. Tubercula pubertatis continuous xviii-xxi, band-shaped, ventral limit dorsal to setal line *b*. Prostate-like pores in xxiii-xxv next to *b*, inconspicuous. Nephridiopores in each segment anterior to *ab*, closer to *a*, starting in segment xiii, very small. First setae in ii, each segment has eight setae closely paired. In x *aa:ab:bc:cd:dd* = 5.00:1.00:5.75:1.00:9.50, in xxx = 5.00:1.00:6.25:1.00:9.50 (Figure 3.8C); *cd* absent in clitellum. Annulation simple. Genital tumescences surrounding *ab* in some or all of xv-xxii, weakly developed. Genital pad absent. Postsetal dark spots visible at the mid-lateral line, in a regular row in i-xiv. Dorsal pit on the peristomium not observed.

Internal Characteristics:

Septa 5/6-8/9 slightly thickened. Pharynx ends in iv, joining a small esophagus. Intestine originates abruptly in ix. Intestinal caeca, typhlosole, calciferous glands and gizzards absent.

Paired spermathecae in vii-ix, without diverticulum. Spermathecal structure well differentiated into ampulla and duct, all of similar size. Duct about as long as or slightly longer than the ampulla, conical, very thin at the ampulla junction. Ampulla globular, smooth, spherical

(Figure 3.8B). Duct connects to the intersegmental furrows level with *cd*. Spermathecae located between a weak muscular fiber originating anteriorly in the intersegmental section of the esophagus and connecting posteriorly to the intersegmental-septum joint. Ovaries in xiii, free, string-like. Oviduct connects in segment xiv, anterior to *a*.

Testes free in x and xi. Epididymis in x and xi, large, iridescent. Seminal vesicles, free, small in x, large and each restricted to a single segment in xi, and xii. Vas deferens within muscular body wall. Tubular, prostate-like glands in xxiii-xxv, small, delicate. Auxiliary prostate-like glands absent.

One pair of meganephridia per segment, starting at xiii. Meganephridia of xiv joint to 13/14 septa, all of similar size. Meganephridia tubular, bladder composed of highly coiled tubules dorsally, ventrally with a wide u-shaped vesicle, longer than the bladder. Stoma not observed.

One pair of moniliform hearts per segment in vii-xi, all of similar size. Dorsal and ventral vessels complete, and simple. Paired lateral vessels present, joining dorsal vessel in xiv, filiform.

Remarks: *GenusA sp7 n. sp.* is similar to *GenusA sp6 n. sp.*, *GenusA sp3 n. sp.* and *GenusA sp5 n. sp.*, but *GenusA sp7* has a shorter clitellum (in xv-xxv), male pores in xxi, and tubercula pubertatis ending in xxi or xxii.

GenusA sp8 n. sp.

Figure 3.9, A-C

Holotype: H-11 One adult. From sediments of Falling Creek, Hitchiti Experimental Forest, Oconee National Forest, Jones Co., GA, USA. Collected from silty sediments at the creek's shore, rich in organic matter, under a slope with some perennial herbs (Coordinates: 33.03521N, 83.71082W). Col. R. Carrera-Martínez, M.K. Taylor. 2.MAY.2017.

Diagnosis:

Length >35 mm. Dorsal pores present postclitellarly, rudimentary. Clitellum annular in xv-xxvii. Tubercula pubertatis continuous in xvii-xxii, band-shaped, ventral limit in *b*. Setae closely paired: $aa < bc$, $ab \approx cd$, $aa < dd$, $bc < dd$. Spermathecal pores between *cd*, in 7/8, small. Prostatic pores in xxiii-xxv. Secondary annulation in ii-xxvii, around the segments. Genital tumescences surrounding *ab* well developed in xxiii-xxvi. Genital markings surrounding *ab* in any of x-xiv, between *ab* in 26/27, between *cd* in 8/9. Genital pad in xxvii, band-shaped. Intestinal origin in ix. One pair of spermathecae in viii, spermathecal structure well differentiated into ampulla and duct, smooth, duct tubular, ampulla ellipsoidal and three times longer than duct. Prostate-like glands in xxiii-xxv and auxiliary prostate-like glands absent. First nephridia in xiii, tubular bladder, wide u-shaped vesicle.

External Characteristics (Figure 3.9A):

Length >35 mm. Diameter in x 2.0 mm, in clitellum, 3.0 mm. Number of segments, >102. Body ovoid in cross-section anteriorly, ventrally flattened at the clitellum, quadrangular in cross-section postclitellarly. Preserved specimens with a yellow-pale coloration, clitellum pink. Cuticle with a strong blue-green iridescence. Prostomium zygotobic. Dorsal pores present postclitellarly, first in 27/28, all rudimentary and discontinuous. Spermathecal pores in 7/8 between *cd*, small but visible. Female pores conspicuous in xiv, anterior to *ab*. Male pores lateral to *b*, within the tubercula pubertatis in 18/19. Clitellum in xv-xxvii annular. Tubercula pubertatis continuous xvii-xxii, band-shaped, ventral limit at *b*, undulated or crested. Prostate-like pores in xxiii-xxv next to *b*, inconspicuous. Nephridiopores anterior to *ab*, closer to *a*, starting in segment xiii, very small. First setae in ii, lumbricine, closely paired. In x $aa:ab:bc:cd:dd = 4.0:1.5:5.0:1.0:7.5$, in xxx $=5.0:1.0:6.0:1.0:9.0$ (Figure 3.9C). Setae *cd* absent in the clitellar segments. Secondary annulation in ii-xxvii all around the segment. Genital tumescences surrounding *ab* in xxv-xxvi, strongly developed. Genital pad in xxvii extending between *bb*, band-shaped. Unpaired genital markings surrounding *ab* in right side of x, left side of xii, xiv, and xv; paired between *cd* in 8/9, level with *ab* in 26/27. Postsetal dark spots visible at the mid-lateral line, in a regular row in i-xiv, in viii-xii a row of up to 5 dark spots can be observed in each segments' equator between *bc* at each side. Dorsal pit on the peristomium not observed.

Internal Characteristics:

Septa 7/8-8/9 thickened. Pharynx ends in iv, joining a small esophagus. Intestine originates abruptly in ix. Intestinal caeca, typhlosole, calciferous glands and gizzards absent.

Paired spermathecae in viii, without diverticulum. Spermathecal structure well differentiated into ampulla and duct. Duct about a third of the ampulla length, tubular. Ampulla globular, smooth, ellipsoidal (Figure 3.9B). Duct connects to the intersegmental furrows level with *cb*. Spermathecae located between a weak muscular fiber originating anteriorly in the intersegmental section of the esophagus and connecting posteriorly to the intersegmental-septum joint. Ovaries in xiii, free, string-like. Oviduct connects in segment xiv, anterior to *a*.

Testes free in x and xi. Epididymis not observed. Seminal vesicles, free, large and each restricted to a single segment in xi, and xii. Vas deferens within muscular body wall. Tubular, prostate-like glands in xxiii-xxv. Auxiliary prostate-like glands absent.

One pair of meganephridia per segment, starting at xiii. Meganephridia of xiv joint to 13/14 septa, all of similar size. Meganephridia tubular, bladder composed of highly coiled tubules dorsally, ventrally with a u-shaped vesicle, longer than the bladder. Bladder with a small, basal caecum. Stoma not observed.

Paired moniliform hearts in vii-xi, first two pairs smallest. Dorsal and ventral vessels complete, and simple. Paired lateral vessels present, joining dorsal vessel in xiv, filiform.

Remarks: *GenusA sp8 n. sp.* is the only species in the family with a single pair of spermathecae, located in viii.

Genus B

Diagnosis: Clitellum beginning on xv or xvi. Male pores in any of xviii-xx. Tubercula pubertatis originating on xix and ending in xxii or xxiii. Genital tumescences surrounding ab or only b present. Auxiliary prostate-like glands in the region of xv-xviii, first pair of primary prostate-like glands in the region of xxii-xxiv. Meganephridia with sack-shaped bladder, tubular distally, J-shaped vesicle (Figure 3.1D).

GenusB sp9 n. sp.

Figure 3.10, A-C

Holotype: H-01 One adult. From sediments of Falling Creek, Hitchiti Experimental Forest, Oconee National Forest, Jones Co., GA, USA. Collected from silty sediments at the creek's shore, rich in organic matter, under a slope with some perennial herbs (Coordinates: 33.03521N, 83.71082W). Col. R. Carrera-Martínez, M.K. Taylor. 2.MAY.2017.

Diagnosis:

Length 45 mm. Dorsal pores absent. Clitellum saddle-shaped in xv-xxvii. Tubercula pubertatis discontinuous in xviii-xxiii as three pairs, one on each of xviii-xix, (1/2)xx-xxi and (1/2)xxii-xxiii, ventral limit just dorsal to setal line *b*. Setae closely paired: $aa < bc$, $ab = cd$, aa or $bc < dd$. Spermathecal pores aliened to *c*, in 6/7-8/9. Prostatic pores in xxiv-xxvii, lateral to *b*, on porophores. Secondary annulation present on i-xxvii. Genital tumescences surrounding *ab* in xv-xviii strong. Intestinal origin in ix. Paired spermathecae in vii-ix, spermathecal structure well differentiated into ampulla and duct, duct tubular, shorter than the spherical ampulla. Prostate-like glands in xxiv-xxvii, auxiliary prostate-like glands in xv-xvii. First nephridia in xiii.

External Characteristics (Figure 3.10A):

Length 45 mm. Diameter in x, 2.0 mm, in clitellum, 3.0 mm. Number of segments, 140. Body ovate in cross-section anteriorly, ventrally flattened at the clitellum, quadrangular postclitellarly, tail lanceolate, anus dorsal. Preserved specimens with a whitish coloration,

clitellum white. Cuticle with a strong yellowish-green iridescence. Prostomium zygotoblic. Dorsal pores absent. Spermathecal pores in 6/7-8/9 level with *c*, conspicuous. Female pores conspicuous in xiv, anterior to *b*. Male pores lateral to *b* in xxi, within the tubercula pubertatis, Postsetal. Clitellum saddle-shaped in xv-xxvii, ventral limit in *b*, less developed in xxv-xxvii. Tubercula pubertatis discontinuous from xviii-xxiii, divided into three pairs in xviii-xix, (1/2)xx-xxi, and (1/2)xxii-xxiii, ventral limit just dorsal to *b*, disk-shaped, very prominent. Prostate-like pores paired xxiv-xxvii and of auxiliary prostate-like glands inconspicuous in xv-xvii lateral to *b* on porophores. Nephridiopores anterior to *a*, starting in segment xiii. First setae in ii, lumbricine, closely paired. In x $aa:ab:bc:cd:dd = 5.0:1.0:6.5:1.0:10.0$, in xxx $=5.0:1.0:6.5:1.0:10.0$ (Figure 3.10C). Secondary annulation present in ii-xxvii, throughout the whole segment, being tetra- or penta-ringed, postclitellarly only slightly. Genital tumescences present in xvi-xxviii surrounding *ab*.

Internal Characteristics:

Septa 7/8-8/9 only slightly thickened. Pharynx ends in vi, connecting to a small oesophagus. Intestine originates gradually in ix. Intestinal caeca, typhlosole, calciferous glands and gizzards absent.

Paired spermathecae in vii-ix, without diverticulum. Spermathecal structure well differentiated into ampulla and duct, all of similar size. Duct tubular, slender, shorter than the ampulla. Ampulla large, globular and spherical, smooth (Figure 3.10B). Duct connects to the intersegmental furrows level with *cb*. Spermathecae located between a weak muscular fiber originating anteriorly in the intersegmental section of the esophagus and connecting posteriorly to

the intersegmental-septum joint. Ovaries in xiii, free, string-like. Oviduct connects in segment xiv, anterior to *b*.

Testes free in x and xi. Epididymis in x and xi, small. Seminal vesicles, free in x, xi, and xii, those in x small, xi-xii large, all almost digitiform with a racemose dorsal end. One pair of digitiform auxiliary genital glands associated to the spermathecae in viii. Vas deferens within muscular body wall. Tubular, prostate-like glands paired in xxiv-xxvii, well developed. Auxiliary prostate-like glands in xv-xvii.

One pair of meganephridia per segment, starting at xiii. Meganephridia of xiv reduced and smaller than xiii. Those of xiii-xiv smaller than xv-xxvii, which are smaller than the following. Meganephridia tubular, highly coiled dorsally, ventrally with a J-shaped vesicle.

One pair of moniliform hearts per segment in vii-xi; all of approximately same size. Dorsal and ventral vessels present, and simple. Paired lateral vessels present, joining dorsal vessel in xiv, filiform.

Remarks: *GenusB sp9 n. sp.* is very similar to *S. komeraki* Reynolds 1980, however it differs from *S. komeraki* by the location of the tubercula pubertatis, having three pairs of spermathecae, the location of the male pores, number of segments with genital tumescences, size and number of segments.

GenusC n. gen.

Diagnosis

Clitellum starting in xv. Dorsal pores and secondary annulation absent. Lateral dark spots present in i-xiv, not forming a regular row. Male pores in xix, not within the tubercula pubertatis. Tubercula pubertatis reduced, covering only two segments, starting after xx. Intestinal origin in ix. Three spermathecal pairs in vii-ix. Auxiliary prostate-like glands absent. Meganephridia with globular bladder, vesicle u-shaped, vesicle duct stomate, bladder with a basal caecum, and multiple blood vessel vacuoles (Figure 3.1E, F).

