Systematic Studies Of Polydesmidan Millipedes (Diplopoda, Polydesmida).

BY

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THESIS
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1. Introduction

1.1 Systematics

The tree of life is a remarkable biological concept based on the accepted notion that every living thing on Earth is related through decent from a common ancestor. In the time since Linnaeus laid the foundation for biological classification, roughly 1.2 million species of plants, animals, fungi and microscopic organisms have been formally described, and millions more remain to be discovered (Mora et al., 2011). All named organisms are ordered into a hierarchical classification that has traditionally been based on morphological similarity. Ideally, the classification system should reflect true evolutionary relatedness through common decent. This goal was significantly advanced by Hennig (1966) with the advent of phylogenetic systematic theory, where relatedness is based on shared traits inherited from a common ancestor. It is the task of the systematist to not only recognize and classify living organisms, but to determine their true evolutionary relationships, thereby untangling the branches on the tree of life. Frequently, the results of such research necessitate restructuring of the hierarchy and reclassification of organisms. A solid systematic foundation forms the framework on which evolutionary, environmental and ecological research is based. Such research not only has heuristic value, but is also essential for the advancement of conservation, agriculture, medicine and other applied fields.

The research contained in this thesis is concerned with one small tip of one small branch in the tree of life.

1.2 Millipedes

Millipedes are terrestrial animals that belong to the phylum Arthropoda, invertebrates characterized by a segmented body, an exoskeleton and jointed appendages. The extant arthropods have been traditionally divided into 4 major groups: Chelicerata (spiders, scorpions, ticks, horseshoe crabs), Hexapoda (insects, springtails), Crustacea (crabs, barnacles, shrimp, isopods) and Myriapoda (millipedes, centipedes). The relationships among these groups has been an ongoing hot topic of research, and several competing hypotheses have been proposed. The popular consensus (Figure 1.1) is that Chelicerata is sister to the remaining arthropods, the Mandibulata. Within the latter, Myriapoda is sister to a Crustacea /Hexapoda clade. Within the myriapods, four classes of extant animals are recognized: Diplopoda (millipedes), Chilopoda (centipedes), Symphyla and Pauropoda. Hypotheses of myriapod relationships also vary. The popular consensus puts Chilopoda as sister to the rest, and Symphyla is sister to Diplopoda and Pauropoda.

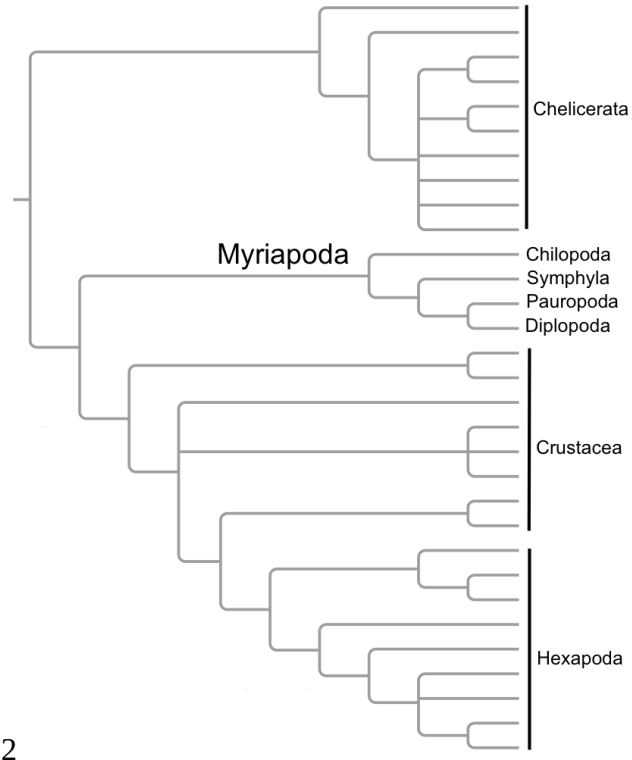

Figure 1.1. Arthropod phylogeny. A popular consensus for the relationships among major arthropod groups and the myriapods. Modified from Giribet and Edgecombe, 2012.

Diplopoda is an extremely diverse, species-rich group of terrestrial arthropods, endemic to every continent (except Antarctica) and many islands. Millipedes are primarily detritivores and can contribute significantly to the decomposition of their local biomass (Cárcamo et al., 2000; Hättenschwiler and Gasser, 2005). They are distinguished by the fusion of adjacent somites into "diplosegments", each of which has two pairs of legs (the first three segments are unfused and have only one leg pair). Body size and shape, as well as the number of legs, varies considerably among millipede species. Over 12,000 species have been formally described, although the number of valid species may be around 7,700 (Brewer et al., 2012a; Shear, 2011). Estimates of how many species actually exist range from 15-20,000 (Brewer et al., 2012a) to 80,000 (Hoffman, 1980). Millipede species are divided among 16 orders (Table 1.1), which are diagnosed mainly by segment number, head morphology, cuticle characteristics and shape and position of sexual organs. The order Polyxenida (bristle-millipedes) consists of minute individuals with uncalcified cuticles covered with tufts of setae. These millipedes have about a dozen segments and no sexually modified legs. The orders Glomeridesmida, Glomerida and Sphaerotherida (collectively called Pentazonia) are generally robust individuals with 12-22 segments and are capable of rolling into a protective ball. One or two pairs of posterior legs in males (telopods) are modified to grasp females during mating.

Most millipedes belong to the remaining group, Helminthomorpha, and are the more familiar forms to most people. Their bodies are long and either tubular or flattened with 20 to 100's of body segments. They have poisonous secretions from pores all along their length for defense, and the males have 1 or 2 leg pairs at the 7 and 8^{th} segments highly modified for sperm transfer (gonopods). Helminthomorpha is divided into the Colobognatha and the Eugnatha. The

Colobognatha (orders Platydesmida, Polyzoniida, Siphonocryptida and Siphonophorida) are distinguished from Eugnatha by their small heads, reduced mouthparts and the position of the gonopods. Eugnatha consist of the Nematophora (orders Callipodida, Chordeumatida and Stemmiulida), the Juliformia (orders Julida, Spirobolida and Spirotreptida) and the Merocheta (order Polydesmida). The Polydesmida is the most species rich (ca. 5,000) order of millipedes and is the focus of this thesis.

Subclass	Infraclass	Subterclass	Superorder	Order
Penicillata				Polyxenida
Chilognatha	Helmintho-morpha	*incertae sedis*		Siphoniulida
		Colobognatha		Platydesmida Polyzoniida Siphonocryptida Siphonophorida
		Eugnatha	Merocheta	Polydesmida
			Juliformia	Julida Spirobolida Spirostreptida
			Nematophora	Callipodida Chordeumatida Stemmiulida
	Pentazonia			Glomeridesmida Glomerida Sphaerotherida

Table 1.1. Taxonomic breakdown of millipede orders.

Polydesmidan millipedes are characterized by the lateral extension of the dorsal tergites (paranota) that give them their flattened appearance. All polydesmidan species have 20 body segments (rarely 19 or 21), exude cyanic defensive secretions, and are completely devoid of

visual organs. All males have the anterior leg pair of the seventh body ring modified into gonopods. Body size varies from a few millimeters to several centimeters in length and coloration varies from dull gray to bright multi-colored patterns. The taxonomic structure of this group, like that of other millipedes, is largely unknown. While several polydesmidan families and genera have been thoroughly studied, most are in a state of taxonomic disorder. Many genera and species are based on very old, sometimes poor, descriptions. Descriptions may be based on single specimens and many type specimens are damaged or missing from their deposited collections.

1.3 Thesis

The overall objective of this thesis is to advance our existing body of knowledge of polydesmidan systematics through three research projects. The first project (Chapter 2) is a review of the genus, *Docodesmus*, which is part of the large, severely understudied family Pyrgodesmidae. The goal is to not only consolidate all information on this group and stabilize its classification, but to provide a template that can be followed in future studies of other pyrgodesmid genera. A complete taxonomic history of the genus is presented for the first time. The diagnostic characters of the genus are evaluated and potential close affinities with several other pyrgodesmid genera are discussed. All 22 species currently assigned to the genus are listed with complete synonymies, citations and diagnoses, and some necessary taxonomic revision is made.

The second project (Chapter 3) is a critical review of the concept of subspecies. Subspecies recognition is a controversial taxonomic practice that stems from the lack of an objective definition of the concept and inconsistent use of the category. The practice of designating

subspecies is assessed here through a thorough review of all subspecies designations of polydesmidan millipedes over a 50-year period. The survey focuses on the justification given for subspecies recognition, the amount of data available for the designation, the handling of nominate subspecies and the criteria used for diagnosis. Several problematic issues are addressed and suggestions to enhance future work are provided. Three examples of subdivided species from the Euryuridae are presented in detail with some taxonomic revision.

The third project, which is spread out over three chapters, is a thorough systematic study of the family Euryuridae. This is one of the better studied polydesmidan families, having been revised by Hoffman (1978) and Shelley (1982b), but has never been subjected to proper phylogenetic analysis nor genetic sequencing. The first part (Chapter 4) concerns the description of a newly discovered species and the dismissal of another controversial one. This chapter also includes an introduction to the taxonomic history of the family name. The second part (Chapter 5) is a phylogenetic study based on genetic sequences and sexual traits. This study suggests relationships of some species based on phylogenetic analyses of different data sets. Although a robust phylogeny could be not be resolved due to discordance of the different data sets, the discordance itself is quite interesting and is discussed. Insight into the biogeographic history based on genetic information and distributional data concludes this part. Finally, Chapter 6 begins with a complete literature review and the taxonomic history of the family and every species ever associated with it. This chapter also comprises a morphological study, which includes SEM images of all body parts and sexual organs of all species and a discussion of morphological characters. All 14 species currently assigned to Euryuridae are listed with complete synonymies, citations and diagnoses.

2. Review of the Caribbean pyrgodesmid genus *Docodesmus* Cook with notes on potentially related genera (Diplopoda, Polydesmida, Pyrgodesmidae)

2.1 Introduction

The polydesmidan family Pyrgodesmidae Silvestri, 1896 contains relatively small (3–15mm) soil-dwelling millipedes with a mostly pantropical distribution, and typically have an enlarged collum (Figure 2.6a, b) that completely conceals the head in dorsal view (Hoffman 1982, listed as diagnostic feature). Other common characters include lobed paranota and granulated or tuberculated tergites, which provide many of these millipedes with a characteristic ornate dorsal texture. Taxonomically, the family is in dire need of monographic revision. The 371 species (including 17 subspecies) are placed in 169 genera, 116 of which are monotypic. The most species-rich genera are *Lophodesmus* Pocock, 1894, *Docodesmus* Cook, 1896, *Myrmecodesmus* Silvestri, 1910, *Calymmodesmus* Carl, 1914, and *Aporodesmus* Porat, 1894, containing 25, 22, 20, 17 and 11 species respectively. All remaining genera contain 8 or fewer species (Figure 2.1).

In light of this situation, and considering that the majority of the genera are poorly defined, potentially monophyletic units cannot be delineated without examination of type specimens of all type species. Since a monographic revision of the entire family is beyond feasibility, we decided to select manageable sets of taxa, such as the genus *Docodesmus*, with geography as the main selection criterion until some putative monophyletic units became discernible. We reviewed all relevant literature and examined every available male type specimen of *Docodesmus* species; we confirmed the unavailability of lost types with the respective curators of the collections. We did not examine all female type specimens (Table 2.1),

but base our discussion of these species on the morphological data presented in the first description of the species. The results we found justify this approach. We were able to provide a robust definition for the group *and* identify potentially related genera. Our research revealed that Pocock's description of the type species was misinterpreted by all subsequent authors, and that diagnostic characters have never been unambiguously identified for the genus. For these reasons we present a review of the genus *Docodesmus* rather than a monographic revision.

SPECIES	Specimen examined	Gonopod type	Tubercles of 4th coxa	Tubercles of 4th sternum
D. alifer	HT (female)	--	--	--
D. amazonicus	HT	type L	reduced	none
D. angustus	HT	type G	present	small swelling
D. brodzinskyi	none	--	--	--
D. centralis	none	?	?	?
D. cooki	HT	type L	present	none
D. coxalis	HT	type G	reduced	small swelling
D. cubensis	HT (female)	type G[1]	?	?
D. eggletoni	none	?	?	?
D. grenadae	HT	type L	present	present
D. griseus	HT	type G	present	none
D. haitiensis	HT	type L	present	small swelling
D. hirudiformis	HT	type L	none	small swelling
D. maculatus	none	?	?	?
D. maldonadoi	none	?	?	?
D. parvior	male	type ?[2]	reduced	none
D. robustus	HT	type L	present	present
D. sculpturatus	HT	type G	present	present
D. semiseptus	HT	type G	present	none
D. trinidadensis	male	type L	present	none
D. vidalius	none	?	?	?
D. vincentii	male	type L	present	present

Table 2.1. Summary of all *Docodesmus* species recognized at the beginning of this study: the material observed, gonopod type and description of tubercles on 5th body ring. Dashes (-) unknown due to species being known from females only. Question marks (?) unknown due to unavailability of material. [1]Inferred from figure 25 of Loomis, 1938. [2]Unknown due to retraction of anterior processes.

Abbreviations

BMNH – The Natural History Museum, London; formerly: British Museum (Natural History)

FMNH – Field Museum of Natural History

FSCA – Florida State Collection of Arthropods

ICZN – International Code of Zoological Nomenclature

INPA – Instituto Nacional de Pesquisas da Amazônia

MACN – Division de Etnomologico, Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina

MCZ – Museum of Comparative Zoology, Harvard University

MSNG – Museo Civico di Storia Naturale "Giacomo Doria", Genova

USNM – National Museum of Natural History, Smithsonian Institution; formerly United States National Museum; types can be searched on-line at: http://collections.si.edu/search/results.jsp

ZMB – Museum für Naturkunde, Berlin

ZMUC – Zoological Museum, University of Copenhagen; types can be searched on-line at: http://www.zmuc.dk/EntoWeb/collections-databaser/diplo-polydesmida.htm

HT – Holotype

PT – Paratype

LAP – long anterior process of gonopod

SAP – short anterior process of gonopod

Specimen designations follow the established collection codes rather than modified names of their respective museums.

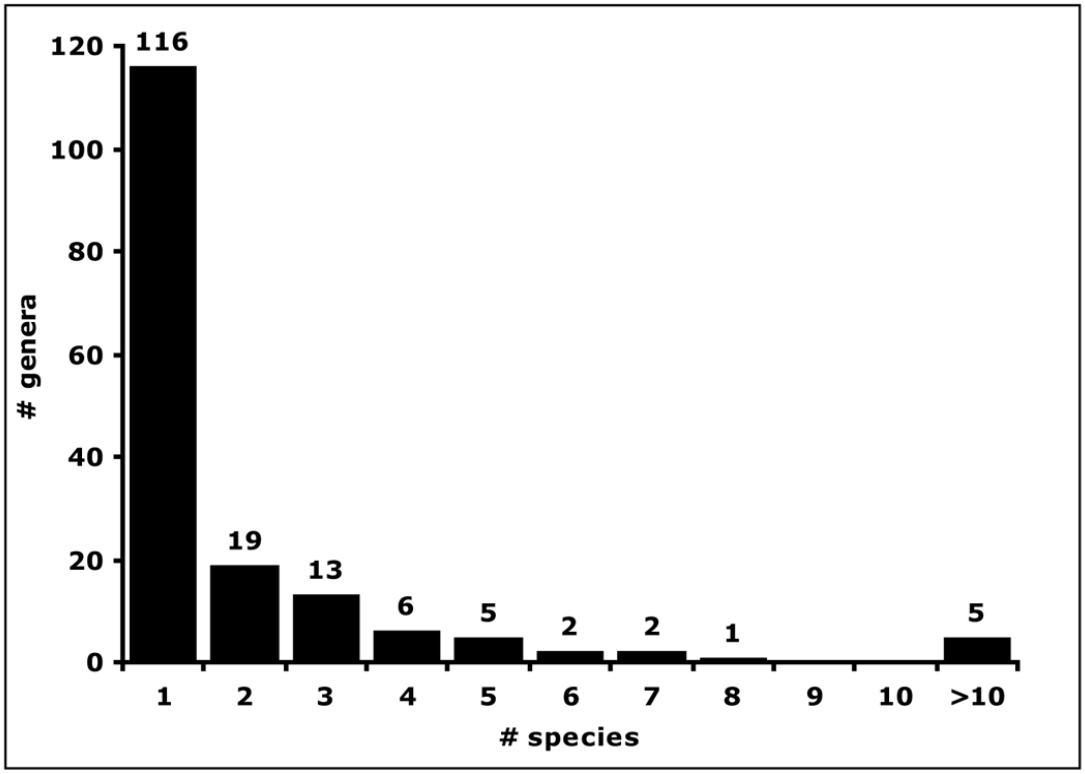

Figure 2.1. Number of species per genus in the family Pyrgodesmidae. 116 of 169 genera or 69% are monotypic. This suggests that the family is greatly oversplit and requires intense taxonomic revision. Data from global millipede database (Sierwald, unpublished).

2.2 Material and Methods

We examined the species descriptions for all species assigned to *Docodesmus*, and defined characters and character states. We organized these in a character matrix, which ensured consistent recording of all characters and character states for all taxa and allowed quick capture of missing data. All variable characters found are listed in Table 2.1

We examined specimens of the following *Docodesmus* species: *alifer* Loomis, 1941 (HT), *amazonicus* Golovatch, 1997 (HT), *angustus* Loomis, 1941 (HT), *centralis* Silvestri, 1898 (HT), *cooki* Loomis, 1969 (HT, PT), *coxalis* Loomis, 1975 (HT, PT), *cubensis* Loomis, 1937

(HT), *grenadae* Chamberlin, 1918 (HT), *griseus* Loomis, 1941 (HT), *haitiensis* Chamberlin, 1918 (HT), *huridiformis* Golovatch, 1999 (HT, PT), *parvior* Chamberlin, 1918, *robustus* Loomis, 1934 (HT, PT), *sculpturatus* Loomis, 1934 (HT, PT), *semiseptus* Loomis, 1936 (HT), *trinidadensis* Chamberlin, 1918 and *vincentii* Pocock, 1894 (PT). For the species to which we did not have access (*brodzinski* Shear, 1981, *maculatus* Bollman, 1888, *eggletoni* Velez, 1967, *maldonadoi* Velez, 1967, *vidalius* Velez, 1967), we relied on the original descriptions. We also examined specimens of the type species of some of the putatively related genera: *Coccoelasma incisura* Loomis, 1936 (HT), *Henicomus septiporus* Loomis, 1941 (HT) and *Jeekelia granulosa* Loomis, 1950 (HT)

Specimens were examined with a Leica MZ8 dissecting microscope. Digital images were taken with a Microptics®-Imaging-System (based at FMNH). Final images were assembled from 6-10 source images taken at different focal lengths using the software package Helicon Focus. SEM images were taken from gold sputter-coated specimens with a LEO Evo 60 SEM.

2.3 History of *Docodesmus*

Genus *Docodesmus* Cook, 1896

Aporodesmus Pocock, 1894: 789. Type species: *Cryptodesmus vincentii* Pocock, 1894, by original designation. Preoccupied by *Aporodesmus* Porat, 1894.

Docodesmus Cook, 1896: 5. Type species: *Cryptodesmus vincentii* Pocock, 1894, by direct substitution. – Loomis, 1937: 224 (key to the 9 then-known species) – Loomis, 1941: 67 (key to West Indian Chytodesmidae genera) – Loomis, 1969: 249 (key to the 13 then-known species).

Schizodira Loomis, 1941: 37. Type species: *Stenonia maculata* Bollman, 1888, by original designation. Synonymized by Loomis, 1950: 165.

Currently, the genus *Docodesmus* contains 22 species, 19 from the Caribbean, and three species from mainland South America.

Pocock (1894b) described *Cryptodesmus vincentii* from the Caribbean Island St. Vincent. *Cryptodesmus* Peters, 1864 (type species *Polydesmus olfersii* Brandt, 1839) became the type genus of the family Cryptodesmidae Karsch, 1880 (1881). Later, Pocock (1894c), designated *vincentii* as the type species for his new genus '*Aporodesmus'* the name of which was preoccupied by *Aporodesmus* Porat, 1894 (type species *Polydesmus gabonensis* Lucas, 1858, from Africa). In a very short note, Cook (1896b) argued that the Caribbean species could not possibly be congeneric with the African species and introduced the new genus *Docodesmus* to accommodate the species *vincentii*.

Earlier, Bollman (1888) had described *Stenonia maculata* from Cuba (*Stenonia* Gray, 1843 is currently placed in the Chelodesmidae). He cited the similarity to *Stenonia fimbriatus* (Peters, 1864, sub *Polydesmus*) as justification for the placement in this genus, despite differences in dorsal tuberculation, crenulation of paranota, anal segment characters and coloration. *Stenonia fimbriatus* became the type species of the genus *Tirodesmus* Cook, 1896 which is currently placed in the Platyrhacidae. Chamberlin (1918a) listed *maculata* under Platyrachidae (sic) as "*Platyrachus* (?) *maculatus"* without further explanation. Loomis (1941b) recognized that *maculata* did not belong in the Platyrhacidae and described the genus *Schizodira* to accommodate the species. However later, having examined the paratype female (USNM), Loomis (1950) confidently placed *maculata* in *Docodesmus,* citing Bollman's misleading

original description for his 'error' in creating *Schizodira.* His justification for placing *maculata* (corrected to *maculatus*) into *Docodesmus* was the lobation pattern of the paranotal margins.

Silvestri (1898) was the first to describe a new species in *Docodesmus – centralis* from La Guaira, Venezuela. The description, however, is brief and contains no justification for placement in the genus. Attems (1899) criticized the introduction of new genera (25 pyrgodesmid and 16 cryptodesmid genera had been described by 1899) and new species supported by sparse descriptions. He assigned *vincentii* to *Aporodesmus* and suggested no placement for *Docodesmus centralis.*

Chamberlin (1918a) described four new Caribbean species in the genus *Docodesmus*: *grenadae*, *haitensis, trinidadensis* and *parvior*. He provided no justification for their placement in *Docodesmus*, but included a few brief comparisons with *D. vincentii*. Subsequently, Loomis described 9 new species: *robustus* and *sculpturatus* (1934), *semiseptus* (1936), *cubensis* (1937), *alifer*, *angustus and griseus (1941a), cooki (1969)* and *coxalis* (1975). Loomis (1937) was the first to address all *Docodesmus* species collectively in a key to 9 of the 10 then recognized species (*centralis* was omitted, as Loomis was concerned with only West Indian species). His most recent species key (1969) addressed 13 species, again omitting *centralis* and, oddly enough, *maculatus* (see above). Additionally, Loomis (1941a) was the first to define generic characters of *Docodesmus* in a key to West Indian Chytodesmidae genera. These included a normal pore formula (5,7,9-10,12-13,15-19); anterior margin of collum rounded, posterior margin angled and simple or indistinctly scalloped; low, often indistinct dorsal tubercles; body slightly arched, paranota (termed keels by Loomis) nearly horizontal; outer and posterior margin of paranota with small scallop-like lobes without deep incisions separating them; and paranota of body rings 7, 9, 10, 12 and 13 with four lobes on the outer margin.

Velez (1967) described three species (*eggletoni, maldonadoi* and *vidalius)* in the genus, but provided no justification for their placement. Shear (1981) described the fossil species *D. brodzinskyi* in the genus, citing its general similarity to Loomis's (1936) *Docodesmus* descriptions as evidence for the placement. Most recently, Golovatch described two South American species in the genus, *D. amazonicus* (1997) and *D. hirudiformis* (1999). He (1997: 328) summarized the generic characters of *Docodesmus*, referring mainly to *vincentii*, most of which are not shared with his two new species, nor with most other species assigned to *Docodesmus* at that time. These discrepancies are apparently due to a misreading of some of Pocock's original descriptions of *vincentii*. *Docodesmus vincentii* has 12, not 10, lobations of the anterior collum margin ("eleven abbreviated grooves radiate from [the border of the first tergite] towards the centre of the plate" (Pocock, 1894b)). The paramedian pair of setiferous tubercles on the anterior sternum and the similar structures on the adjacent coxae of males (Figures 6 e,f), assumed by later authors to occur on the 8th body ring and 10th leg pair, occur in fact on the 5th body ring and the 4th leg pair. Apparently, Pocock's use of the term '8th somite' referred to the 5th body ring (assuming diplosegments and defining somites as individual segments) and not to the 8th body ring. Golovatch (1997) also provided an up to date listing of *Docodesmus* species where he commented on the "shaky" status of *D. maculatus*, citing an unexplained transfer by Torre (1974). Apparently, Torre was unaware of the previously mentioned work by Loomis (1941b, 1950) and claimed to transfer *maculatus* from *Platyrachus* to *Docodesmus*. However, Loomis (1950) had already placed *maculatus* in *Docodesmus* twenty-four years prior and provided justification for the transfer (see above).