Type species: *GenusC sp10* **n. sp.**

Etymology: from Latin *vacuola* and *nephridia*, describing the nephridial structure of the genus only species.

Remarks: *GenusC* **n. gen.** accommodates a single species, *GenusC sp10* **n. sp.**, which is unique in having a drastic posterior displacement and reduction of the tubercula pubertatis, male pores anterior to the tubercula pubertatis, and nephridia with multiple bladder vacuoles.

GenusC sp10 n. sp.

Figure 3.11, A-C

Holotype: H-02 One adult. From sediments of Falling Creek, Hitchiti Experimental Forest, Oconee National Forest, Jones Co., GA, USA. Collected from silty sediments at the creek's shore, rich in organic matter, under a slope with some perennial herbs (Coordinates: 33.03521N, 83.71082W). Col. R. Carrera-Martínez, M.K. Taylor. 2.MAY.2017.

Diagnosis:

Length 90mm. Dorsal pores absent. Clitellum saddle-shaped in xv-xxvi, ventral limit in *a*. Tubercula pubertatis continuous in xxi-xxii, bilobate, ventral limit just dorsal to *b*. Setae closely paired: $aa < bc$, $ab = cd$, aa or $bc < dd$. Spermathecal pores leveled to *c*, in 6/7-8/9. Prostatic pores absent. Secondary annulation present in i-xv. Genital tumescences surrounding *ab* in xv-xvii and xxii-xxvi all weakly developed. Intestinal origin in ix. One pair of spermathecae per segment in vii-ix, spermathecal structure poorly differentiated into ampulla and duct, duct tubular, as long as the elongated, ellipsoidal ampulla. Prostate-like glands and auxiliary prostate-like glands absent. First nephridia in xiii.

External Characteristics (Figure 3.11A):

Length 90 mm. Diameter in x 2.5 mm, in clitellum 3.5 mm. Number of segments, 249. Body ovate in cross-section anteriorly, dorsoventrally flattened at the clitellum, quadrangular postclitellarly, tail quadrangular and dorsoventrally flattened, anus dorsal. Preserved specimens

with a pale-whitish coloration, reddish-pink anteriorly, clitellum pinkish-white. Cuticle with a strong yellowish-green iridescence. Prostomium zygotoblastic. Dorsal pores absent. Spermathecal pores in 6/7-8/9 level with *c*, minute or inconspicuous. Female pores minute in xiv, anterior to *a*. Male pores lateral to *b* in xix close to 19/20, aligned with the tubercula pubertatis. Clitellum in xv-xxvi, saddle-shaped but almost annular, ventral limit in *a*. Tubercula pubertatis continuous xxi-xxii, bilobate, ventral limit dorsal to *b*. Prostate-like pores absent. Nephridiopores anterior to *b*, starting in segment xiii. First setae in ii, lumbricine, closely paired. In x $aa:ab:bc:cd:dd = 5.5:1.0:6.5:1.0:10.0$, in xxx $=5.5:1:7.5:1.0:10.0$ (Figure 3.11C); *cd* and most of the *ab* absent in clitellum. Secondary annulation present in ii-xv, throughout the whole segment, tetra-ringed; postclitellarly only slightly. Genital tumescences strong in xv, weak in xvi-xvii and xxii-xxvi surrounding *ab*. Lateral dark spots present at or above the mid-lateral line, in an irregular row in i-xiv. Dorsal pit absent.

Internal Characteristics:

Septa 7/8-8/9 only slightly thickened. Pharynx ends in vi, paired glands in iii-iv. Intestine originates gradually in ix. Intestinal caeca, typhlosole, calciferous glands and gizzards absent.

One paired spermathecae in each of vii-ix, without diverticulum. Spermathecal structure poorly differentiated into ampulla and duct, all of similar size. Duct tubular, slender, as long as the ampulla. Ampulla elongated, slender, ellipsoidal, smooth, tip digitiform (Figure 3.11B). Duct connects to the intersegmental furrows level with *cb*. Spermathecae located between a weak muscular fiber originating anteriorly in the intersegmental section of the esophagus and connecting posteriorly to the intersegmental-septum joint. Ovaries in xiii, free, string-like. Oviduct connects in segment xiv, anterior to *b*.

Testes free in x and xi. Epididymis not observed. Seminal vesicles, free in xi, and xii, large, filling most of the coelomic cavity, ramous. Vas deferens within muscular body wall. Tubular, prostate-like glands and auxiliary prostate-like glands absent.

One pair of meganephridia per segment, starting at xiii. Meganephridia of xiv reduced and smaller than xiii. Those of xiii of same size as the ones in xv-xxvii, but smaller than those in xviii and afterwards. Bladder globular, with a “caecum” at the base of the bladder, ventrally with a U- to J-shaped vesicle. Base of the duct considerably widened, connected to 1-3 vascular stoma. Bladder connected to the lateral blood vessels (Figure 3.1E, F).

One pair of moniliform heart in each of vii-xi; all of approximately same size. Dorsal and ventral vessels present, and simple. Paired lateral vessels present, joining dorsal vessel in xiv, filiform.

Remarks: *GenusC sp10 n. sp.* seems closely related to *S. gatesi* Reynolds 1980, and *S. helenae* Reynolds 1980 by having a reduction of the tubercula pubertatis (being absent in *S. helenae* and in xxii-xxiv in *S. gatesi*). However, it differs from these species by having an intestinal origin in ix (viii in *S. helenae* and x in *S. gatesi*), and by size, clitellum extension and extension of the tubercula pubertatis. These species differ so much from the other known sparganophilids that they might be placed either within *GenusC n. gen.* or in a separate genus in the future, when new DNA sequences and adequate morphological descriptions are available for them. However, few individuals of *GenusC sp10* have been collected and *S. gatesi* is known only from the lost type specimen (Reynolds 1980, 2008), suggesting that this peculiar species might be rare to encounter.

Discussion

Reynolds (2008) recognizes 13 species of sparganophilids, but later Rota et al (2016) demonstrated that two of these species (*S. eiseni* and *S. langi*) are junior synonyms of *S. tamesis*. However, difficulties arise in the study of the eleven valid species, as their descriptions are mostly incomplete and vague. Reynolds (1975, 1977, 1980) described seven of the valid species, but these descriptions were based on single specimens, macerated or damaged specimens, many of which have been lost and currently unavailable for study. Additionally, Reynolds only provided the ‘diagnostic’ characteristics for each species. Further, Jamieson (1978) generalized the description of *S. tamesis* including various morphologies that are currently known to represent different species and even different genera (Ikeda *et al.*, unpublished data). However, the present study demonstrated that ignored characters, such as nephridial shape and composition, presence or absence of lateral dark spots, auxiliary prostate-like glands and auxiliary genital glands, genital markings, pads and tumescence, and spermathecal shape, can be used to not only discriminate between species, but also between genera. Even though the description of the eleven species are incomplete, each of the species described here differ from these eleven species in at least three or more characters, making their classification as one of the previously described species unjustified.

The genus *GenusA* is closely related to *Sparganophilus* and, thus, morphologically similar. *GenusA* differs from *Sparganophilus* by having lateral dark spots on all segments of i-xiv, usually having dorsal pores and secondary annulation. *GenusA* additionally holds the majority of the species in the family, with most species collected in freshwater systems from the Midwest to the East Coast of the US (Ikeda *et al.*, unpublished data). The species here described can morphologically form three species groups: *GenusA sp3 n. sp.* and *GenusA sp4 n. sp.* with annular clitellum and three pairs of spermathecae, *GenusA sp8 n. sp.* with an annular clitellum and a single

pair of spermathecae, and *GenusA sp6*, *GenusA sp5*, *GenusA sp7*, *GenusA sp2*, and *GenusA sp1* with a saddle-shaped clitellum and three pairs of spermathecae. Table 1 summarizes morphological differences between these species.

GenusB differs from both *GenusA* and *Sparganophilus* by having auxiliary prostate-like glands in the region of xv-xviii. The discovery of *GenusB sp9 n. sp.* is geographically challenging to explain, as it belongs to the *GenusB* species group with interrupted tubercula pubertatis, so far found only in Mexico and California (Ikeda et al, unpublished data). However, Reynolds (1980) described *Sparganophilus komeraki* from a series of specimens collected in Vidalia, GA, about 150 km from the collection of *GenusB sp9*. *Sparganophilus komeraki* resembles in many ways *GenusB sp9*, however, because of the poor description of *S. komeraki*, it is not possible to determine whether it belongs to *GenusB* or not. Nonetheless, *S. komeraki* differs from *GenusB sp9* by having only two pairs of spermathecae, the location of the male pores and extension of the tubercula pubertatis.

The last genus *GenusC n. gen.* differs from all known genera of sparganophilids by having a considerable reduction and posterior displacement of the tubercula pubertatis, and by having nephridia with multiple blood vessel vacuoles. However, *GenusC* does possess lateral dark spots, which suggests a closer affiliation to *GenusA* and *Sparganophilus* than with *GenusB*. However, *GenusC sp10 n. sp.* seems to share close affinities to *S. gatesi* Reynolds, 1980, and *S. helenae* Reynolds, 1980, in having a reduced and posteriorly displaced tubercula pubertatis (in the case of *S. helenae*, being completely absent). Unfortunately, it is currently unknown if *S. gatesi* and *S. helenae* should be placed in *GenusC*, as Reynolds' descriptions do not mention important generic characters, which are now considered phylogenetically important, including the nephridial structure, presence or absence of auxiliary prostate-like glands and lateral dark spots.

With the description of the present species, our knowledge of the sparganophilids increases and opens the door for ecological studies of the group. However, the ten species described here are not represented in Ikeda *et al.*'s (unpublished data) collection of 100-120 species. This suggests that the real number of species of Sparganophilidae may be much higher than the estimated 120 that are still lacking description. Because of this, as many more areas are sampled and modern techniques are used together with detailed and completed descriptions, the family Sparganophilidae diversity is expected to increase dramatically.

Acknowledgements

I am grateful to Dr. Samuel James for taking the time to teach me earthworm taxonomy, and to Melanie K. Taylor, Gregg Chapman, Christie Hawley, Stephen Ruswick, and Dexter Stother for the help provided in the field. This research was funded in part by the National Science Foundation (NSF) through the Geosciences Directorate's Division of Earth Sciences Critical Zone Observatory program (EAR-1331846)

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Table 3.1. Major morphological differences between the newly described species of *Genusa*.

Species	Length (mm)	Clitellum	Tubercula Pubertatis, and ventral maring	Dorsal pores	Male Pores	Secondary Annulation
<i>Genusa sp1</i> n. sp.	45-55	Saddle-shaped, xv-xxiii, xxiv, (1/n)xxv	(1/n)xvii-xxii, dorsal to b	Absent	xix	none
<i>Genusa sp2</i> n. sp.	31	Saddle-shaped, xv-xxiii	xviii-xix, xx dorsal to b	Absent	xix	none
<i>Genusa sp3</i> n. sp.	>40	Annular, xv-xvii	(1/2)xvii, xviii-xxii, at a	Absent	xix	none
<i>Genusa sp4</i> n. sp.	65	Annular, xiv-xv	(1/n)xvii-xxii, at a	Absent	xix	none
<i>Genusa sp5</i> n. sp.	>57	Saddle-shaped, xv-xxvi	xvii-xxii, at b	First in 2/3	xix	none
<i>Genusa sp6</i> n. sp.	45-80	Saddle-shaped, xv-xxvi, xxvii	xvii-xxii, at b	Usually absent	xix	none
<i>Genusa sp7</i> n. sp.	>55	Saddle-shaped, xv-xxv	xviii-xxi, xxii dorsal to b	Absent	xxi	none
<i>Genusa sp8</i> n. sp.	>35	Annular, xv-xvii	xvii-xxii, at b	Posteriorly	xix	ii-xxvii
Species	Genital Markings	Genital Pads	Genital Tumescence	Spermatheca	Prostate-like glands	Intestinal origin
<i>Genusa sp1</i> n. sp.	none	None	<i>ab</i> xiv-xxiii, xxv	vii, viii, ix	xxiii-xxv, or absent	Ix
<i>Genusa sp2</i> n. sp.	none	None	<i>ab</i> xx-xxii	vi, vii, viii	xx-xxiii	Viii
<i>Genusa sp3</i> n. sp.	none	xxvii-xxvii	none	vii, viii, ix	xxiii-xxvi	Ix
<i>Genusa sp4</i> n. sp.	none	xxv-xxvi	none	vii, viii, ix	xxi-xxiii	Ix
<i>Genusa sp5</i> n. sp.	none	xxvii-xxvii	<i>ab</i> xv-xxii	vii, viii, ix	xxiii-xxvi	Ix
<i>Genusa sp6</i> n. sp.	none, or <i>ab</i> x	xxvii-xxvii	<i>ab</i> xxiii-xxv	vii, viii, ix	xxiii-xxv, rarely in xxvi	Ix
<i>Genusa sp7</i> n. sp.	none	None	<i>ab</i> xv-xxii	vii, viii, ix	xxiii-xxv	Ix
<i>Genusa sp8</i> n. sp.	<i>ab</i> in any of x-xiv, 26/27; <i>cd</i> on 8/9	xxvii-xxvii	none	viii	xxiii-xxv	Ix

Figure 3.1. Nephridial structures of different genera of Sparganophilidae: (A) *Sparganophilus*, (B) *GenusA sp1 n. sp.* (without vacuole), (C) *GenusA sp2 n. sp.* (with vacuole), (D) *Mckeyiella*, and (E) *GenusC n. gen.* (F) picture of the nephridia of *V. ikedae n. sp.* In A-E, blue: bladder, green: vesicle, and magenta: vacuole. In F, blue arrows: blood vessel, red arrows: vacuoles, black arrow: nephridial tubule, V: vesicle, C: caecum, and B: bladder.

Figure 3.2. *GenusA sp1 n. sp.* (A) external ventral view, (B) spermatheca, (C) setal formula. ♀: ovipores, P: prostatic pores, ♂: male pores, N: first nephridiopore, GP: genital pad, GM: genital marking, GT: genital tumescences.

Figure 3.3. *GenusA sp2 n. sp.* (A) external ventral view, (B) spermatheca, (C) setal formula. ♀: ovipores, P: prostatic pores, ♂: male pores, N: first nephridiopore, GP: genital pad, GM: genital marking, GT: genital tumescences.

Figure 3.4. *GenusA sp3 n. sp.* (A) external ventral view, (B) spermatheca, (C) setal formula. ♀: ovipores, P: prostatic pores, ♂: male pores, N: first nephridiopore, GP: genital pad, GM: genital marking, GT: genital tumescences.

Figure 3.5. *GenusA sp4 n. sp.* (A) external ventral view, (B) spermatheca, (C) setal formula. ♀: ovipores, P: prostatic pores, ♂: male pores, N: first nephridiopore, GP: genital pad, GM: genital marking, GT: genital tumescences.

Figure 3.6. *GenusA sp5 n. sp.* (A) external ventral view, (B) spermatheca, (C) setal formula. ♀: ovipores, P: prostatic pores, ♂: male pores, N: first nephridiopore, GP: genital pad, GM: genital marking, GT: genital tumescences.

Figure 3.7. *GenusA sp6 n. sp.* (A) external ventral view, (B) spermatheca, (C) setal formula. ♀: ovipores, P: prostatic pores, ♂: male pores, N: first nephridiopore, GP: genital pad, GM: genital marking, GT: genital tumescences.

Figure 3.8. *GenusA sp7 n. sp.* (A) external ventral view, (B) spermatheca, (C) setal formula. ♀: ovipores, P: prostatic pores, ♂: male pores, N: first nephridiopore, GP: genital pad, GT: genital tumescences.

Figure 3.9. *GenusA sp8 n. sp.* (A) external ventral view, (B) spermatheca, (C) setal formula. ♀: ovipores, P: prostatic pores, ♂: male pores, N: first nephridiopore, GP: genital pad, GM: genital marking, GT: genital tumescences.

Figure 3.10. *GenusB sp9 n. sp.* (A) external ventral view, (B) spermatheca, (C) setal formula. ♀: ovipores, P: prostatic pores, ♂: male pores, N: first nephridiopore, GT: genital tumescences.

Figure 3.11. *GenusC sp10 n. sp.* (A) external ventral view, (B) spermatheca, (C) setal formula. ♀: ovipores, P: prostatic pores, ♂: male pores, N: first nephridiopore, GM: genital marking, GT: genital tumescences.

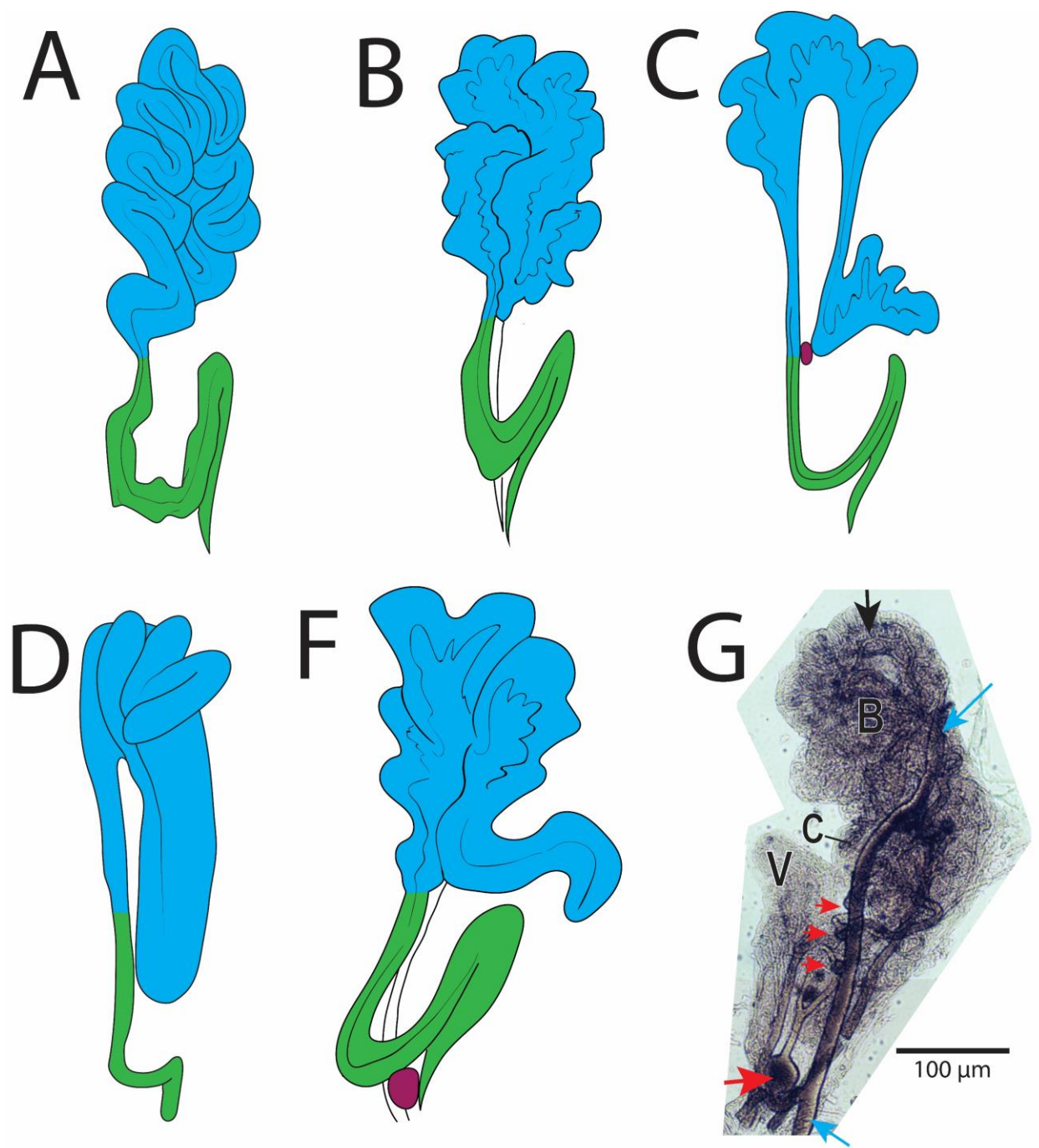


Figure 3.1.

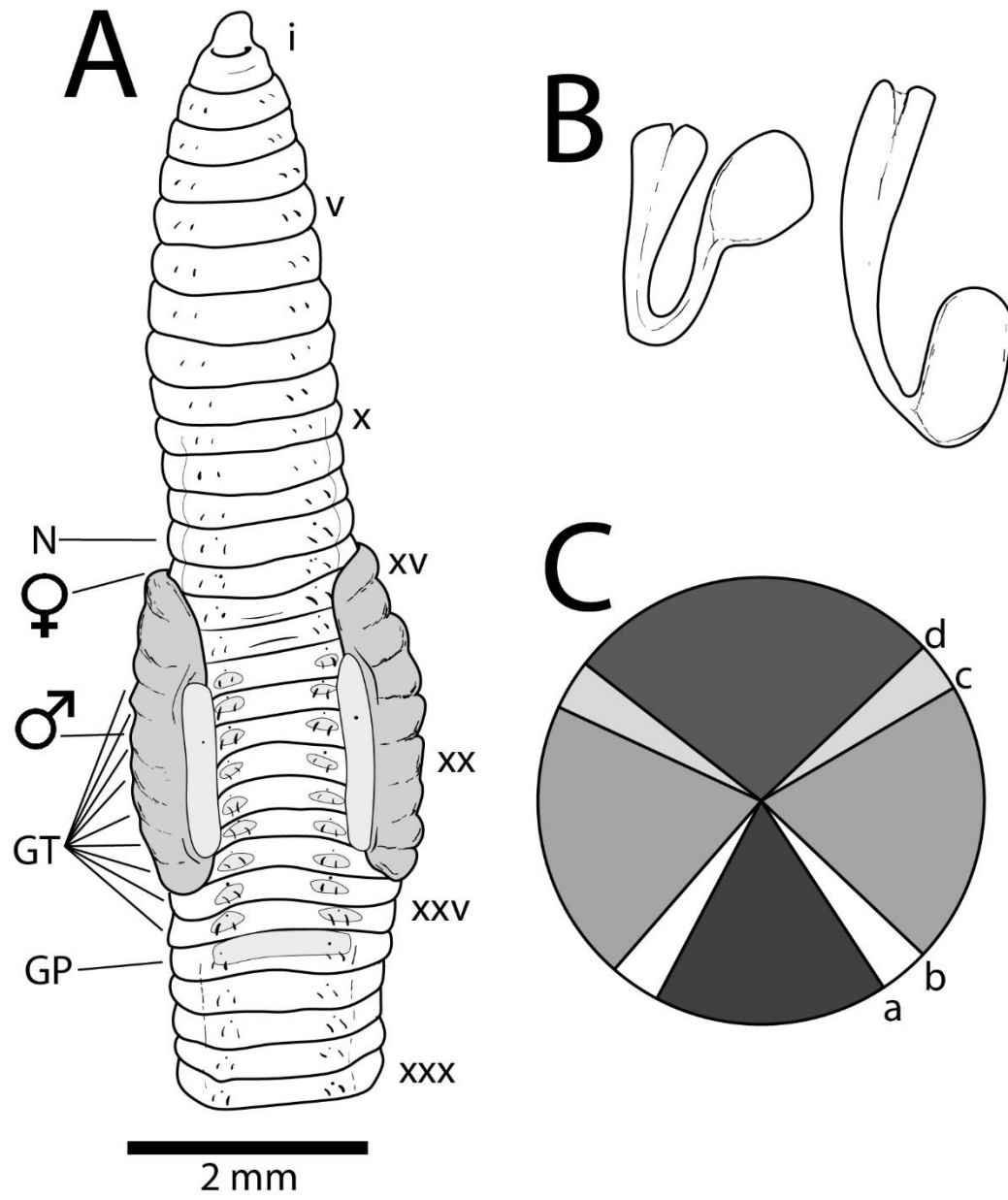


Figure 3.2.

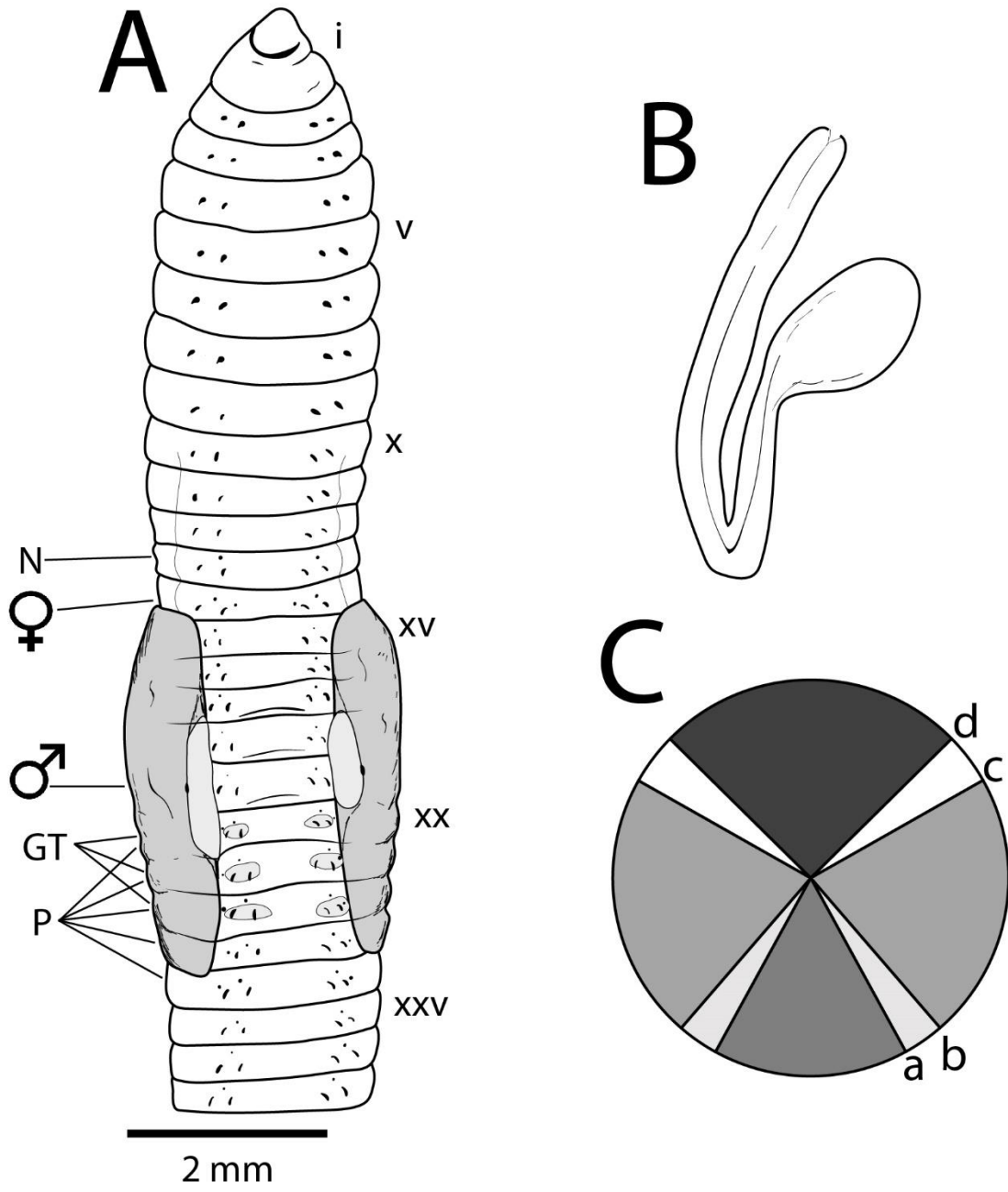


Figure 3.3.