2.4 Characters of the genus *Docodesmus*

All members of the genus share a common pattern of tergite lobation (see diagnosis below and Figures 2.2, 2.6). These lobes are formed by indentations in the tergal margins, which can be apparent or indistinct. On occasion, individuals were observed with certain body rings deviating from the general pattern, likely due to developmental defect or injury; for example, the type specimen of *D. griseus* has asymmetrical lateral lobation on several body rings. The genus *Docodesmus*, as other members of the Pyrgodesmidae, is currently defined by somatic features only, no putative apomorphic characters from the male gonopods have been identified to date.

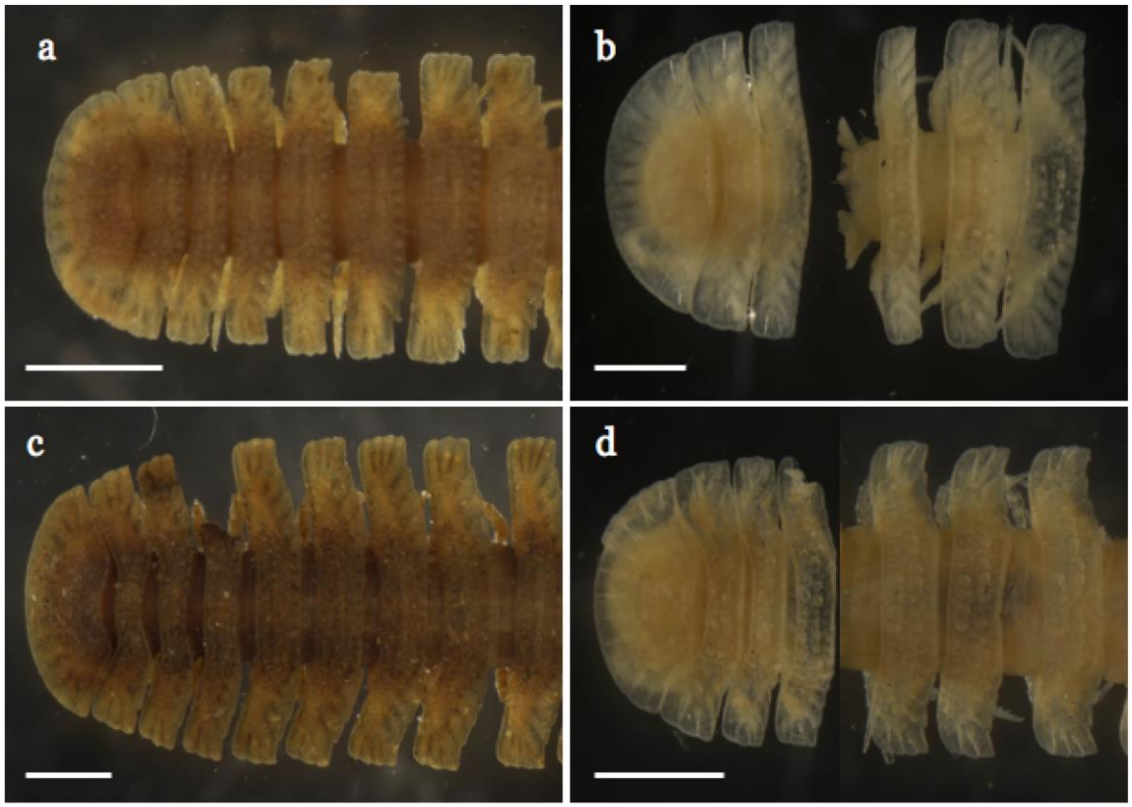

Figure 2.2. Digital Microptics® images of four *Docodesmus* species. Variation in dorsal granulation and tuberculation is difficult to capture due to their small size, differing degrees of translucence and encrusting of soil particulates. Lobation pattern, however, is clearer and shared by all *Docodesmus* species. a) *D. trinidadensis* male specimen, b) *D. haitiensis* male holotype, c) *D. robustus* female paratype, d) *D. cubensis* female holotype. Scale bars 1 mm.

Gonopod description first requires a clarification of terminology. Polydesmidan gonopods consist of a basal coxite and a distal telopodite. Telopodites are highly modified among different taxa usually resulting in several distinct processes or branches. No universally accepted set of terms exists for these structures throughout the order, or within the family Pyrgodesmidae, resulting in the use of multiple terms for the same structure, and use of the same term for different structures (see table 2 in Rowe and Sierwald 2006). Sections of the telopodite have been labeled variously as the podomeres of walking legs (e.g. prefemur, pre-femoral process, tibiotarsus), yet primary homology hypotheses for these sections with the podomeres have not been established. It must also be noted that usage of the terms pre-femur and femur to denote proximal and more distal telopodite sections changed over time; earlier authors (e.g., Attems and Brölemann) used the term femorite for the proximal section, which is currently denoted as prefemur, which typically carries setae, whereas the more distal sections of the telopodite are smooth. This shift in terminology is confusing and hinders the use of gonopods in phylogenetic analyses. The problem is compounded in the Pyrgodesmidae by the overall complexity of the gonopods and by the lack of any revisionary studies in the group. While we offer no solution to this problem, we avoid implying homology by employing descriptive terms for some gonopod structures.

The gonopods of *Docodesmus* (Figures 2.3-2.5) consist of large, bulbous coxae and much smaller, mesally oriented telopodites. Each telopodite has a basal setiferous prefemur (*pf* in Figures 3-4) and three distal processes. The posterior-most distal process is comparably larger, blunt and cylindrical (termed here, ‘cylinder’, *cyl* in Figures 2.3-2.5). The two anterior processes, LAP (long anterior process) and SAP (short anterior process), are long and spear-like (Figures 2.3-2.5). The prostatic groove is carried on the LAP. The association between the

cylinder and anterior processes divides *Docodesmus* species into two groups, which corresponds to a geographic pattern (Figure 2.5). In the group found on the South American mainland, several of the Lesser Antilles, and on Hispaniola, the cylinder is separated from the anterior processes ('type L' – Lesser Antilles, Figure 2.5). In the other group, found in the Bahamas, Cuba, Jamaica and Hispaniola, the base of the larger anterior process is continuous with the margin of the cylinder ('type G' – Greater Antilles, Figures 2.3-2.5). Three species from Puerto Rico – *eggletoni*, *maldonadoi* and *vidalius,* likely fall into these categories; unfortunately the type specimens are unavailable (Agnarsson in litt.) and the original work lacks detailed gonopod descriptions and clear drawings (Velez 1967).

The scanning electron microscope study revealed that *Docodesmus coxalis* possesses four spinneret-like structures on the epiproct (Figure 2.6d). Shear (2008) reviewed the occurrence of spinnerets in millipedes, with a focus on Polydesmida, and confirmed that spinnerets are common in many families of Polydesmida. He observed that families with comparatively larger body sizes tend to have putatively vestigial spinnerets that appear to be ordinary setae. Families with smaller individuals (including Pyrgodesmidae) appear to have functioning spinnerets with each seta set in a depression and nested within a short sleeve, as is shown in Figure 2.6d.

Most *Docodesmus* males have distinct setiferous tubercles on the anterior face of the 4th coxae (Figure 2.6e-f). A similar structure is also found on the adjacent sternum in some species. These structures vary among species in size and pilosity (Table 2.1).

Species differences within the genus are chiefly associated with size, tergite form and gonopod structure.

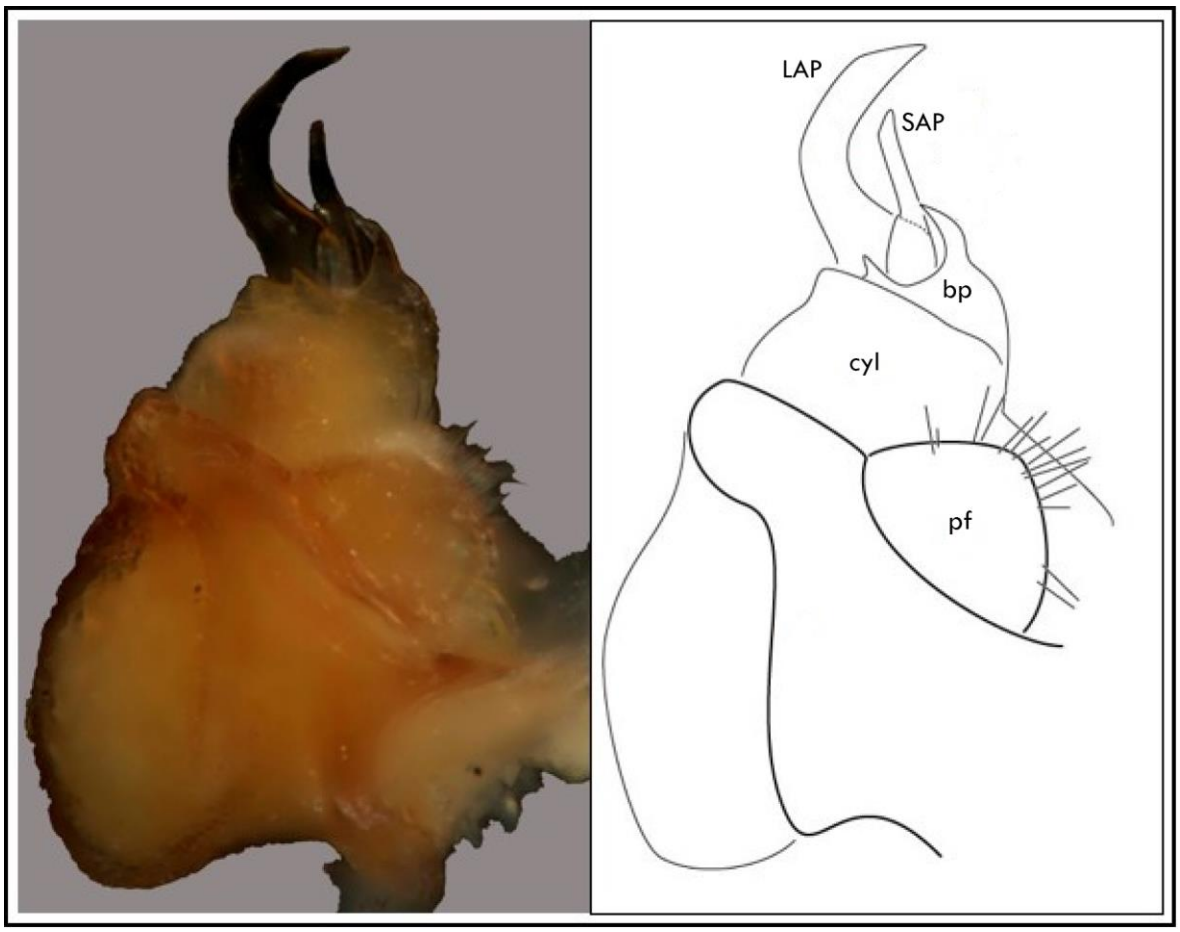

Figure 2.3. Digital Microptics® image of *D. coxalis* gonopod used as template for Adobe Illustrator drawing on right, gonopod type G. LAP - long anterior process, SAP - short anterior process, cyl - "cylinder", pf - prefemur, bp - bifid process formed at margin of cylinder (found only in *D. coxalis* and *D. cubensis*).

2.4.1 Intraspecific variation and species delimiting characters

Original descriptions of *Docodesmus* species rely in their diagnoses on comparisons with other, previously described species assumed to be congeneric. A survey of approximately 50 paratype specimens of *D. coxalis* revealed a significant amount of intraspecific variation in some of the characters used in these comparisons. These specimens were collected at the same time and location as the holotype, and all males can be confidently identified as conspecific due to the

presence of the bifid process of the cylinder (Figures 2.3-2.4). The color of the specimens varies from yellow to reddish to brown, the distinctness of the marginal lobes and dorsal tubercles varies from well defined to barely noticeable, and the paranota vary from being nearly flat to dipping acutely ventrad. Therefore, these characters were not included in any of our diagnoses.