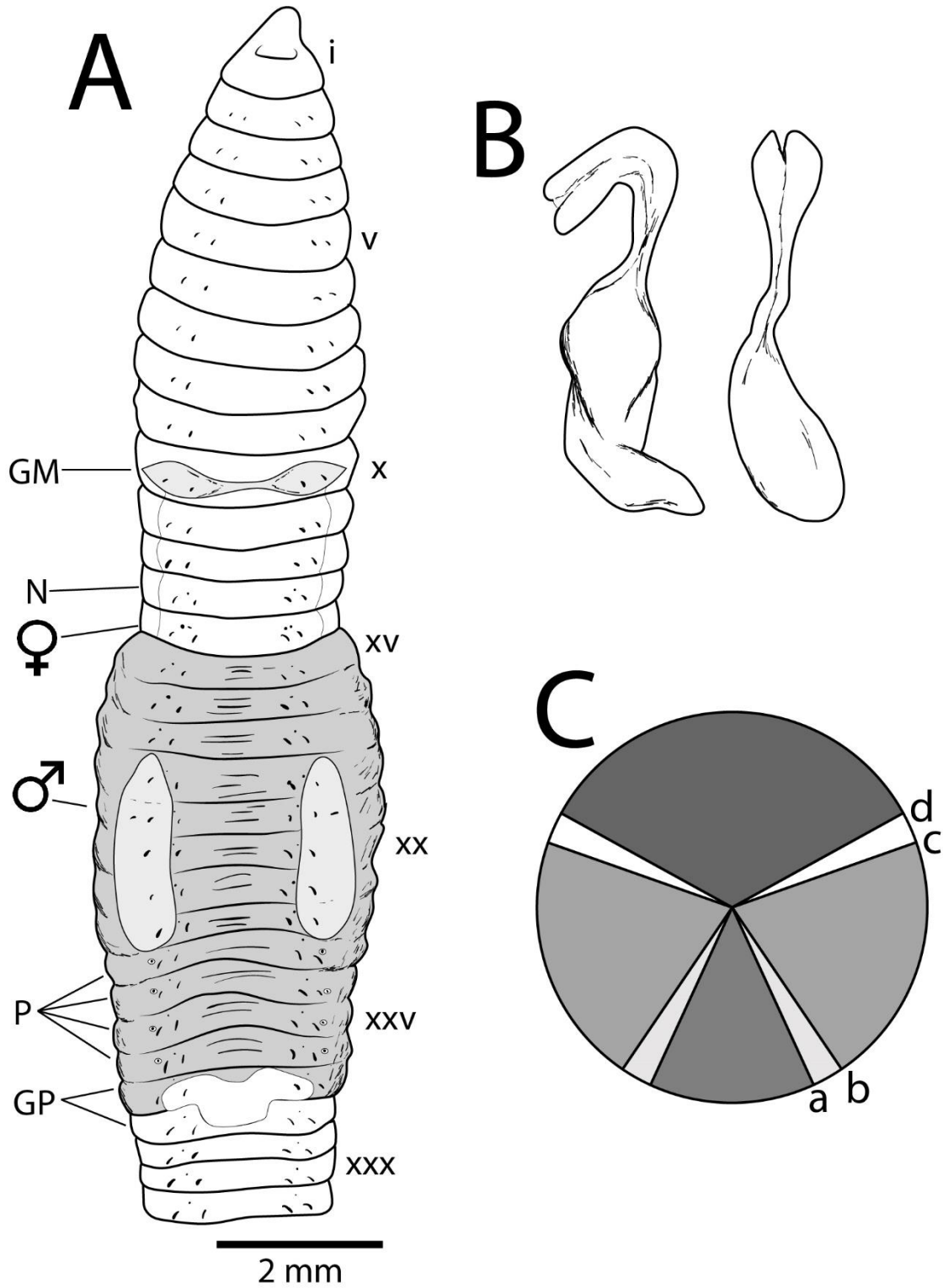


Figure 3.4.

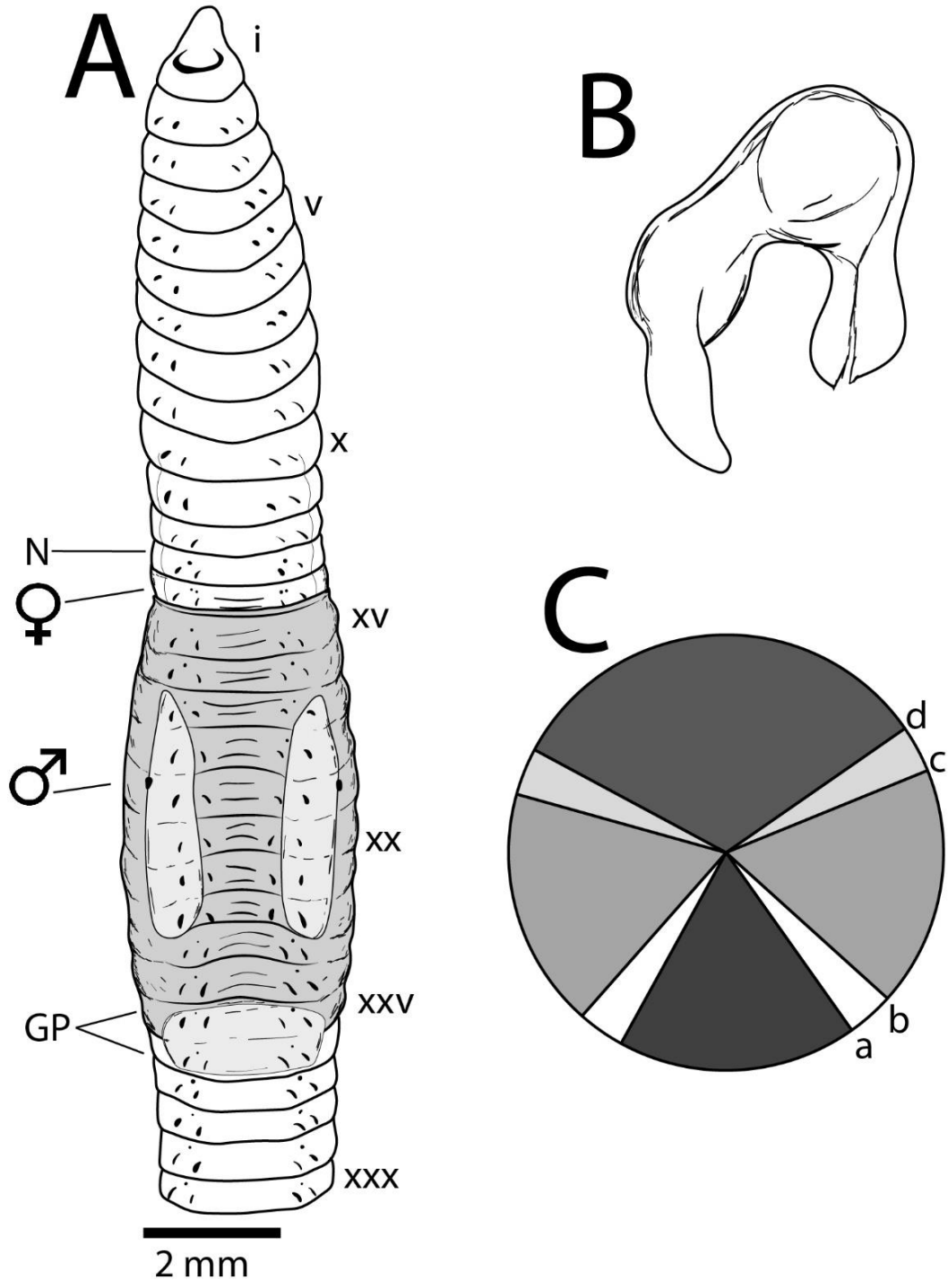


Figure 3.5.