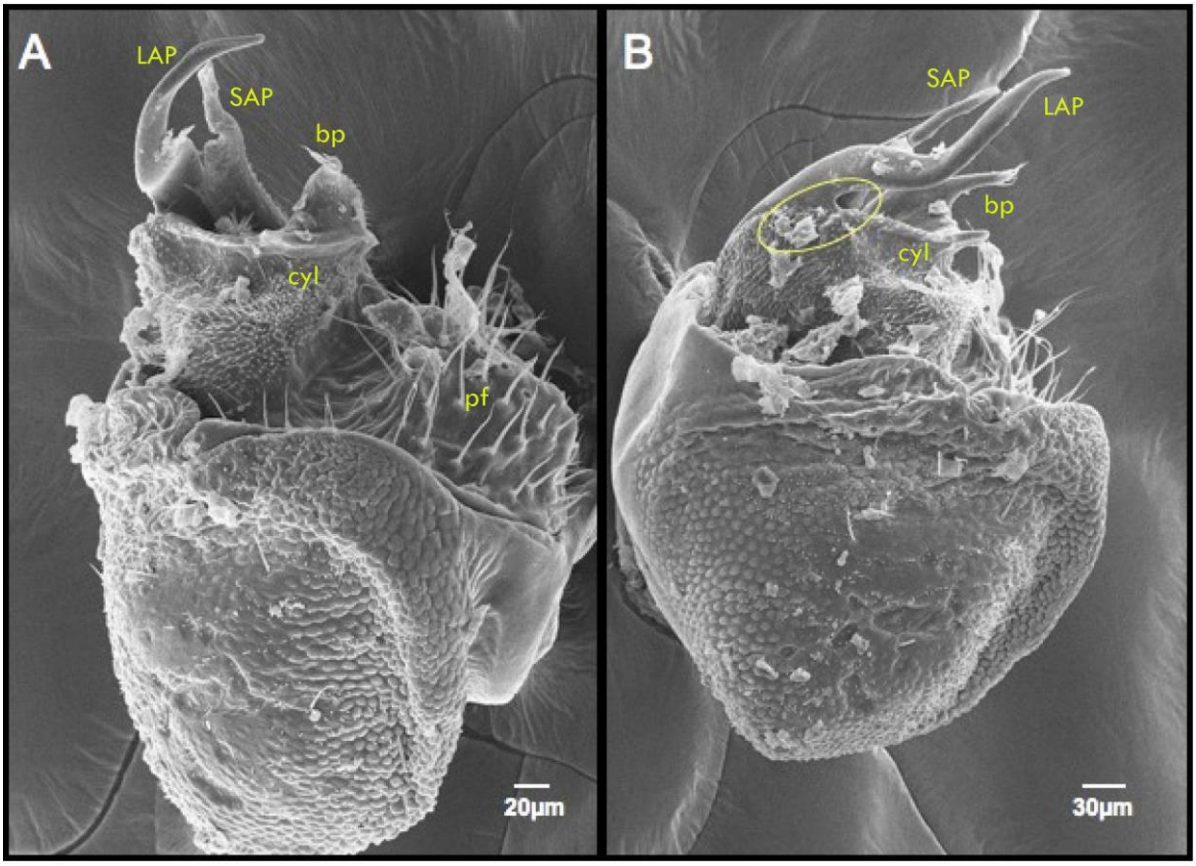

Figure 2.4. SEM images of *D. coxalis* right gonopod A) ventro-anterior view, B) ventral view. Anterior processes are oriented mesad *in situ*. Abbreviations same as Figure 2.3. Ellipse highlights area where LAP is continuous with cylinder (type G).

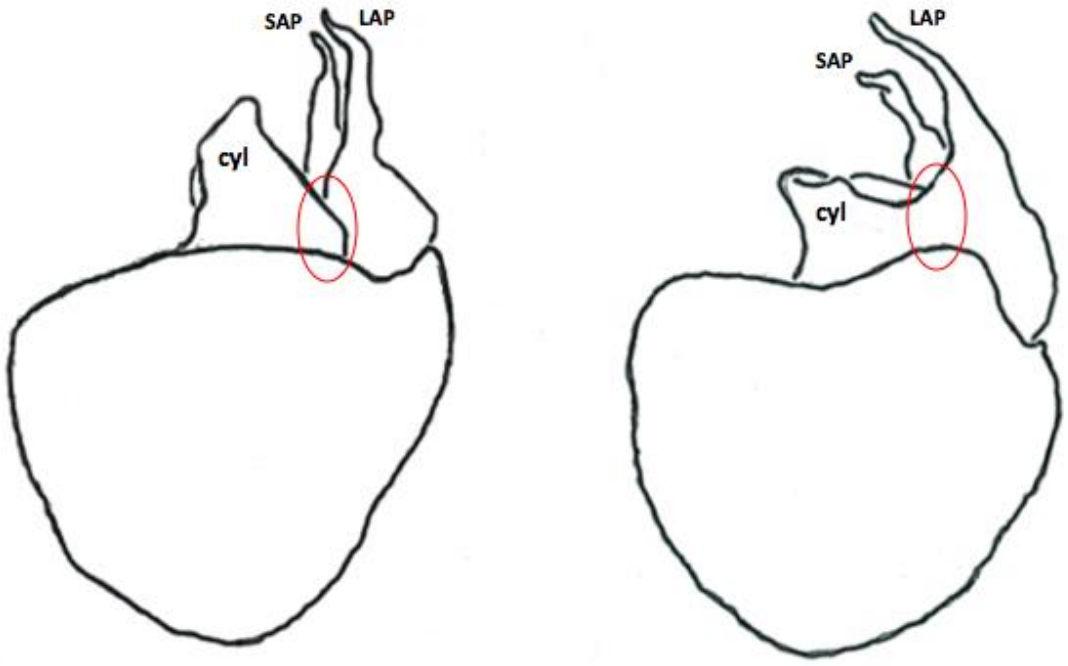

Figure 2.5. Left gonopods of *D. haitiensis* (left) and *D. angustus*, ventral view, illustrating the difference between type L and type G gonopods. The cylinder (cyl) of Type L species like *D. haitiensis* is separated from the LAP. In Type G species like *D. angustus*, the cylinder and LAP are continuous.

2.4.2 Diagnosis

The anterior margin of the first tergite (collum) is semi-circular and has 12 lobes (Figures 2.6a, b). The posterior margin of this segment is straight and unlobed. The ensuing tergites have an unlobed anterior margin and 16 strictly posterior lobes, quite distinct on the paranota, less so toward the body midline. Paranota have 3 lateral lobes on the non-poriferous body rings plus ring 5 (2-6, 8, 11, 14). The remaining body rings (7, 9-10, 12-13, 15-19) have 4 lateral lobes (Figure 2.7). Gonopods with large, bulbous coxites and mesally oriented telopodites consisting of one posterior 'cylindrical' process and two anterior spear-like processes.

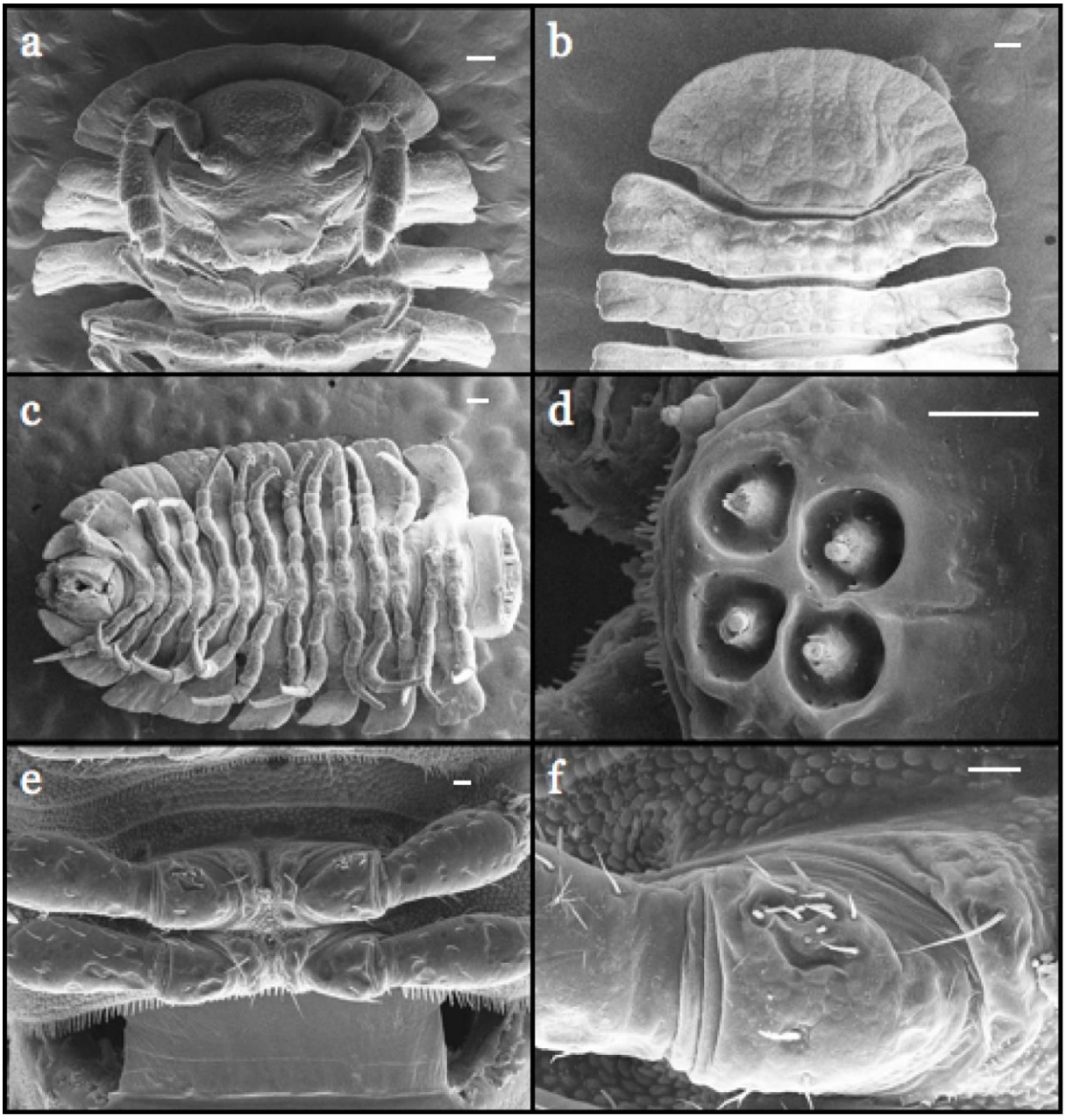

Figure 2.6. SEM images of *D. coxalis* male. a) ventral and b) dorsal views of anterior end. c) ventral view of posterior end. d) 'spinnerets' on tip of epiproct. e) ventral view of 4th and 5th leg pair and f) close up of tubercles of 4th leg pair coxa (up=anterior). Males of most *Docodesmus* species have such structures, varying in size and pilosity. Scale bars: a-c 100 µm, d-f 20 µm.

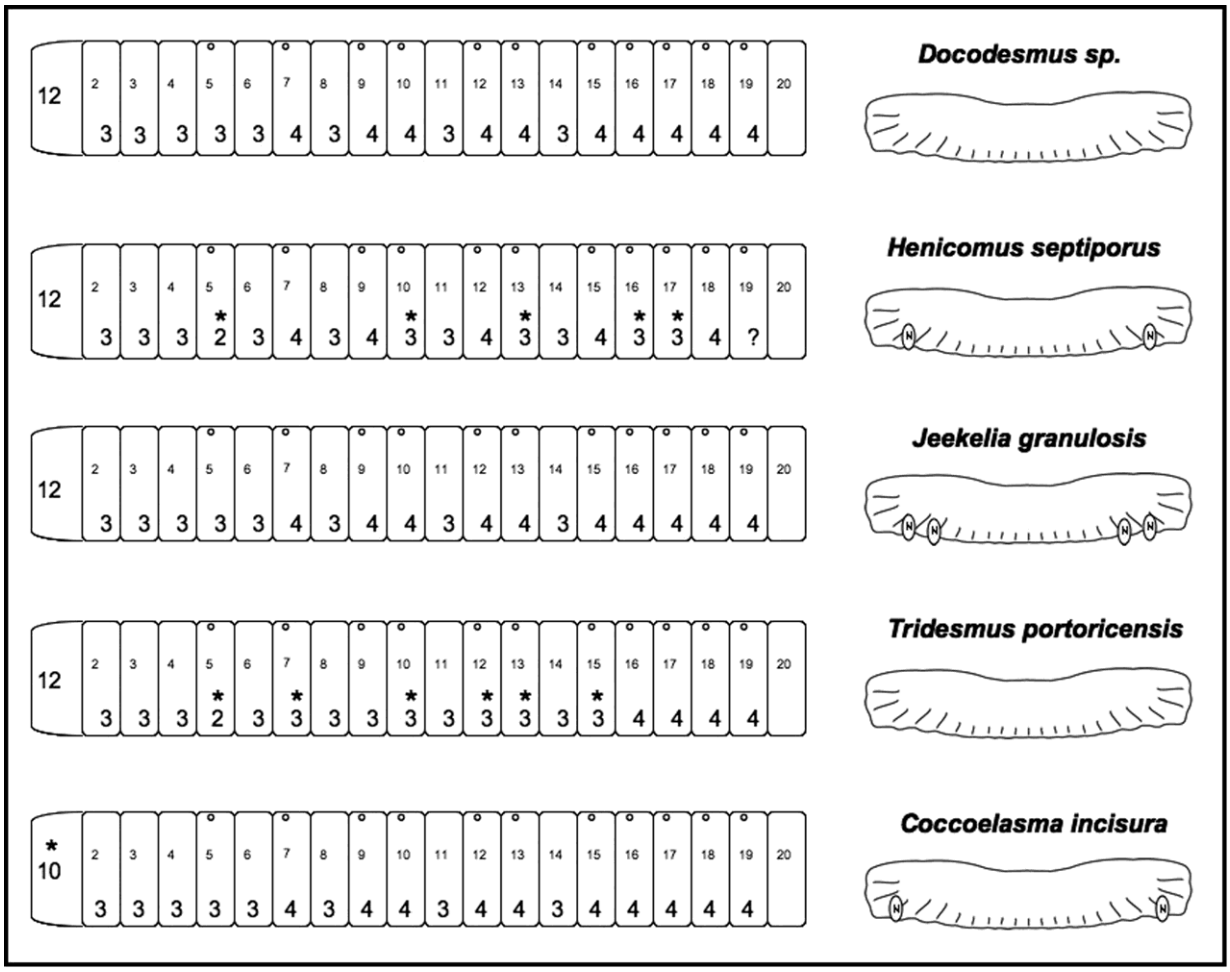

Figure 2.7. Comparison of lobation patterns of five Caribbean pyrgodesmid genera. Small numbers = ring number; large numbers = number of lobes, stars indicate difference from *Docodesmus*; ovals represent posterior notches.

2.5 Affinities of *Docodesmus*

Golovatch (1997) suggested, without citing characters that the genera *Leuritus* Chamberlin, 1923, *Coccoelasma* Loomis, 1936, *Cyphotylus* Loomis, 1936 and *Lobodesmus* Loomis, 1936 might be close relatives of *Docodesmus* when he discussed placement for his new species *Docodesmus amazonicus*. Three other Caribbean pyrgodesmid genera were cited by other authors as having a close affinity with *Docodesmus*: *Henicomus* Loomis, 1941, *Jeekelia*

Loomis, 1950 and *Tridesmus* Cook, 1896. Currently, the delineation of *Docodesmus* rests on the lobe patterns of the collum, the paranota and the posterior tergal margins. Due to insufficient descriptions and figures of potentially closely related taxa, no gonopodal apomorphies for *Docodesmus* can be defined at this point. Furthermore, mature male specimens have been identified for only three of the above genera: *Tridesmus* (one single male), *Lobodesmus* and *Leuritus*. After examination of the characters described, the illustrations of the type species, and examination of type material, we conclude that at this time none of the seven genera listed above can be unequivocally synonymized with *Docodesmus*. Except for *Tridesmus,* the other six genera are currently monotypic. Four genera, *Henicomus*, *Jeekelia*, *Tridesmus* and *Coccoelasma*, may have close affinity with *Docodesmus* based on their lobation pattern as illustrated in Figure 2.7.

2.5.1 *Tridesmus* Cook, 1896

The history of the genus name *Tridesmus* Cook, 1896 exemplifies the nomenclatorial confusion hindering the systematic treatment of many millipede taxa, especially in, but certainly not restricted to the Pyrgodesmidae. The genus *Tridesmus* can essentially be considered a phantom genus. It was described by Cook (1896a: 21), including the type species *Tridesmus* 'sectilis'. However, as Silvestri correctly noted (1908: 577), Cook did not actually describe the type species; he merely listed the name, a specimen of unspecified gender from Puerto Rico deposited in the Berlin Museum. According to ICZN, Article 12.3, the species is not validly described and thus the species name 'sectilis' is not available. Nevertheless, subsequent authors cited 'sectilis' as a valid species and placed other species into *Tridesmus*, without ever designating a type species for the genus. Currently, *Tridesmus* consists of two species from Puerto Rico: *T. portoricensis* Silvestri, 1908, and *T. guilarteus* Chamberlin, 1950, plus the Berlin

specimen Cook mentioned as ‘sectilis’, and four species from South America: *T. serratus* Silvestri, 1898, *T. cognatus* Silvestri, 1898, *T. ortonedae* Silverstri, 1898, and *T. perucola* Chamberlin, 1955. The type specimens of *portoricoensis* and *guilarteus* are female; the genders of the type specimens for *T. cognatus*, *T. perucola* and *T. serratus* were not specified in the descriptions, no figures of gonopods were given, nor were males mentioned in the descriptions. Silvestri mentions a male specimen for *T. ortonedae*. According to ICZN Article 67.2.2 the three species placed into *Tridesmus* by Silvestri in 1898 “are deemed to be the only originally included species.” A type species by subsequent designation should be selected from these three. *Tridesmus ortonedae*, being one of the first validly described species included in the genus *Tridesmus*, would qualify as the type species. However, it is questionable whether the South American and Puerto Rican species are congeneric. We postpone the designation of the type species until all available specimens assigned to *Tridesmus* have been re-examined.

The somatic features are best described in *T. portoricensis*, which has a 12-lobed collum and 16 lobes at the posterior margin of the tergites. The lateral lobation pattern is similar to *Docodesmus*, differing by poriferous paranota having one less lobe, but with the addition of a distinct structure, likely pore bearing, at the posterior tip of each paranotum. Cook (1896a) noted the similarity of *Tridesmus* to *Docodesmus* in size and shape and the differences in dorsal sculpture and poriferous paranota. Thus, the data that can be gleaned from the published literature, listed completely below, does not provide sufficient evidence to evaluate the possible relationship of any species placed into *Tridesmus* to *Docodesmus*.

Genus ***Tridesmus*** Cook, 1896

Tridesmus Cook 1896a: 21. Type species: listed as *Tridesmus* sectilis Cook, 1896a from Puerto Rico, name not available. Silvestri (1908: 577) noted that the proposed type species of the genus was still undescribed.

Tridesmus cognatus Silvestri

Tridesmus cognatus Silvestri, 1898: 63, no figures. HT (ZMUC) of unspecified gender from Venezuela. Type specimen listed as available at: http://www.zmuc.dk/EntoWeb/collections-databaser/diplo-polydesmida.htm

Cryptodesmus ? cognatus: Attems, 1899: 368. Placed into *Cryptodesmus* by Attems without reference to characters nor examination of specimens.

Tridesmus guilarteus Chamberlin

Tridesmus guilarteus Chamberlin, 1950: 148, no figures. Female HT from Puerto Rico in Coll. Chamberlin, type specimen available in USNM

Tridesmus guilarteus: Hoffman, 1999: 499. – Shelley, 2004: 1161.

Tridesmus ortonedae Silvestri

Tridesmus ortonedae Silvestri 1898: 63, no figures. Male HT from Guayaquil, Ecuador in Coll. Silvestri, possibly deposited in MFS (Portici) or at MACN.

Cryptodesmus ortonedae: Attems, 1899: 368. Placed in *Cryptodesmus* by Attems without reference to characters nor examination of specimens.

Tridesmus perucola Chamberlin

Tridesmus perucola Chamberlin, 1955: 42, no figures. Female HT from Peru deposited in Coll. Chamberlin, type specimen availabe at USNM.

Tridesmus portoricensis Silvestri

Tridesmus portoricensis Silvestri, 1908: 577, figures X, XI. Female HT from Puerto Rico deposited possibly at AMNH.

Tridesmus portoricensis: Chamberlin, 1918: 220. – Hoffman, 1999: 500. Holotype cited as male. – Shelley, 2004: 1161.

***Tridesmus* sectilis** Cook**,** name not available

Tridesmus sectilis Cook, 1896a: 21, no figures, no description, gender of specimen not recorded; locality: Puerto Rico, deposited at ZMB (listed as male syntype in Moritz & Fischer, 1978).

Cryptodesmus? sectilis: Attems, 1899: 367. Placed into *Cryptodesmus* by Attems without reference to characters nor examination of specimens, cited as an available name.

Tridesmus sectilis: Silvestri, 1908: 577, notes that the species has not yet been described. – Chamberlin, 1918: 219. (cited as an available name). – Hoffman, 1999: 500. (cited as an available name; holotype cited as male).

Tridesmus sectile [sic]: Shelley, 2004: 1161. (cited as available name).

Tridesmus serratus Silvestri

Tridesmus serratus Silvestri, 1898: 63, no figures. HT (ZMUC) of unspecified gender from Puerto Rico. Type specimen listed as available at: http://www.zmuc.dk/EntoWeb/collections-databaser/diplo-polydesmida.htm

Cryptodesmus? serratus: Attems, 1899: 368. Placed in *Cryptodesmus* by Attems without reference to characters nor examination of specimens.

2.5.2 *Coccoelasma* Loomis, 1936

Coccoelasma has the same number of lateral and posterior lobes as *Docodesmus*, but only ten on the collum. Loomis (1936) remarked on the association of *Coccoelasma* with *Docodesmus* when he created the genus and described its sole species, *C. incisura* of Hispaniola. He offered proportions of body and antennae, location of pores, squamate areas of dorsum, and gonopod structure as evidence of this association. Contrasting the genera, he described *Coccoelasma* as having a more convex dorsum covered with fine granules, first segment narrower than second and with a narrow anterior margin, ensuing body rings with 3 instead of 2 areas in the longitudinal rows, and a deep incision in the posterior margin of each paranotum. The gonopods were neither described nor figured, but merely mentioned 'as in *Docodesmus*,' from which Attems (1940: 237) infers that there are two telopodite branches. Since the delineation of the genus *Docodesmus* currently rests on somatic features, such as the 12-lobed collum, genera such as *Coccoelasma* with a 10-lobed collum cannot be synonymized until gonopodal or other apomorphies have been defined.

Coccoelasma incisura Loomis

Coccoelasma incisura Loomis, 1936: 170, figure 71, and plate 3, figure 4. Male HT (USNM) and female PT (MCZ) from Haiti, *vidi*.

Coccoelasma incisura: Attems, 1940: 237, figures and description after Loomis. – Hoffman, 1999: 478.

2.5.3 *Cyphotylus* Loomis, 1936

Golovatch (1997) suggested a close relationship with *Docodesmus* without discussion of characters. Loomis placed the genus close to *Coccoelasma*, most likely based on the 10-lobed collum. The pronounced dorsal tuberculation of the specimen's tergites, as illustrated by Loomis in figure 72, is strikingly different from the tuberculation in any *Docodesmus* species. Since the holotype is an immature male, gonopodal characters cannot be assessed.

Cyphotylus prolatus Loomis

Cyphotylus prolatus Loomis, 1936: 172, figure 72. Immature male HT (USNM) from Haiti.

Cyphotylus prolatus: Attems, 1940: 253, figures and descriptions after Loomis.

2.5.4 *Leuritus* Chamberlin, 1923

Leuritus displays several unique, most likely autapomorphic features, which do not support affinities with *Docodesmus*. The type species is densly setose, the epiproct is broad, the gonopodal prefemur has a small process, the telopodite has two long slender branches.

Leuritus termitophilus Chamberlin

Leuritus termitophilus Chamberlin, 1923: 413, plate 25, figures 1-7 (plate is incorrectly labeled). Male HT from Guyana, in Coll. Chamberlin, not listed in USNM millipede type collection.

Leuritus termitophilus: Attems, 1940: 238, figures and descriptions after Chamberlin. – Silvestri, 1948: 16, figure VIII.

2.5.5 *Jeekelia* Loomis, 1950

The genus *Jeekelia* contains the single species *J. granulosa* from the Dominican Republic. The original genus name for this species was *Melanodesmus*, however this name is preoccupied by the Colombian chelodesmid genus *Melanodesmus* Carl, 1914. Loomis later (1950) established *Jeekelia* to accommodate *granulosa*. This species also shares the anterior collum and lateral paranotal lobe patterns of *Docodesmus* but has fewer posterior lobes with two pronounced posterior notches on each side. Again, Loomis mentioned a possible close relationship to *Docodesmus*, noting the similar shape and proportions. The major differences noted by Loomis are the dorsal texture and the posterior tergite notches. Gonopod characters cannot be assessed as the type of *J. granulosa* is female.