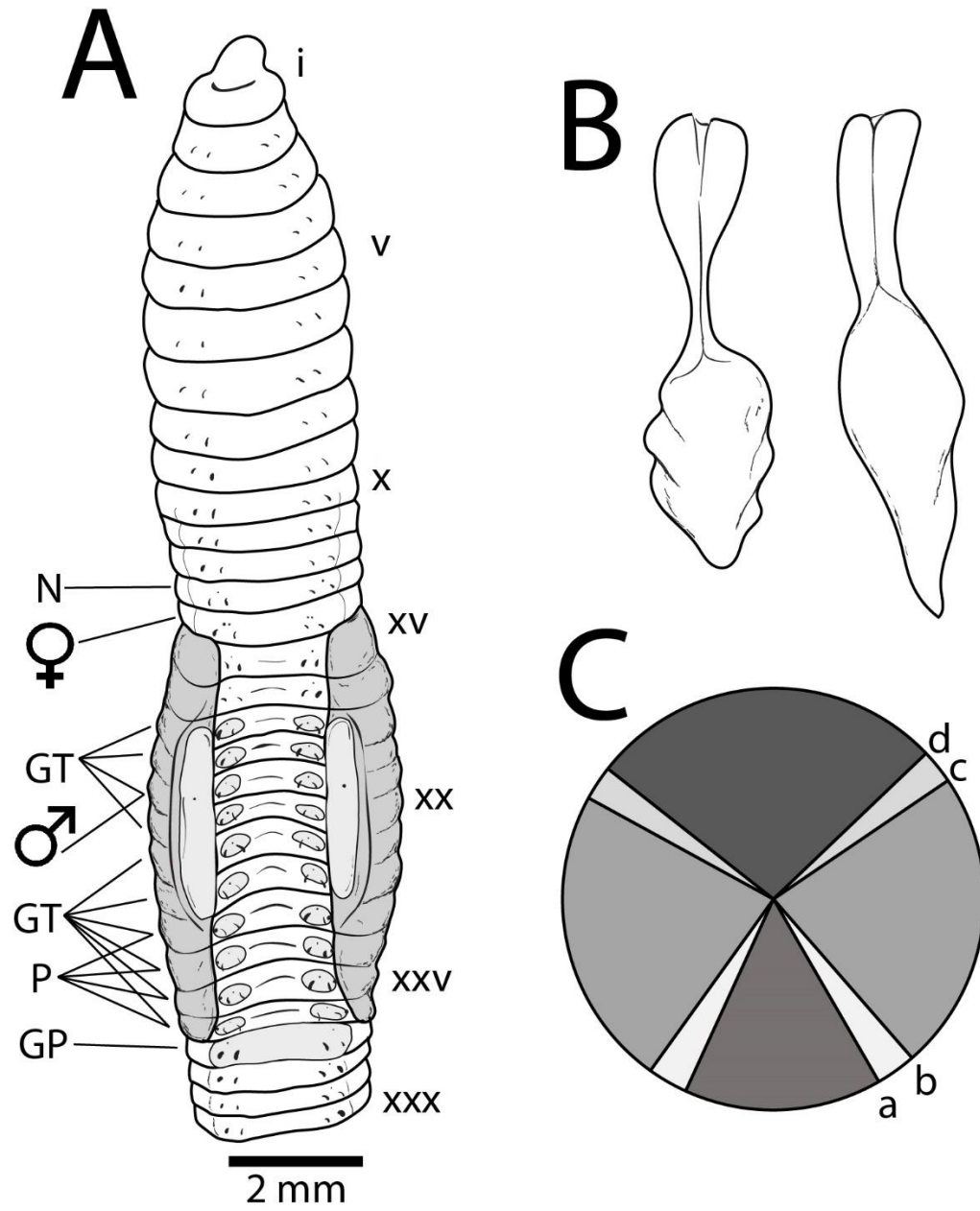


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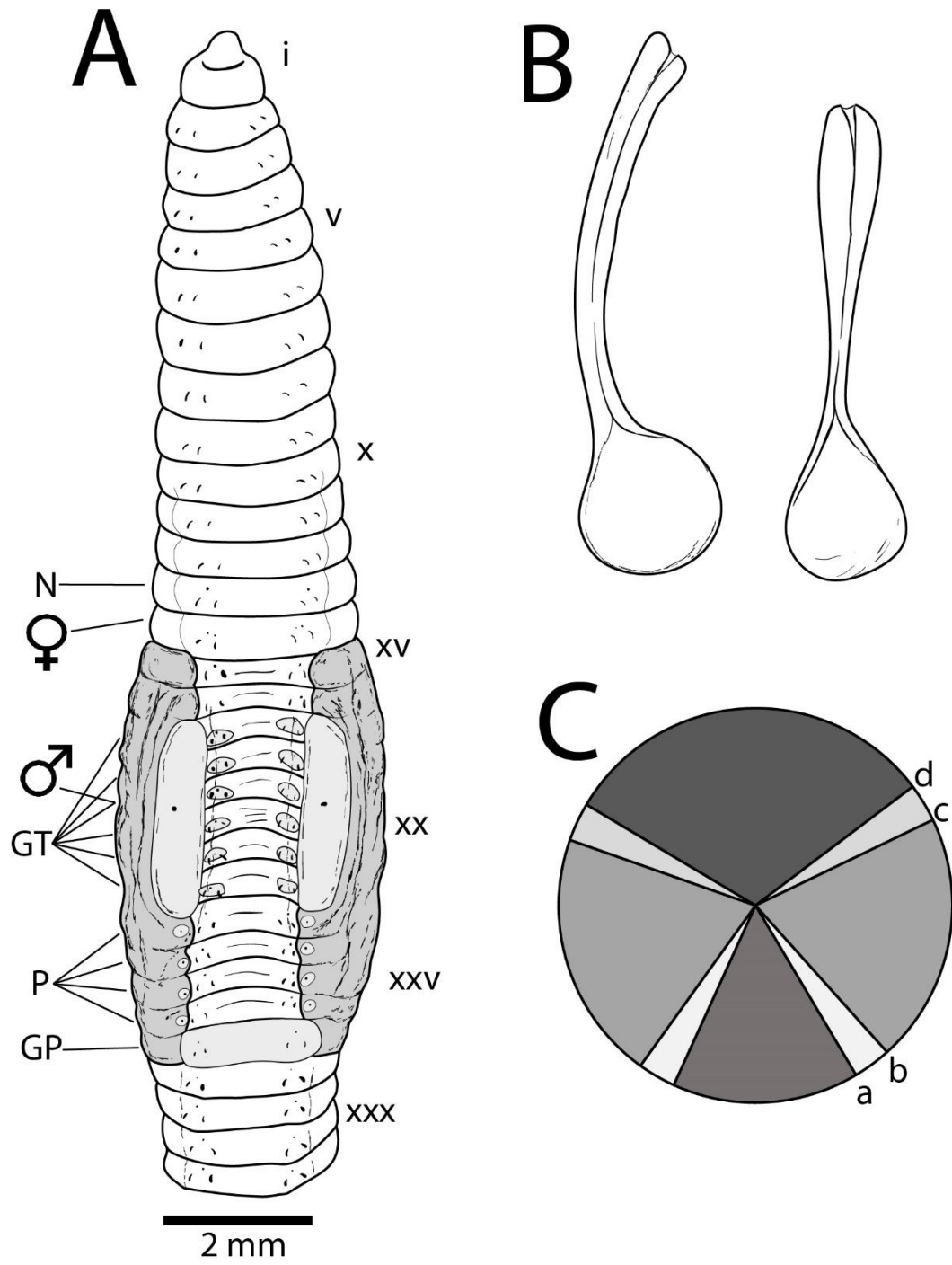


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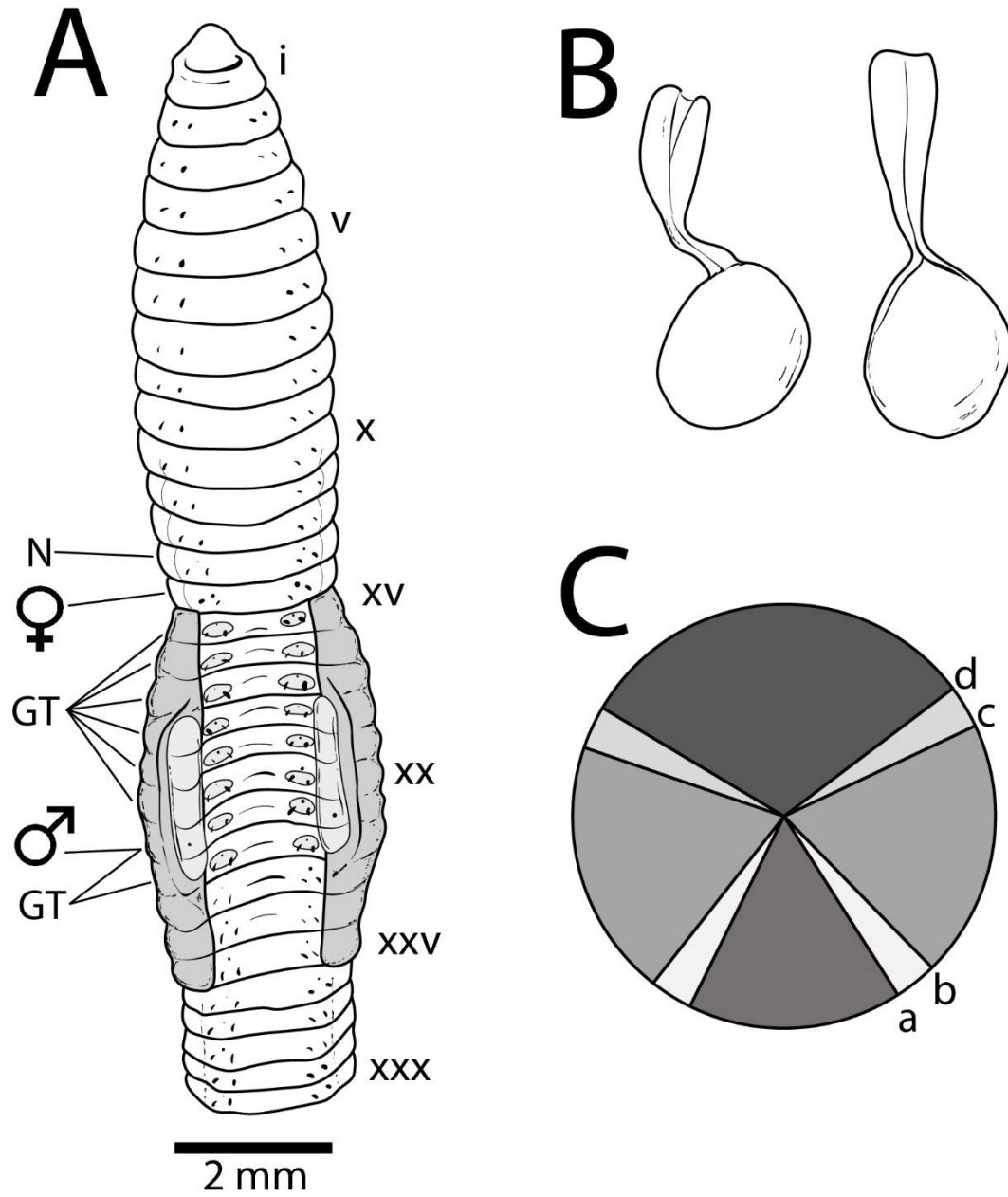


Figure 3.8.

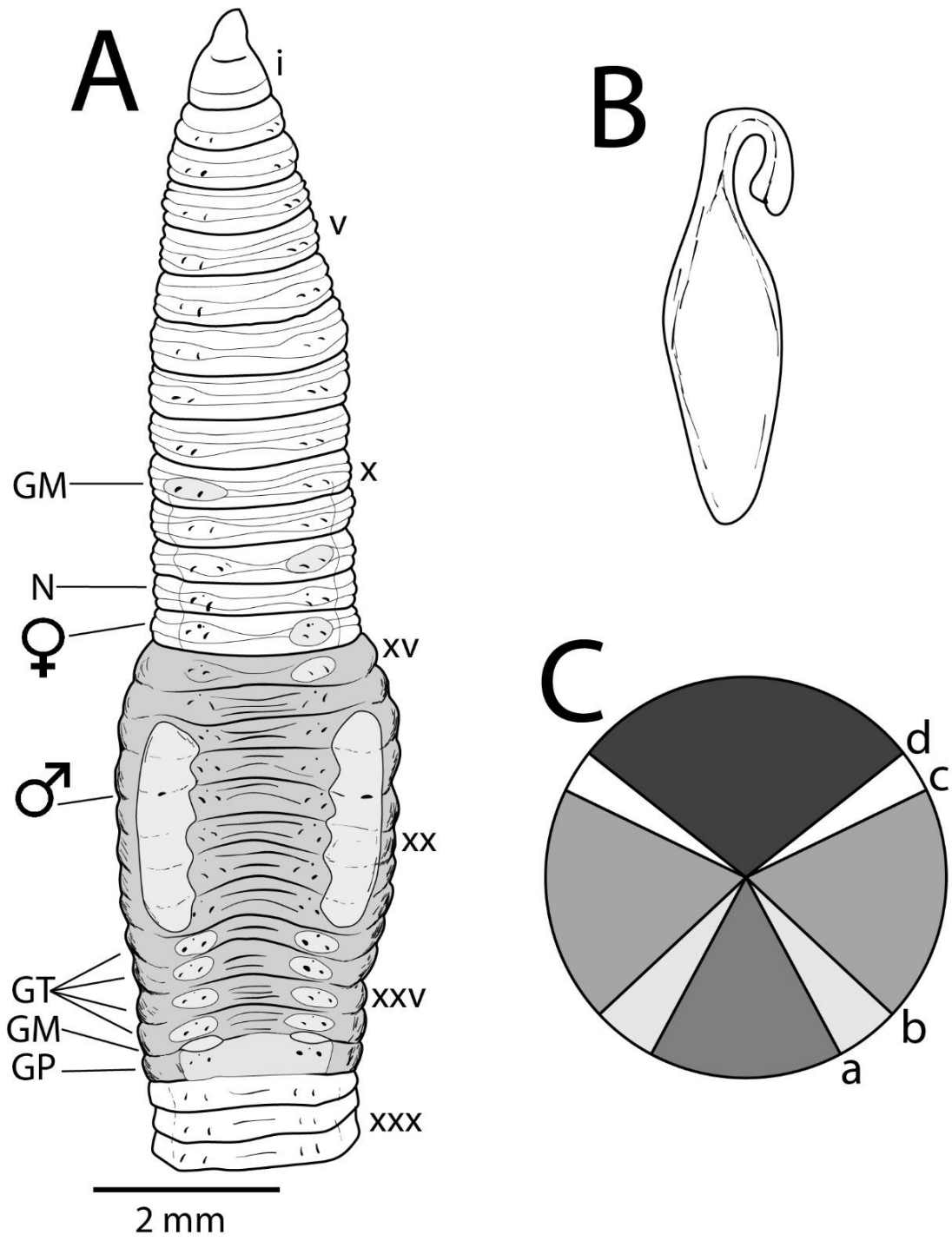


Figure 3.9.

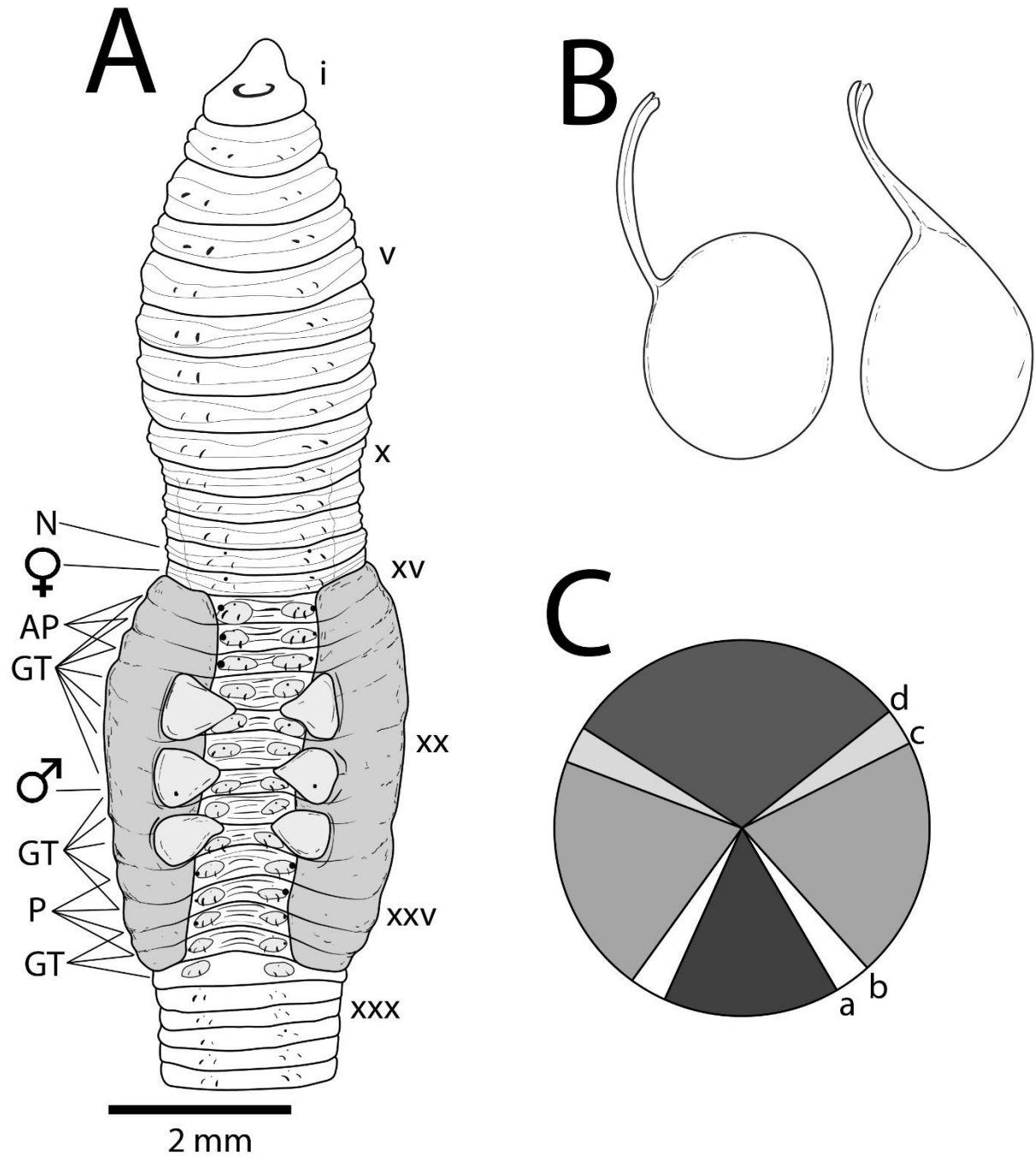


Figure 3.10.