Jeekelia granulosa (Loomis)

Melanodesmus granulosus Loomis, 1941a: 74, no figures. Female HT (MCZ) from Puerto Rico, *vidi*.

Jeekelia granulosa : Loomis, 1950: 166.

2.5.6 *Lobodesmus* Loomis, 1936

Loomis placed *Lobodesmus* in close relationship with *Tridesmus*, citing the trilobed non-poriferous paranota. The original description and figures provide no evidence of a close affinity with *Docodesmus*; the posterior edge of the collum features 8 lobes, and the posterior edge of the tergites is marked by 4 large lobes. The gonopod illustration does not show resemblance with gonopods in *Docodesmus*.

Lobodesmus granosus Loomis

Lobodesmus granosus Loomis, 1936: 165, figure 70. Male HT (MCZ) from Morne La Hotte, Haiti.

Lobodesmus granosus: Attems, 1940: 249, figures and description after Loomis. – Hoffman, 1999: 488.

2.5.7 *Henicomus* Loomis 1941

Henicomus is another monotypic genus described by Loomis (1941a) from the Dominican Republic, containing *H. septiporus*. This species has a 12-lobed collum and a comparable lateral paranotal lobe pattern. Paranota of rings 5, 10, 13 and 16-17 have one less lateral lobe than the respective rings in *Docodesmus*, however the posterior-most of these lobes are quite large and consist of a circular structure containing the ozopore. The posterior margin of each tergite has 14 lobes instead of 16, in addition to a slight posterior notch on each paranotum. Loomis remarked on the similarity of *Henicomus* to *Docodesmus* in general form and sculpture, but noted the more convex dorsum, descending paranota, and uneven sterna of each body ring as distinct differences. The most "outstanding feature" of *Henicomus* according to Loomis, is the

unique pore formula (5, 10, 13, 16-19), however, ozopores of pyrgodesmids can be quite cryptic. Additionally, the type specimen for *H. septiporus* is female, thus no comparison of gonopod structure is possible.

Henicomus septiporus Loomis

Henicomus septiporus Loomis, 1941a: 79, figure 33. Female HT (MCZ) from Dominican Republic, *vidi*.

Henicomus septiporus: Hoffman, 1999: 485.

2.6 The Species of *Docodesmus*

Docodesmus alifer Loomis

Docodesmus alifer Loomis, 1941a: 68, figures. 26a-c. Female HT, *vidi* and female PT, *vidi* (MCZ) from Pico del Yaque, Loma Rucilla, Dominican Republic.

Docodesmus alifer: Loomis, 1969: 249 – Golovatch, 1997: 328 – Hoffman, 1999: 482

Diagnosis: The prominently elevated paranota distinguish this species from all other congeners. Gonopod structure is unknown, as this species is known from only two female specimens. It is not unreasonable to suspect that these are aberrant specimens of one of the other four Hispaniola species. Length 15 mm, width 3 mm.

Specimens examined: Two fragmented females including holotype (MCZ).

Docodesmus amazonicus Golovatch

Docodesmus amazonicus Golovatch, 1997: 327, figures. 17-21. Male HT, *vidi* (INPA) from Rio Tarumã Mirím, Manaus, Brazil.

Diagnosis: Gonopod (Golovatch, 1997: figures. 20-21) is type L, with LAP significantly longer and more robust than in all island species. SAP is absent or fused with LAP. LAP splits into two branches at the distal third, with the solenomere being the shorter branch. Longer branch distally flat and retrorse. This single, flat tip distinguishes this species from the other Amazonian species *D. hirudiformis*, the tip of which terminates in two flat processes. Length 7 mm, width 1.5 mm.

Specimen examined: Male holotype.

Docodesmus angustus Loomis

Docodesmus angustus Loomis, 1941a: 71, figures. 29a-d. Male HT, *vidi* (MCZ) from Valle Nuevo, southeast of Constanza, Dominican Republic.

Docodesmus angustus: Loomis, 1969: 250 – Golovatch, 1997: 328 – Hoffman, 1999: 482

Docodesmsus griseus Loomis, 1941a: 69, figure 27. Male HT, *vidi* (MCZ) from Sanchez, Dominican Republic (**syn.n.)**. Loomis, 1969: 249 – Golovatch, 1997: 328 – Hoffman 1999: 483

Diagnosis: Gonopods (Loomis, 1941a: figure 29d) are type G. Cylinder is very distinct without additional processes. LAP with distal 90° bend and single acute tip. SAP with slight bend and single acute tip. Distinguished from other *Docodesmus* species of Hispaniola by the cylinder of the gonopod being continuous with LAP and having no additional processes (opposed to *D. haitiensis*), and by its larger size (compared to *D. parvior* and *D. semiseptus*). HT Length 14mm, width 3mm. From original description: largest male length 15 mm; largest female length 18 mm. *D. griseus* HT length 14 mm, width 2.5 mm.

Docodesmus griseus is a junior subjective synonym of *D. angustus*. In the original description, figure 27 gives a rather inaccurate illustration of the *D. griseus* gonopod. The cylinder appears detached from the LAP in this illustration, which is not the case. The illustration of *D. angustus* in figure 29d is more accurate. The holotype of *D. griseus* appears to be an aberrant specimen in which certain body rings have different numbers of lobes on each paranotum. As first revisors, and to avoid having an aberrant holotype for this species, we have selected *angustus* as the senior synonym despite *griseus* having a two-page priority.

Specimens examined: Male holotypes of *D. angustus* and *D. griseus*.

Docodesmus brodzinskyi Shear

Docodesmus brodzinskyi Shear, 1981: 53, figures 1, 2. Female HT, *non vidi* (collection of J. Brodzinsky, Santo Domingo, D.R.) from an uncertain locality in the Dominican Republic.

Docodesmus brodzinskyi: Golovatch, 1997: 330 – Hoffman, 1999: 482

This is a fossil specimen in amber, thought to be of Oligocene age (30-35mya). The two figures from the original description suggest that the lobes of the collum and the lateral lobes of tergites 2, 3 and 11 are consistent with our diagnosis of *Docodesmus*. Length 9.5 mm, width 1.25 mm.

Docodesmus centralis Silvestri

Docodesmus centralis Silvestri, 1898: 62. Male HT, *vidi* (deposited in MSNG) from La Guaira, Venezuela.

Docodesmus centralis: Attems, 1899: 373 – Golovatch, 1997: 328

The holotype male (the only known specimen for this species) has a lobation pattern inconsistent with all other *Docodesmus* species. The collum has 10 lobes and all paranota have 3 lateral lobes. The ozopore bearing paranota also have a porostele on the caudal lobe. The gonopods are missing from the specimen's vial. The original gonopod description is vague and contains nothing that suggests *Docodesmus*. Length 5 mm, width 1 mm.
We conclude that *centralis* does not belong in genus *Docodesmus*, but have no suggestion for placement at this time and leave it *incertae sedis.*

Specimen examined: Holotype (fragmented, gonopods missing). Listed in the original description as being deposited in ZMUC.

Docodesmus coxalis Loomis

Docodesmus coxalis Loomis, 1975: 170, figure 4. Male HT, *vidi* (FSCA) from one mile south of Claremont, St. Ann Parish, Jamaica.

Docodesmus coxalis: Golovatch, 1997: 328 – Hoffman, 1999: 483

Diagnosis: Gonopods are type G. Cylinder is very distinct with a small additional bifid process on the margin opposite the LAP. LAP with distal 90° bend and single acute tip. SAP with variable bends and curves and single acute tip. Distinguished from other type G *Docodesmus* species by the presence of the additional bifid process on the cylinder. Males assigned to *D. cubensis* have a similar process, as illustrated by Loomis (1938: figure 25). However, *D. cubensis* individuals are much larger. HT length 7 mm, width 1.8 mm. PT males range in length from 7-7.5 mm, in width from 1.5-2 mm. PT females range in length from 7-8 mm, in width from 1.8-2 mm.

Specimens examined: Male holotype, ca. 50 paratypes, all from type locality (all FSCA).

Docodesmus cubensis Loomis

Docodesmus cubensis Loomis, 1937: 225, figures 13, 14. Female HT, *vidi* (MCZ) from Soledad, Prov. Cienfuegos, Cuba.

Docodesmus cubensis: Loomis, 1938: 473, figure 25. – Loomis, 1950: 166 – Loomis, 1969: 250 – Torre, 1974: 8. – Loomis, 1975: 170, 172 – Golovatch, 1997: 328 – Hoffman, 1999: 483

Diagnosis: The type is female and no male specimens were available to us. Loomis's drawing (1938, figure 25) of a male gonopod assigned to *cubensis* shows a gonopod nearly identical to that of *D. coxalis.* Distinguished from other *Docodesmus* species except *D. coxalis* by the presence of the additional bifid process on the cylinder, however *D. coxalis* individuals are much smaller. Length 11 mm, width 2.5 mm.

Specimen examined: Female holotype (fragmented).

Docodesmus eggletoni Velez

Docodesmus eggletoni Velez, 1967: 28, figures 7-9, map II, tbl. III. Male HT, *non vidi* (USNM) from Hy. 119, nine km north of San German, Puerto Rico.

Docodesmus eggletoni: Golovatch, 1997: 328 – Hoffman 1999: 483

The type specimens of *Docodesmus eggletoni*, *D. maldonadoi* and *D. vidalius* were not available for this study. The holotypes (USNM) and paratypes (University of Puerto Rico, Rio Piedras) are apparently missing from their respective depositories (DeRoche, *in litt*. Agnarsson, *in litt*.). The only literature treatment is the original description (all Velez 1967). The descriptions and illustrations do not provide enough information for diagnoses or comparisons with congeners. The presence of a cylinder, LAP and SAP is apparent, but whether they are type G, type L or something else cannot be discerned. In spite of this, the descriptions clearly show that these three species have a lobe pattern consistent with our diagnosis for *Docodesmus*.

Docodesmus grenadae Chamberlin

Docodesmus grenadae Chamberlin, 1918: 218, 259. Male HT, *vidi* (MCZ) from Grand Etang, Grenada, Lesser Antilles.

Docodesmus grenadae: Loomis, 1937: 226, figures 15, 16. – Loomis, 1969: 250 – Golovatch, 1997: 328 – Hoffman 1999: 483

Diagnosis: Gonopods are type L. The cylinder is reduced to a rounded knob. The LAP is long, flattened and distally bent 90°. The SAP is straight and needle-like and in complete or near-complete contact with the LAP along its entire length. Distinguished from other type L *Docodesmus* species by the complete contact of LAP and SAP. This character is shared only with *D. trinidadensis*, but the two species are distinguished by the tip of the LAP (needle-like in *trinidadensis*). Length 13 mm, width 3 mm.

Specimen examined: Male holotype (fragmented), male specimen from Grenada (BMNH).

Docodesmus haitiensis Chamberlin

Docodesmus haitiensis Chamberlin, 1918: 216, 259. Male HT, *vidi* (MCZ) from Diquini, Haiti.

Docodesmus haitiensis: Loomis, 1934: 45, plate 3, figures 1, 2. – Loomis, 1936: 162. – Loomis, 1937: 225 – Loomis 1941a: 71. figures 28a, b. – Loomis, 1969: 250 – Loomis, 1975: 170 – Golovatch, 1997: 328 – Hoffman 1999: 483

Docodesmus cooki Loomis, 1969: 248, figures 8-10. Male HT, *vidi* (USNM) labeled Etowah, Tennessee (**syn.n.**). – Golovatch, 1997: 328 – Hoffman, 1999: 482. – Shelley, 2004: 1161.

Diagnosis: Gonopod is type L. Cylinder very prominent with a short, flat and blunt extension at the posterior-most margin. LAP with distal 90° bend or slight curve. SAP slightly shorter with bends and curves varying among specimens. Distinguished from other *Docodesmus* of Hispaniola by the cylinder being discontinuous with the LAP (type L). All other Hispaniola species are type G. HT length 14 mm, width 3.5 mm. Other specimens range in length 14-18 mm and width 3.5-4 mm in both sexes.

Docodesmus cooki is a junior subjective synonym of *D. haitiensis* based on our examination of the type specimens. The mystery still remains, as discussed by Loomis (1969) and Shelley (2004), of how two *Docodesmus* specimens turned up in a jar of Tennessee millipedes.

Specimens examined: Male holotype (fragmented), ca. 7 fragmented topotypes (MCZ); 1 male and 1 female from Cape Haitien, Haiti, det. Loomis (USNM); 1 male and 1 female from Pétionville, Haiti, det. Loomis (FSCA); *D. cooki* HT and PT (USNM). Also known from Dominican Republic (Loomis 1941a: 71).

Docodesmus hirudiformis Golovatch

Docodesmus hirudiformis Golovatch, 1999: 224. Male HT, *vidi* (INPA) from the environs of Manaus, Brazil.

Diagnosis: Gonopod is type L, with LAP significantly longer and more robust than in all island species. SAP is absent or fused with LAP. Solenomere branches from LAP at distal third. Remaining branch splits into two flattened processes. These two processes distinguish this species from the other Amazonian species, *D. amazonicus*. HT length 6.5 mm, width 2 mm. Male PT length 8 mm, width 2 mm. Female PT length 6-7 mm, width 1.5 mm.

Specimens examined: Male holotype. One male, 2 female paratypes (INPA).

Docodesmus maculatus (Bollman)

Stenonia maculata Bollman, 1888: 336. Male HT, *non vidi* (USNM, lost, deRoche *in litt*.), from Cuba, without further locality data.

Platyrhachus maculatus: Pocock, 1894b: 511.

Platyrachus ? maculatus: Chamberlin, 1918: 216, 259.

Schizodira maculata: Loomis, 1941b: 37.

Docodesmus maculatus: Loomis, 1950: 165 – Torre, 1974: 9 (cited again as a new combination without mentioning Loomis's placement). – Golovatch, 1997: 328 – Hoffman 1999: 483.

Known only from male HT and female PT specimens, which are apparently lost (DeRoche, *in litt*.). No description of the gonopod structure has been published. This may be the same species as *D. cubensis*, but was assigned to Platyrhacidae at the time of *cubensis*' description. Hence, we designate *Docodesmus maculatus* a *nomen dubium*.

Docodesmus maldonadoi Velez

Docodesmus maldonadoi Velez, 1967: 27, figure 6, map II. Male HT, *non vidi* (USNM, not located in collection) from Km 4.4 on the Sabana Road at 1,850 ft, near El Yunque, Puerto Rico.

Docodesmus maldonadoi: Golovatch, 1997: 328 – Hoffman 1999: 484

See treatment of *D. eggletoni* above.

Docodesmus parvior Chamberlin

Docodesmus parvior Chamberlin, 1918: 218, 259. Female HT, *non vidi* (MCZ) from Furcy, Haiti.

Docodesmus parvior: Loomis, 1936: 162, plate 3, figure 3. – Loomis, 1937: 224. – Loomis, 1941a: 73. – Loomis, 1941c: 194. – Loomis, 1969: 250 – Loomis, 1975: 170. – Golovatch, 1997: 328. – Hoffman 1999: 484.

Diagnosis: Gonopods with telopodite apparently retracted into the coxae, resulting in anterior processes appearing shorter than in other species. Cylinder with a short, flat and blunt extension on posterior end. LAP is flattened and slightly longer than SAP. HT length 8.5 mm, width 2 mm. Other specimens: Female length 8 mm, width 1.5 mm. Male length 8.5 mm, width 2 mm. Length can reach 10 mm (Loomis 1936).

Specimens examined: One male, 2 females from Petionville, Haiti, det. Loomis (FSCA).

The male examined for this diagnosis and several others were identified by Loomis (1936, 1941), but no explanation was given as to how they were identified as such. The similarity of gonopods with *haitiensis* suggests synonymy, but there is a considerable difference in size between the two species.

Docodesmus robustus Loomis

Docodesmus robustus Loomis, 1934:47, figure 24, plate 4, figure 3. Male HT, *vidi* (USNM) from King's Bay, Tobago Island.

Docodesmus robustus: Loomis, 1937: 224 – Loomis, 1969: 249. – Golovatch 1997:328

Diagnosis: Gonopods are type L. The LAP is flat and broadens at the midpoint where it then abruptly narrows and bends 90°. A small process is present near the tip. SAP is considerably shorter than the LAP, needle-like with a slight bend. Known from two specimens. Distinguished from other type L *Docodesmus* species by the broad basal half of the LAP. The other species known from Tobago, *trinidadensis*, has a needle-like LAP tip, and has the LAP and SAP in total contact along their lengths. Length 13 mm, width 3.7 mm.

Specimen examined: Male holotype and female paratype (USNM).

Docodesmus sculpturatus Loomis

Docodesmus sculpturatus Loomis, 1934: 45, figure 22, plate 4, figure 1. Male HT, *vidi* (USNM) from a "banana hole" three or four miles from Nassau, New Providence, Bahama Islands.

Docodesmus sculpturatus: Loomis, 1937: 225, 227. – Velez, 1967: 29, map II. – Loomis, 1969: 250. – Golovatch, 1997: 328. – Hoffman 1999: 484.

Diagnosis: Gonopods are type G. Cylinder is reduced but still apparent. LAP is flattened, distally bent and promptly tapers to a point. SAP is straight and nearly equal in length as LAP. Distinguished from other type G *Docodesmus* species by the combination of a reduced cylinder and a flattened LAP. Length 5-8 mm, width 1-1.5 mm.

Specimens examined: Male holotype (USNM), 3 male paratypes (FSCA).

Also known from Puerto Rico (Velez, 1967, map II).

Docodesmus semiseptus Loomis

Docodesmus semiseptus Loomis, 1936: 163, figure 69. Male HT, *vidi* (USNM) from Morne Pilboreau, above Ennery, Haiti.

Docodesmus semiseptus: Loomis, 1937: 224. – Loomis 1969: 249. – Golovatch, 1997: 328. – Hoffman 1999: 484.

Diagnosis: Gonopods are type G with short, flat extension of posterior margin of cylinder. LAP wide at the base with a slight distal curve. SAP with a slight bend. Distinguished from other type G Hispaniola species by the flat extension on the posterior margin of the cylinder. Length 8 mm, width 1.7 mm.

Specimen examined: Male holotype.

Docodesmus trinidadensis Chamberlin

Docodesmus trinidadensis Chamberlin 1918: 219. Female HT, *non vidi* (MCZ) from Port of Spain, Trinidad.

Docodesmus trinidadensis: Loomis, 1934: 46, figure 23, plate 4 figure 2. – Loomis, 1937: 224-227. – Loomis, 1969: 250. – Golovatch, 1997: 328.

Diagnosis: Gonopods are type L. LAP long, straight and wide for most of its length, then abruptly narrowing to a needle-like point. A small knob present on side of needle. SAP is straight and in complete contact with the LAP along its entire length. Distinguished from all other *Docodesmus* species by the abrupt needle-like tip of the LAP. HT length 13.2 mm. Other specimens: 2 males length 9 mm, width 2 mm; 2 females length 10 mm, width 2 mm.

Specimens examines: Two males and 2 females from Arena Forest, Trinidad, det. Loomis (FSCA).

Docodesmus vidalius Velez

Docodesmus vidalius Velez, 1967: 24, figures 2-5, map II, tbl. II. Male HT, *non vidi* (USNM, not located in collection) from Km 10.7 on Hy. 146, about 10 km southwest of Ciuales, Puerto Rico.

Docodesmus vidalius: Golovatch, 1997: 328 – Hoffman 1999: 484.

See treatment of *D. eggletoni* above.

Docodesmus vincentii (Pocock)

Cryptodesmus vincentii Pocock, 1894b: 510, plate 39, figures 2-2d. HT, *non vidi* (BMNH) from St. Vincent, Lesser Antilles.

Aporodesmus vincentii: Pocock, 1894c. – Attems, 1899: 372

Docodesmus vincenti [sic]: Chamberlin, 1918: 216, 259.

Docodesmus vincentii: Cook, 1896: 5, 20. – Loomis, 1936: 161. – Loomis, 1937: 225. – Velez, 1967: 26. – Loomis, 1969: 250. – Golovatch, 1997: 328. – Hoffman 1999: 484.

Diagnosis: Gonopods are type L and very similar to *D. grenadae*. They differ from *grenadae* by having a 90° torsion at the distal bend of the LAP. Short, rounded process present at bend.

Type material listed as deposited BMNH. We received on loan from BMNH 8 vials identified on the loan invoice as paratypes. Although all specimens are conspecific, no vial

contained any information on type status. Two vials had label information similar to that found in the original description ("Forest below 1500 ft., under rotting leaves; pretty common."): one vial with one adult female, one adult male (gonopods missing), 2 juveniles, plus additional pieces; one vial with 3 small juveniles and 3 immature females.

Specimens examined: 5 males, 11 females from St. Vincent (BMNH).

3. A review of subspecies recognition in polydesmidan millipedes (Diplopoda) with a revision of the subspecies of Euryuridae (Xystodesmoidea).

"...the subspecies is a device of convenience for the taxonomist. It is nothing more, nothing less." – Ernst Mayr (in Inger 1961)

3.1 Introduction

The practice of recognizing subspecies and assigning trinomials dates to the infancy of Linnaean taxonomy. Up until the late 19th century, however, the subspecies was undefined and often designated on individual variants and simple syntopic polymorphisms (Mayr and Ashlock, 1991). After Darwin, biologists began to think of taxonomic hierarchy from a more evolutionary perspective and viewed subspecies as natural groups with real taxonomic relevance. Even so, many continued to designate trinomials indiscriminately, causing the legitimacy of the practice to come into question (Wilson and Brown, 1953). To this day, the scientific community is divided among those who think subspecies have merit and those who would discard the whole practice.

Those who support the practice stress the importance of recognizing all distinct forms of life and thus providing a more thorough and accurate picture of the planet's biodiversity (Fox, 1955; Mayr, 1982; Mulcahy, 2008). This is not only heuristically desirable, but can be valuable for conservation policy (Haig et al., 2006; Phillimore and Owens, 2006; Ryder, 1986) and studies of evolutionary processes (Amadon and Short, 1976; Barton, 1993; Smith and White, 1956). Supporters tend to be taxonomic specialists of well-known animal groups (birds, mammals)

where discovery of new species is rare and most taxonomic work is focused on improving the existing classifications. Critics are concerned with misrepresentation of biodiversity due to inconsistent taxonomic practices and the complexity of variation within and among species (Gillham, 1956; Wilson and Brown, 1953) and how this may mislead conservation efforts (Haig et al., 2006; Zink, 2004). There is also concern that the plethora of trinomials and the priority rules of their authorships ultimately clutter up the taxonomic literature (Edwards, 1954). Given such disparate points of view, any consensus among scientists on how to handle subspecies is unlikely to ever form and may not even be necessary (Fitzpatrick, 2010). Regardless of their stance on the issue, however, most authors agree that the practice requires some level of revision.

3.1.1 Subspecies definitions

One underlying dilemma of the debate is the unresolved status of the subspecies as an evolutionarily unit. Subspecies have been identified in parapatry, allopatry and along a cline (Frost and Hillis, 1990). Sometimes they have secondary contact with interbreeding (Hewitt, 1989). They could be incipient species and/or the result of adaptation to their local environments (Mayr, 1982). The subspecies designation is given to populations resulting from any of several evolutionarily significant phenomena. However, due to the varied frameworks within which subspecies are recognized, there is no actual subspeciation phenomenon itself. From this point of view, the subspecies is not a natural entity, but serves as a convenient taxonomic tool (Mayr, 1982). Whatever one thinks of the subspecies, it resides clearly in the gray area between a single monotypic species and two full species. How one works within this gray area is undoubtedly influenced by how one defines full species and by what purpose subspecies would serve. Despite his labeling of subspecies as merely a taxonomic tool, Mayr still saw value in subspecies use and

proposed how to define them (Mayr, 1942). His definition has been frequently cited in zoological literature; the most recent phrasing being: "*an aggregate of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of that species and differing taxonomically from other populations of that species*" (Mayr and Ashlock, 1991). He defined "taxonomically" as "*by sufficient diagnostic morphological characters*". Simply put, two subspecies should be similar, but not too similar, and different, but not too different. Drawing this distinction can only be left to the expertise of the specialist and, ideally, some level of a consensus will exist within the community of experts for that group. It is important to keep in mind that, ontologically, this definition falls within the framework of the biological species concept (BSC); "*a group of interbreeding natural populations that is reproductively isolated from other such groups*" (Mayr and Ashlock, 1991). But systematists typically do not design experiments to test whether individuals can mate and produce fertile offspring. Operationally, species are usually identified based on morphological comparisons. Thus, close similarity is often assumed to act as a proxy for reproductive compatibility, which in turn defines species. However, the most significant and objective part of Mayr's definition is undoubtedly the geographic element. Subspecies collectively make up the entire range of the species, with each inhabiting a definable portion of it. Within this range, there may be significant interbreeding between the different subspecies if they come into contact and individuals may exhibit characters from both subspecies (Mayr and Ashlock, 1991). This seems to be the most objective diagnostic to differentiate them from full species, which, whether allopatric or sympatric, exhibit minimal, if any, hybridization. Even so, identification of distinct populations with a subspecific relationship can be erroneous if sampling is low and unrepresentative of the species' whole range. There have been cases where recognized subspecies are nothing more than two extremes

of a gradual cline with no clear break in the defining characteristic (Gillham, 1956; James, 2010; van Son, 1955). Likewise, multiple character differences may not be congruent with each other geographically, causing delimitation of subspecies populations to be dependent on which character is considered most important (Inger, 1961; Wilson and Brown, 1953). Therefore, a thorough understanding of range and the expression of variation throughout that range are fundamental to any interpretation of a species' subdivision.