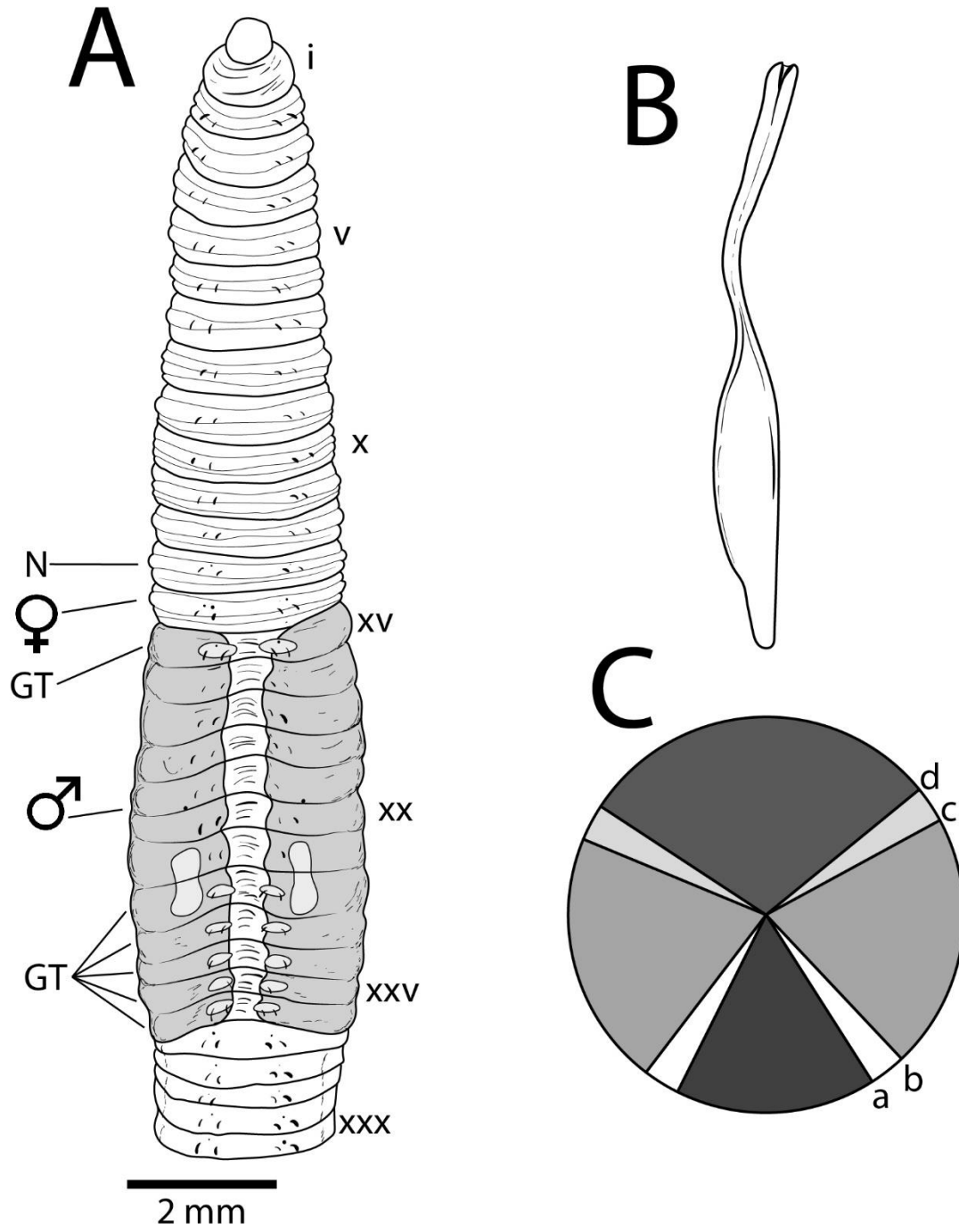


Figure 3.11.

CHAPTER 4

CONCLUSION AND SUMMARY OF FINDINGS

With only three seasons of sampling in the Piedmont of the Southeast USA, it was possible to almost double the number of species known within the semiaquatic earthworm family Sparganophilidae, from 11 to 21 species. Further, it was possible to identify three additional genera within the Sparganophilidae. Considering that this research was conducted at three sites, and that none of the sites shared species, it is expected that the real number of sparganophilid species may be multiple times that of the known species. Moreover, it suggests a high level of endemism of these semiaquatic earthworms within a single river system, and sometimes, a small watershed within a system. Similar observations on the underestimation of earthworm diversity have been reported from French Guiana, where researchers found 48 species of earthworms with only 36 plots in two sample sites (44 of which potentially represent undescribed species), within a country with only 22-23 recorded species (Decaëns *et al.* 2016). A similar pattern has also been observed with the semiaquatic earthworm genus *Glyphidrilus* Horst, 1889 (Almidae), where most species are found to occur within a single river system, with only a handful present in more than one system (Chanabun *et al.* 2013, 2017).

The discovery of such a high diversity of sparganophilid earthworms is not necessarily surprising, as many experts have not studied the group because of their habitat species expertise: terrestrial oligochaetes experts suggest that it should be the work of the aquatic oligochaetes experts and vice versa (Reynolds 1980). Additional trouble arises from the fact that most known

species are poorly described and some species type material is either lost or destroyed (Reynolds 1980, 2008). Future studies should focus on re-describing the known species in addition to the undescribed species.

From an ecological standpoint, a strong phenology was also observed for the group. Wherein adult sparganophilids were collected in greater abundance during the spring, becoming less abundant during the summer, to being completely absent during the fall the juvenile sparganophilids were always present, but increasingly common during the summer and fall. These patterns suggest that sparganophilid earthworms in this region reach adulthood and reproduce during winter and spring to early summer, and the offspring hatch during the summer and fall. This confirms the strong phenology observed previously (Hague 1923; Harman 1965), but with each documented study differing in the duration and timing of the observed phenological stages. This suggests that reproductive cycles differ between species and geographic areas.

This work also provides the basis of detailed evolutionary studies, and emphasizes the potential of the family to serve as an eco-evolutionary model. The high endemism of sparganophilids in a small area, within the same habitat, raises questions about their evolutionary history. Understanding how sympatric species can coexist in stable communities has been a major area of research in both evolutionary and ecological fields (MacArthur 1970; Werner 1977). For example, species packing theory states that the number of species present will be dependent on the amount of resources available and the distance between their niches; while the niche expansion theory suggests that with greater resources, new unexploited niches will arise and species will be able to utilize them (Kremer & Klausmeier 2017; MacArthur 1970; Pellissier *et al.* 2018; Werner 1977). Since the sparganophilids are relatively easy to collect, are present all year, and inhabit a very specific habitat, they provide a good study model to address these questions in the field and

in the laboratory. Understanding how diversity is maintained is critical, because it will allow us to predict how disturbances like introduction of invasive species, climate change, and habitat loss will affect native biodiversity, and how its loss can be prevented.

Many ecological and functional characteristics are still virtually unknown for the sparganophilids. For example, terrestrial oligochaetes are usually classified in ecological functional groups defined by ingesta, burrowing, and feeding behavior, and morphology (Bouché 1977). This concept, however, has been recently questioned (Chang *et al.* 2016b). Nonetheless, in the case of semiaquatic taxa, this classification is not applicable since no obvious horizonation is necessarily present in the sediments these organisms occupy (Gasparini *et al.* 2004), the leaf litter layer may not be on the surface being buried during as erosion occurs (Metzler & Smock 1990), and some sparganophilids earthworm have been collected completely submerged under water (Reynolds 1980). Further, the feeding preferences and behaviors of these taxa are currently unknown, and little intestinal material was found during the dissections performed in Chapter 3, thus providing few insights.

In times that climate change and habitat loss are current threats, known and unknown biodiversity is currently being lost. However, most efforts in soil ecology are concentrated in nutrient cycles, gas exchange, carbon sequestration, and microbial diversity, ignoring the ecological roles, diversity and function of the fauna (including earthworms) that inhabit soils. This present work provides a basis for further study on the ecology of the sparganophilid earthworms, and also highlights how little we, as scientists, know and understand about soil faunal diversity of all kinds and how much is still to be learned.

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APPENDIX A

COMPETITION BETWEEN TWO NORTH AMERICAN NATIVE *DIPLOCARDIA* (ACANTHODRILIDAE) EARTHWORMS AND THE EUROPEAN *APORRECTODEA* *CALIGINOSA* (LUMBRICIDAE) AS AFFECTED BY SOIL pH

Introduction:

European lumbricid species are known to be antagonistic to North American native ecosystems. These antagonistic effects include native flora (Derouard *et al.* 1997; Larson *et al.* 2009; Loss & Blair 2014) and invertebrate loss (Burtis *et al.* 2014; Eisenhauer *et al.* 2007; Hale *et al.* 2006; Rätty 2004; Schlaghamerský *et al.* 2014), nutrient cycle shifts (Bohlen *et al.* 2004a; b; James & Seastedt 1986), acceleration of leaf litter decomposition (González *et al.* 2003), changes in soil structure (Hale *et al.* 2005; Snyder *et al.* 2011), and declines in ground nesting birds (Loss & Blair 2011, 2014). Recently, new evidence suggests that invasive earthworms and invasive plants might be aiding each other when colonizing a novel habitat together, following the invasional meltdown hypothesis (Heneghan *et al.* 2007; Kourtev *et al.* 1999; Madritch & Lindroth 2009). However, not all evidence supports this hypothesis (Wyckoff *et al.* 2014), and the mechanisms behind these invasion relationships are still poorly identified.

One possible case of invasional meltdown is the invasion of the shrub, Chinese privet (*Ligustrum sinense*) which appears to facilitate co-invasion by European lumbricids in the southeastern US. One case reported a decline of native earthworms, *Diplocardia* spp., when invasive earthworms were more abundant in the presence of privet (Lobe *et al.* 2014). The genus

Diplocardia includes about 50 species that are distributed throughout parts of the United States and Mexico that were not covered by ice during the last glaciation (Fender 1995; James 1995), with many additional species awaiting a formal description (for example, see Fragoso & Rojas 2014). The mechanisms behind these declines are unknown. One possible mechanism includes competitive exclusion of the native *Diplocardia* by the invasive lumbricids. However, until very recently, competition between native and invasive earthworms has not been experimentally tested (Chang *et al.* 2016). Furthermore, there is a general lack of knowledge on the basic biological and ecological aspects of the species within the *Diplocardia* genus to inform us about possible competitive interactions, and most of the European lumbricid invasion studies in the US have not taken interactions with native earthworms into account.

Lobe *et al.* (2014) suggested that the decline of the *Diplocardia* spp. and subsequent dominance of the invasive lumbricids might be a response to soil acidity. In their study, soils in sites invaded by Chinese privet were relatively more basic (i.e., higher pH: 5.0) in comparison with soils that had not been invaded (of 4.6). These more basic soils were dominated by the invasive lumbricids, while the acidic native sites were dominated by the native *Diplocardia* spp. Additionally, the removal of privet was sufficient to shift the earthworm community back to a *Diplocardia*-dominated one, and to generate a coincident decline in soil pH (Lobe *et al.* 2014). Because of this and the known pH preferences of some of the invasive lumbricids (Baker & Whitby 2003), it was suggested that pH might have an important role in determining the outcome of competitive interactions between native and invasive earthworms (Lobe *et al.* 2014).

It is likely that the invasion of privet could potentially alter other soil properties (such as nutrient availability, microbial diversity and activity, and soil pH), but for this study we focused on the soil pH changes associated with privet invasion effects, and how these changes might affect

earthworm competition. The objectives of this project were to determine if competitive exclusion will occur between *Diplocardia* and *Aporrectodea caliginosa* in response to manipulations of soil pH. We tested the probability of competitive exclusion with the Lotka-Volterra competitive exclusion models. These models used three parameters to predict the outcome of competition between two species: the carrying capacity of both species (k_1 and k_2), the effect of species 1 on species 2 (α_{12}) and the effect of species 2 on species 1 (α_{21}). There are four possible outcomes of this model: (1) species 1 competitively excludes species 2, (2) species 2 competitively excludes species 1, (3) both species coexist in a stable point, and (4) they are in a constant state of instability with multiple attractors.

Methods:

Specimen collection: All specimens were collected at or near the Calhoun Experimental Forest and Critical Zone Observatory, Union Co., South Carolina. The specimens were extracted from the soil by a combination of methods: grunting, turning rocks and logs or digging and hand-sorting soil. The specimens were maintained alive in 1L containers filled with moist soils at room temperature, until the beginning of the experiment. Collected *Aporrectodea caliginosa* adults and the two most abundant *Diplocardia* morphotypes were used during the experiment: *Diplocardia* cf. *singularis* and an undescribed species (herein referred as *Diplocardia* sp2). These *Diplocardia* morphotypes differed in size, pigmentation and arrangement of genital markings.

Experimental design: All mesocosms used in this study consisted of a PVC tube of 10 cm diameter holding at least 15 cm depth of soil. The soil was collected from the top 20 cm depth at the site collection of the specimens. This soil was passed through a 4-mm sieve to homogenize its texture and eliminate any rock and debris fragments mixed in the soil. The top and bottom of each

mesocosm were covered by a 0.50 mm mesh to avoid earthworm escapes. All mesocosms held 350 g of soil and were watered once a week and kept at field capacity once the experiment started.

Two soil pH treatments were employed: unmodified soil with a natural 4.5-5.0 pH range and limed soil with a pH of 6.0-6.3. Soil pH was modified by adding calcium carbonate (CaCO_3) and diluted sulfuric acid as needed in order to reach a pH within the range of 6.0-6.3. Soil pH was measured following Jones (2001), with a solution of 1:1 soil and deionized water.

Competition experiment: To apply the Lotka-Volterra competition model, we estimated the carrying capacity (k), the effect of taxon 1 on taxon 2 (α_{12}) and of taxon 2 on taxon 1 (α_{21}). Each of these parameters were estimated as earthworm proportional weight change and survival compared to when a single specimen of each taxon occurred alone. To estimate k , two earthworms of the same species or morphotype were placed together. To estimate the α_{12} and α_{21} one *D. cf. singularis* was placed together with one *A. caliginosa* and one *Diplocardia* sp2, with one *A. caliginosa*. Competition treatments are summarized in Table A1. High k is represented by higher mass gain (or lower mass lost) when both specimens of the same taxon co-occur. A small effect of taxa 1 on 2 (α_{12}) will be reflected as a smaller mass change (non-significant) on taxa 2, while a big effect, as a greater (significant) mass lost, compared to when taxa 2 occurs alone. All mesocosm were incubated for 90 days. There were five replicate soil cores for each competition and pH treatment combination.

Statistical analyses: To determine if there were significant differences between the proportion of mass lost for each of *D. cf. singularis*, *Diplocardia* sp2 and *A. caliginosa* in all competition treatments and pH treatments, an AxB factorial ANOVA was conducted, followed by a Tukey's Honestly Significant Difference test to determine which groups were significantly different, given that no soil pH and competition treatment interactions were found. These

ANOVAs were conducted twice: with earthworm death (1) represented as missing data and (2) represented as a 100% mass lost. To determine the likelihood of mortality of each of the earthworm species depended on the competition treatments and/or pH treatments, a logistic regression was conducted. All statistical analyses were conducted in RStudio ver. 9.1.0 (R Core Team 2016).

Results:

Mortality of earthworms was low (8%) for *Aporrectodea caliginosa* and high for both native species: *Diplocardia cf. singularis* and *Diplocardia* sp2 had a mortality of 44% and 70%, respectively. The logistic regressions found no effect of competition treatments on mortality. The only factor appearing to affect the mortality of *D. cf. singularis* was pH: higher mortality was detected at lower, natural occurring pH (60%) than that of lime-treated, higher pH soil (30%). Soil pH did not explain the mortality of *A. caliginosa* nor *Diplocardia* sp2 (Table A2).

All surviving earthworms registered a considerable proportion of mass lost, between 15-88%. The ANOVAs found no significant difference between the proportion of mass lost and competition treatments nor an interaction between soil pH and competition treatments, regardless of whether mortality was included as a 100% mass lost or not (Table A3). Higher mass loss of *A. caliginosa* was observed in lower pH soils, but only when death was not included in the analysis as 100% mass lost. Similarly, higher mass lost was detected for *D. cf. singularis* but only when earthworm death was equivalent to a 100% mass lost (Table A3).

Discussion:

Competition between earthworm species have been experimentally attempted only on two occasions (Chang *et al.* 2016; Winsome *et al.* 2006), all of which assessed competition between

native North American and invasive taxa. However, we failed to detect any effect of pH on the competition of the native species of *Diplocardia* and the invasive European *Aporrectodea caliginosa*. High mortality of both native species was observed, with *D. cf. singularis* sustaining a mortality of 44% and *Diplocardia* sp2., 70%, while few *A. caliginosa* died (8%). Even though high mortality could be attributed to competitive exclusion (e.g. Griffen & Delaney 2007; Lohrer *et al.* 2002), logistic regression suggests that in our experiment, mortality was not attributable to any of the experimental treatments. The only exception is *D. cf. singularis*, which registered a higher mortality in soils with naturally occurring pH than on lime-treated soils (Table A2), with a mortality of 30% in lime-treated soils and 60% in not modified soils. However, *D. cf. singularis* mortality was independent of the competition treatments, and the detected higher mortality of the species may be a result of low replication (n = 5).

All surviving earthworms lost a considerable proportion of their weight (between 25-85% of their initial mass). Weight lost did not differ between competition treatments, regardless of whether death was considered as a 100% weight lost or as missing data. Soil pH had a significant effect on the weight loss of *A. caliginosa* but only when death was not considered as a 100% weight lost, and in *D. cf. singularis*, when death was considered as a 100% weight lost. Higher weight lost in soils with relative pH of less than 4.5 has been observed for *Aporrectodea* species (Baker & Whitby 2003). Since the surviving *D. cf. singularis* did not differ between treatments or relative pH of soils, we cannot attribute the higher mortality of *D. cf. singularis* to the relative pH of the soil, especially when all specimens of the species and the soils used in this experiment were collected on the same site.

Failure to detect any effect of soil pH on the competition between the native *Diplocardia* species and *A. caliginosa* may be due to three factors, which are not necessarily mutually exclusive.

Firstly, the climate conditions in the laboratory where the incubation of these mesocosms took place were extremely variable, with oscillations of temperature between 21 and 32°C that likely accelerated the desiccation of the mesocosms at the higher temperatures, and that promoted saturation of the mesocosms at the lower temperatures (Personal observation). This climate instability could have created conditions too stressful for the native *Diplocardia* species, while many of the European earthworms are known to be capable of aestivation (Garnsey 1994), allowing them to survive through unfavorable conditions. It is currently unknown whether the native North American species are capable to undergo aestivation, or which survival mechanisms they employ to survive hostile environments.

Secondly, the life history of North American native earthworms is virtually unknown for almost all taxa. Basic biological data on life span, for example, are still unavailable. There is a possibility that the native species died because they were close to the end of their life cycle. However, this is unlikely since early juveniles, presumably of both taxa, were collected with the adults. Additionally, the few confirmed annual species of earthworm complete their life cycle in late fall, surviving the winter only as cocoons (Callahan *et al.* 2003; Görres *et al.* 2014, 2016).

Thirdly, pH may not have an effect on earthworm competition. This third explanation is highly unlikely since it is widely accepted that earthworm (at least the Lumbricidae, which includes *A. caliginosa*) have strong preferences for a specific range of soil pH, which may differ between species. The European *Aporrectodea* has been reported to prefer soils with pH higher than 4.5, reflecting higher mortality, lesser reproduction and development on soils with a pH lower than 4.5 (Baker & Whitby 2003). Our data support this preference of *A. caliginosa* for alkaline soils, and indirect field observations suggest that their dominance in the ecosystem may be dictated by soil pH (Lobe *et al.* 2014). Contrasting the possibility of pH having no effect on competition, it is

possible that competition took place at different intensities at both pH levels and for all species combinations, but due to the length of the experiment and additional stress due to lack of climate control, was not reflected at the time of harvest.

Although most *Diplocardia* species are generally referred as endogeic species (Callaham *et al.* 2001), as well as *A. caliginosa* (Bouché 1977), endogeic is a considerably broad ecological classification, in which species can be found to exploit resources at different depths (Callaham *et al.* 2001). Our specimens of *D. cf. singularis* were small (<35 mm long), pigmented and were collected in the soil, which suggests an epi-endogeic classification, while *Diplocardia* sp2 is larger (70-90 mm long), unpigmented and their ingesta was dominated by mineral soil (personal observation), which suggests endogeic classification (Bouché 1977). However, it is unlikely that these species were able to avoid competition by niche displacement since they only had one food source: the soil.

Work on earthworm ecology is particularly limited by our general lack of understanding of their diversity. This is reflected in our study as the native *Diplocardia* sp2 is an undescribed species, of which the current study represents its first ecological study. Similarly, *D. cf. singularis* may potentially represent an undescribed species, as it does not strictly fit the description of *D. singularis* (*sensus stricto*), an already recognized unresolved species complex (Gates 1977). In order to advance ecological studies of earthworms and their specific ecological roles, it is first necessary to understand their diversity.

Conclusion:

Even though competition between native and invasive earthworms has been demonstrated to occur in previous reports, we failed to observe competition during the course of this experiment.

This is likely due to the unexpected environmental instability of the laboratory where this experiment was incubated, making our data, at best, inconclusive. Future work should take place in infrastructure with better and constant climate control.

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Table A1. Competition treatments between *Diplocarcia cf. singularis* and *Aporrectodea caliginosa* and *Diplocarcia* sp2 and *A. caliginosa*.

Competitor	<i>Diplocarcia cf. singularis</i>	<i>Diplocarcia</i> sp2	<i>Aporrectodea caliginosa</i>
No competition	Single specimen	Single specimen	Single specimen
Intraspecific competition	Two specimens	Two specimens	Two specimens
Interspecific competition with <i>A. caliginosa</i>	One <i>D. cf. singularis</i> One <i>A. caliginosa</i>	One <i>Diplocarcia</i> sp2 One <i>A. caliginosa</i>	NA

Table A2. Logistic regressions of earthworm mortality for *Aporrectodea caliginosa*, *Diplocardia cf. singularis* and *Diplocardia sp2*. Significant P values are bold.

	Estimate (+/- SE)	Z value	P value
<i>Aporrectodea caliginosa</i>			
Intercept	-1.739 (1.12)	-1.555	0.120
Interspecific competition	-0.763 (1.49)	-0.513	0.608
Vs. <i>D. cf. singularis</i>	6.957x10 ⁻¹⁵ (1.51)	<0.001	1.000
Vs. <i>Diplocardia sp2</i>	3.977x10 ⁻¹⁶ (1.51)	<0.001	1.000
Lime-treated soil	-1.195 (1.20)	-0.999	0.318
<i>Diplocardia cf. singularis</i>			
Intercept	0.759 (0.76)	0.996	0.319
Interspecific competition	0.466 (0.97)	0.480	0.631
Vs. <i>A. caliginosa</i>	-0.466 (0.84)	-0.556	0.578
Lime-treated soil	-1.519 (0.69)	-2.188	0.029
<i>Diplocardia sp2</i>			
Intercept	0.817 (0.77)	1.060	0.289
Interspecific competition	0.717 (0.84)	0.851	0.395
Vs. <i>A. caliginosa</i>	1.012 (1.04)	0.975	0.329
Lime-treated soil	-0.791 (0.74)	-1.066	0.286

Table A3. ANOVAs results for proportion of mass change for each of *Aporrectodea caliginosa*, *Diplocardia cf. singularis* and *Diplocardia* sp2 as a function of competition treatments and soil pH. Significant P values are bold.

Factor	<i>Aporrectodea caliginosa</i>		<i>Diplocardia cf. singularis</i>		<i>Diplocardia</i> sp2	
Death as missing values						
Competition treatments	F _{3,29} = 1.209	P = 0.324	F _{2,11} = 0.100	P = 0.906	F _{2,6} = 0.061	P = 0.941
Soil pH	F _{1,29} = 4.304	P = 0.047	F _{1,11} = 0.923	P = 0.357	F _{2,6} = 0.011	P = 0.921
Competiton * soil pH	F _{3,29} = 0.867	P = 0.469	F _{2,11} = 1.292	P = 0.313	F _{2,6} = 1.901	P = 0.229
Death as 100% mass lost						
Competition treatments	F _{3,32} = 0.153	P = 0.927	F _{2,24} = 1.135	P = 0.338	F _{2,24} = 0.446	P = 0.646
Soil pH	F _{1,32} = 3.069	P = 0.089	F _{2,24} = 4.590	P = 0.043	F _{2,24} = 1.895	P = 0.181
Competiton * soil pH	F _{3,32} = 0.908	P = 0.448	F _{2,24} = 0.162	P = 0.851	F _{2,24} = 0.548	P = 0.585

APENDIX B

NUMBER OF EARTHWORMS COLLECTED AND CASTINGS COUNTED AT EACH SITE AND COLLECTION TIME FROM CHAPTER 2

This section contains the castings counts and earthworm abundance analyzed in Chapter 2. The abbreviations in all tables are as follows: EW., Earthworms; Sparg., Sparganophilidae; S.S., Scull Shoals; Cal., Calhoun; Hit., Hitchiti; Cast., average number of castings; EW., total earthworm abundance; T.S., total abundance of Sparganiphilidae; J.S., juvenile sparganophilids; A.S., adult sparganiphilids; E., *Eukerria saltensis* abundance; O., *Octolasion tyrtaeum* abundance; A., *Amyntas* spp. abundance. The number of castings is here represented by the average of the counts made by two or three different investigators. Table B1 contains the data collected during the spring, Table B2, summer, and Table B3, fall 2017.

Table B1. Number of castings by earthworm taxa at each plot of the Spring.

Site	Stream	Plot	Date	Cast.	EW.	T.S.	J.S.	A.S.	E.	O.	A.
S.S.	Small	1	17-Apr-17	4.33	8	3	3	0	5	0	0
S.S.	Small	2	17-Apr-17	12.33	7	4	4	0	3	0	0
S.S.	Small	3	17-Apr-17	4.67	8	0	0	0	5	3	0
S.S.	Small	4	17-Apr-17	32.33	10	7	7	0	2	0	1
S.S.	Small	5	17-Apr-17	24.00	1	1	0	1	0	0	0
S.S.	Medium	1	18-Apr-17	38.33	7	5	2	3	2	0	0
S.S.	Medium	2	18-Apr-17	1.33	0	0	0	0	0	0	0
S.S.	Medium	3	18-Apr-17	0.00	0	0	0	0	0	0	0
S.S.	Medium	4	18-Apr-17	0.00	0	0	0	0	0	0	0
S.S.	Medium	5	18-Apr-17	18.67	0	0	0	0	0	0	0
S.S.	Large	1	19-Apr-17	0.00	0	0	0	0	0	0	0
S.S.	Large	2	19-Apr-17	0.00	0	0	0	0	0	0	0
S.S.	Large	3	19-Apr-17	0.00	0	0	0	0	0	0	0
S.S.	Large	4	19-Apr-17	0.00	0	0	0	0	0	0	0
S.S.	Large	5	19-Apr-17	0.00	0	0	0	0	0	0	0
Cal.	Small	1	27-Apr-17	3.00	5	5	5	0	0	0	0
Cal.	Small	2	27-Apr-17	0.00	1	1	0	1	0	0	0
Cal.	Small	3	27-Apr-17	0.00	0	0	0	0	0	0	0
Cal.	Small	4	27-Apr-17	2.00	1	1	0	1	0	0	0
Cal.	Small	5	27-Apr-17	0.00	0	0	0	0	0	0	0
Cal.	Medium	1	27-Apr-17	3.00	14	10	4	6	4	0	0
Cal.	Medium	2	27-Apr-17	2.33	0	0	0	0	0	0	0
Cal.	Medium	3	27-Apr-17	0.00	0	0	0	0	0	0	0
Cal.	Medium	4	27-Apr-17	0.00	0	0	0	0	0	0	0
Cal.	Medium	5	27-Apr-17	0.00	7	0	0	0	7	0	0
Hit.	Small	1	1-May-17	0.00	0	0	0	0	0	0	0
Hit.	Small	2	1-May-17	0.00	0	0	0	0	0	0	0
Hit.	Small	3	1-May-17	0.00	0	0	0	0	0	0	0
Hit.	Small	4	1-May-17	0.00	0	0	0	0	0	0	0
Hit.	Small	5	1-May-17	0.00	0	0	0	0	0	0	0
Hit.	Medium	1	2-May-17	0.00	0	0	0	0	0	0	0
Hit.	Medium	2	2-May-17	0.00	1	0	0	0	1	0	0
Hit.	Medium	3	2-May-17	0.00	0	0	0	0	0	0	0
Hit.	Medium	4	2-May-17	16.00	7	5	2	3	2	0	0
Hit.	Medium	5	2-May-17	20.00	25	25	7	18	0	0	0

Continuation to Table B1.

Site	Stream	Plot	Date	Cast.	EW.	T.S.	J.S.	A.S.	E.	O.	A.
Cal.	Large	1	18-May-17	3.00	1	1	0	1	0	0	0
Cal.	Large	2	18-May-17	102.00	3	3	3	0	0	0	0
Cal.	Large	3	18-May-17	14.50	0	0	0	0	0	0	0
Cal.	Large	4	18-May-17	18.50	2	1	1	0	1	0	0
Cal.	Large	5	18-May-17	91.50	21	21	14	7	0	0	0

Table B2. Number of castings by earthworm taxa at each plot of the Summer.

Site	Stream	Plot	Date	Cast.	EW.	T.S.	J.S.	A.S.	E.	O.	A.
S.S.	Small	1	6-Jul-17	34.50	0	0	0	0	0	0	0
S.S.	Small	2	6-Jul-17	148.00	4	0	0	0	4	0	0
S.S.	Small	3	6-Jul-17	0.00	0	0	0	0	0	0	0
S.S.	Small	4	6-Jul-17	16.50	1	1	0	1	0	0	0
S.S.	Small	5	6-Jul-17	0.00	0	0	0	0	0	0	0
S.S.	Medium	1	6-Jul-17	0.00	0	0	0	0	0	0	0
S.S.	Medium	2	6-Jul-17	7.00	0	0	0	0	0	0	0
S.S.	Medium	3	6-Jul-17	3.00	5	2	1	1	1	0	2
S.S.	Medium	4	6-Jul-17	0.00	1	1	1	0	0	0	0
S.S.	Medium	5	6-Jul-17	0.50	1	1	1	0	0	0	0
S.S.	Large	1	24-Jul-17	0.00	0	0	0	0	0	0	0
S.S.	Large	2	24-Jul-17	0.00	3	0	0	0	3	0	0
S.S.	Large	3	25-Jul-17	0.00	0	0	0	0	0	0	0
S.S.	Large	4	25-Jul-17	0.00	0	0	0	0	0	0	0
S.S.	Large	5	25-Jul-17	1.00	0	0	0	0	0	0	0
Cal.	Small	1	20-Jul-17	0.00	0	0	0	0	0	0	0
Cal.	Small	2	20-Jul-17	4.00	0	0	0	0	0	0	0
Cal.	Small	3	20-Jul-17	0.00	5	5	5	0	0	0	0
Cal.	Small	4	20-Jul-17	9.33	0	0	0	0	0	0	0
Cal.	Small	5	20-Jul-17	0.00	0	0	0	0	0	0	0
Cal.	Medium	1	20-Jul-17	0.00	0	0	0	0	0	0	0
Cal.	Medium	2	20-Jul-17	1.00	0	0	0	0	0	0	0
Cal.	Medium	3	20-Jul-17	0.67	3	1	0	1	0	0	2
Cal.	Medium	4	20-Jul-17	0.00	0	0	0	0	0	0	0
Cal.	Medium	5	20-Jul-17	3.00	0	0	0	0	0	0	0
Hit.	Small	1	11-Jul-17	0.00	0	0	0	0	0	0	0
Hit.	Small	2	11-Jul-17	0.00	0	0	0	0	0	0	0
Hit.	Small	3	11-Jul-17	0.00	0	0	0	0	0	0	0
Hit.	Small	4	11-Jul-17	0.00	0	0	0	0	0	0	0
Hit.	Small	5	11-Jul-17	0.00	0	0	0	0	0	0	0
Hit.	Medium	1	11-Jul-17	0.00	0	0	0	0	0	0	0
Hit.	Medium	2	11-Jul-17	0.00	0	0	0	0	0	0	0
Hit.	Medium	3	11-Jul-17	0.00	0	0	0	0	0	0	0
Hit.	Medium	4	11-Jul-17	0.00	4	2	2	2	2	0	0

Continuation to Table B2.

Site	Stream	Plot	Date	Cast.	EW.	T.S.	J.S.	A.S.	E.	O.	A.
Hit.	Medium	5	11-Jul-17	9.00	23	23	23	0	0	0	0
Cal.	Large	1	19-Jul-17	0.00	0	0	0	0	0	0	0
Cal.	Large	2	19-Jul-17	31.67	0	0	0	0	0	0	0
Cal.	Large	3	19-Jul-17	0.33	1	1	1	0	0	0	0
Cal.	Large	4	19-Jul-17	0.33	14	14	14	0	0	0	0
Cal.	Large	5	19-Jul-17	11.00	5	5	4	1	0	0	0
Hit.	Large	1	12-Jul-17	0.00	0	0	0	0	0	0	0
Hit.	Large	2	12-Jul-17	0.00	0	0	0	0	0	0	0
Hit.	Large	3	12-Jul-17	0.00	0	0	0	0	0	0	0
Hit.	Large	4	12-Jul-17	0.00	0	0	0	0	0	0	0
Hit.	Large	5	12-Jul-17	0.00	0	0	0	0	0	0	0
Hit.	Small	6	11-Jul-17	11.50	0	1	1	0	0	0	0

Table B3. Number of castings by earthworm taxa at each plot of the Fall.

Site	Stream	Plot	Date	Cast.	EW.	T.S.	J.S.	A.S.	E.	O.	A.
S.S.	Small	1	21-Sep-17	3.00	0	0	0	0	0	0	0
S.S.	Small	2	21-Sep-17	12.00	2	1	1	0	1	0	0
S.S.	Small	3	21-Sep-17	0.00	0	0	0	0	0	0	0
S.S.	Small	4	21-Sep-17	0.00	0	0	0	0	0	0	0
S.S.	Small	5	21-Sep-17	0.00	0	0	0	0	0	0	0
S.S.	Medium	1	21-Sep-17	4.00	2	0	0	0	2	0	0
S.S.	Medium	2	21-Sep-17	0.00	0	0	0	0	0	0	0
S.S.	Medium	3	21-Sep-17	0.00	0	0	0	0	0	0	0
S.S.	Medium	4	21-Sep-17	0.00	0	0	0	0	0	0	0
S.S.	Medium	5	21-Sep-17	5.50	0	0	0	0	0	0	0
S.S.	Large	1	19-Sep-17	2.00	4	1	1	0	3	0	0
S.S.	Large	2	19-Sep-17	2.00	0	0	0	0	0	0	0
S.S.	Large	3	19-Sep-17	0.00	0	0	0	0	0	0	0
S.S.	Large	4	19-Sep-17	0.00	0	0	0	0	0	0	0
S.S.	Large	5	19-Sep-17	0.00	0	0	0	0	0	0	0
Cal.	Small	1	12-Oct-17	0.33	0	0	0	0	0	0	0
Cal.	Small	2	12-Oct-17	2.33	0	0	0	0	0	0	0
Cal.	Small	3	12-Oct-17	0.00	0	0	0	0	0	0	0
Cal.	Small	4	12-Oct-17	0.33	0	0	0	0	0	0	0
Cal.	Small	5	12-Oct-17	0.67	0	0	0	0	0	0	0
Cal.	Medium	1	12-Oct-17	0.00	0	0	0	0	0	0	0
Cal.	Medium	2	12-Oct-17	0.00	2	1	1	0	1	0	0
Cal.	Medium	3	12-Oct-17	2.33	2	1	1	0	0	0	0
Cal.	Medium	4	12-Oct-17	0.00	7	0	0	0	6	0	1
Cal.	Medium	5	12-Oct-17	0.33	2	2	2	0	0	0	0
Hit.	Small	1	6-Oct-17	0.00	0	0	0	0	0	0	0
Hit.	Small	2	6-Oct-17	0.00	0	0	0	0	0	0	0
Hit.	Small	3	6-Oct-17	4.50	1	1	1	0	0	0	0
Hit.	Small	4	6-Oct-17	2.67	0	0	0	0	0	0	0
Hit.	Small	5	6-Oct-17	0.00	0	0	0	0	0	0	0
Hit.	Medium	1	6-Oct-17	33.33	26	26	26	0	0	0	0
Hit.	Medium	2	6-Oct-17	42.00	7	7	7	0	0	0	0
Hit.	Medium	3	6-Oct-17	0.00	0	0	0	0	0	0	0
Hit.	Medium	4	6-Oct-17	0.00	0	0	0	0	0	0	0

Continuation to Table B3.

Site	Stream	Plot	Date	Cast.	EW.	T.S.	J.S.	A.S.	E.	O.	A.
Hit.	Medium	5	6-Oct-17	0.00	0	0	0	0	0	0	0
Cal.	Large	1	12-Oct-17	0.00	0	0	0	0	0	0	0
Cal.	Large	2	12-Oct-17	28.67	17	17	17	0	0	0	0
Cal.	Large	3	12-Oct-17	9.67	18	18	18	0	0	0	0
Cal.	Large	4	12-Oct-17	4.33	5	5	5	0	0	0	0
Cal.	Large	5	12-Oct-17	52.00	15	15	15	0	0	0	0
Hit.	Large	1	6-Oct-17	12.50	0	0	0	0	0	0	0
Hit.	Large	2	6-Oct-17	7.50	0	0	0	0	0	0	0
Hit.	Large	3	6-Oct-17	37.50	1	1	1	0	0	0	0
Hit.	Large	4	6-Oct-17	22.00	4	4	4	0	0	0	0
Hit.	Large	5	6-Oct-17	30.00	1	1	1	0	0	0	0