As mentioned, Mayr's definition is based on the BSC, but phylogenetic species delimitation is becoming more common due to the increasing availability of genetic data. Phylogenetic species concepts vary in operational criteria but rely heavily on genetic information and typically view species as the smallest, indivisible natural unit. Indeed, genetic data is often utilized to test species and subspecies boundaries, sometimes resulting in the dismissal of subspecific taxa by synonymization with the nominate subspecies or elevation to full species (Manier, 2004; Mulcahy, 2008; Yeung et al., 2009). However, there may still be a place for trinomials in a strictly phylogenetic context. With molecular information, there are more and more discoveries of populations that are separated by deep phylogenetic breaks yet still manage to interbreed in secondary contact. Populations identified as such through genetic data are potential new candidates for subspecies status and could be defined as "*groups of actually or potentially interbreeding populations phylogenetically distinguishable from, but reproductively compatible with, other such groups*" (Avise and Ball, 1990). This definition goes on to stress the importance of the genetic evidence coming from multiple independent loci with congruent geographic distributions. Unfortunately though, most authors of species level taxa have not had genetic data at their disposal, so utilization of such with existing subspecies would require too much field and lab work to make it immediately practical.

Still, if subspecies are simply a tool for the taxonomist, a single set of criteria is irrelevant. The author will define it any way he or she wants. Several other categories of subspecies have been proposed in addition to the above. They include ecological subspecies defined as "*distinctively different macrogeographically sympatric infraspecific populations….which are isolated microgeographically, but whose members would cross-breed rather freely and normally if the populations were to become microgeographically sympatric under natural conditions*" (Edwards, 1954). There are also several temporal definitions of subspecies, based on daily, seasonal or annual activity (Edwards, 1954). These subspecies definitions, though differing in diagnostic criteria, all have one attribute in common: they generally resemble corresponding full species concepts with the added element of actual or potential interbreeding.

The present study is not an attempt to resolve the issue, but to look objectively at how the practice is handled. First, an exhaustive survey of the literature on an exclusive taxonomic group, polydesmidan millipedes, will be presented. The main objective was to compile details on how individual subspecies designations were implemented and then develop recommendations to help refine the practice. Our assumption is that the findings of this study will mirror those found in any similar studies of other animal groups. To the best of our knowledge, such a survey has not been published before. The subject has been addressed in the context of different animal groups, most notably birds (e.g. Ornithological Monographs vol. 67, 2010), but usually only to critique one or two cases. Second, a revision of the currently recognized subspecies of the polydesmidan family Euryuridae is presented in light of new morphological data to serve as an illustration of the results of the first objective.

3.1.2 Millipedes

Over 12,000 species of millipedes (Diplopoda) have been described (Brewer et al., 2012a; Sierwald and Bond, 2007), with roughly half belonging to the order Polydesmida. Polydesmidans are typically flat with their dorsal sclerites forming prominent lateral extensions (paranota). Other distinguishing characteristics are 20 body rings (rarely 19 or 21) and a complete lack of visual organs. Coloration ranges from dull earthy colors to bright, multicolored aposematic patterns (Marek and Bond, 2009). One of the most interesting anatomical structures of polydesmidans (and most other millipedes) is the pair of gonopods – the intromittent organs of males. These are heavily modified walking legs located on the seventh body ring replacing the eighth leg-pair. The gonopods are "charged" with sperm from gonopores located on the second body ring, then used to inseminate the female via reproductive openings of her second body ring. Variation in gonopod morphology is astounding, with many adorned with multiple accessory processes of unknown function. This variation makes gonopod morphology most useful in species/subspecies and other taxon recognition. In fact, in many cases gonopod morphology is the only varying character among species and most subspecies. Furthermore, gonopod morphology can be a potential indicator of reproductive compatibility between populations (Tanabe et al., 2001).

However, millipede taxonomy is a small field and has only recently received attention from molecular systematists, especially at the species level (eg. Brewer, Spruill, *et al.* 2012; Frederiksen *et al.* 2012; Marek & Bond 2007; Walker *et al*. 2009). The majority of millipede systematists rely solely on morphological and geographical data, thus Mayr's subspecies definition is the most practical, with emphasis on reproductive compatibility, vis-à-vis the BSC. The best evidence for such compatibility is the presence of morphologically intermediate

individuals (intergrades) in a zone of contact. Under these circumstances, full species status would ideally be chosen for new taxa when there is no clear evidence of reproductive compatability.

Within this framework, we will address the status of euryurid subspecies to 1) provide necessary taxonomic revision, and 2) illustrate the findings of the survey with detailed examples. Euryuridae Pocock, 1909, is a family of polydesmidan millipedes endemic to the eastern United States. Euryurids are characterized by a black and orange dorsal color pattern and the broadened caudal extension (epiproct) of the last body segment. The family is divided into two genera, *Euryurus* Koch, 1847 and *Auturus* Chamberlin, 1942, which are distinguished by major differences in gonopod anatomy. Currently, there are three subdivided species within Euryuridae. *Euryurus leachii* (Gray, 1832) was subdivided by Hoffman (1978) on the basis of a difference in gonopod anatomy. The gonopods terminate with two acicular processes: the solenomere, which functions in sperm transfer, and the subterminal process, with unknown function (Figure 3.1). These processes from specimens in the southern part of the range were nearly equal in length, whereas the solenomere was noticeably longer in the north. However, several specimens in the Field Museum collection were not properly labeled by the proposed diagnostic of Hoffman and instead seemed identified based on collection locality (Jorgensen pers obs). This prompted the present investigation into the status of this millipede species. The other two subdivided euryurids, *Auturus erythropygos* (Brandt, 1839) and *A. louisianus* (Chamberlin, 1918), also consist of two subspecies, each based on gonopod anatomy.

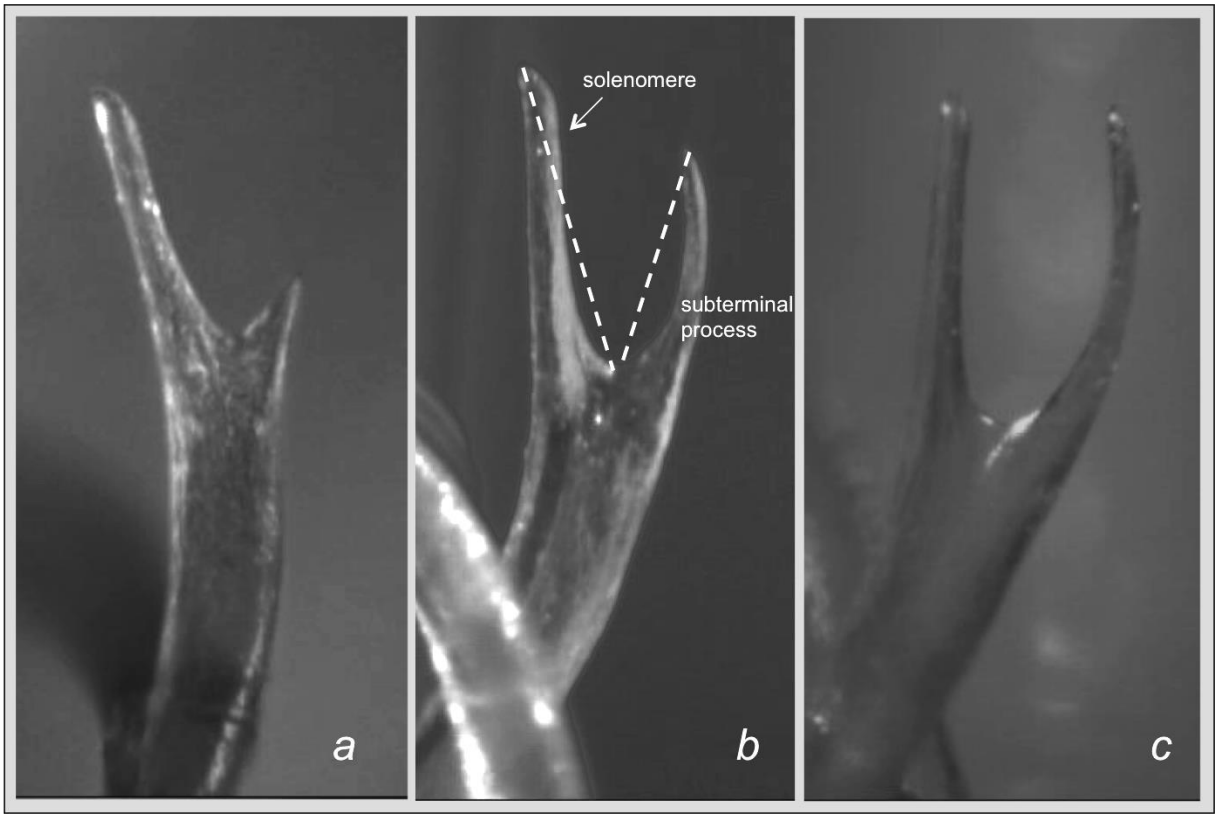

Figure 3.1. Distal portion of gonopod from three *E. leachii* specimens illustrates the range of process ratio (Rp) variation found in this species. R_p ranged from 0.30 (*a* from Lawrence Co. IL) to 1.0 (*c* from Cullman Co. AL), with a mean of 0.68 (as in *b* from Lawrence Co. IL). Perforated lines show measurements for calculation of process ratio. (R_p) = measurement of the subterminal process (right in all three photos) divided by the solenomere measurement.

3.2 Methods

This paper consists of two parts. First, an exhaustive survey of all recently published subspecies designations of Polydesmida was conducted to evaluate the practice. Second, the three subdivided species of Euryuridae were evaluated as case studies, in light of our recommendations arising from the literature survey. This included a quantitative assessment of *E. leachii* gonopods to test the validity of recognizing its subspecies.

3.2.1 Literature survey

Subspecies designations have three basic sources: original description at the subspecies level, formal rank reduction of a full species, and implied rank reduction of a full species. An implied reduction results from the designation of a new subspecies that consequently reduces what becomes the nominate subspecies. All three were included in the survey regardless of the taxon's current status. Only species of the millipede order Polydesmida (total described species ca. 5000) were used to keep the data set at a manageable size. Only papers published 1950-1999 (50 year span) were selected. Prior to this time, descriptions and taxonomic practices were too inconsistent to address along with more current work. The World Millipede Catalogue (Sierwald unpublished) was queried for all entries of polydesmidan subspecies and the publications were collected. It is possible that some qualified subspecies designations were overlooked during the database construction and did not make it into the survey; we assume that any such inadvertent omissions do not bias the results of the survey. The following questions were posed.

** Was any justification for subspecies over full species rank given? Recognizing multiple forms is obviously the rationale for designating new taxa, but why did the author choose to place them at the subspecific level instead of specific? Any attempt at justification was counted, regardless of how "convincing" it was.*

** How many specimens were observed for the original descriptions? When the diagnostic character was solely of the gonopod, only male specimens were counted. Only original descriptions were surveyed for this information. Rank reductions can be done observing few specimens if the species is already well described.*

** Is the nominate subspecies formally reduced or must it be implied that its status has changed?*

** Were data pertaining to the different subspecies distributions demonstrated? This requires more than type locality information.*

** What characters were used to distinguish the different subspecies? Characters (if mentioned) were variations in gonopods, peripheral (non-sexual) traits, color, size or some combination.*

3.2.2 Reassessment of *Euryurus leachii*

One hundred and one male specimens of *E. leachii* were observed, regardless of subspecies identification. Specimens came from the collections of the Field Museum, North Carolina Natural History Museum, Florida State Collection of Arthropods and the Illinois Natural History Survey. The specimens represent the entire range of the species, with an extra concentration from southern Illinois to look at smaller scale variation. Most specimens were collected within the last 30 years.

As mentioned, the two subspecies of *E. leachii* are distinguished by a difference in the relative lengths of the two apical processes of the gonopod (Figure 3.1). To quantify this character, the process ratio (R_p), was calculated for each specimen. For each gonopod, the length of the shorter process (subterminal process) was divided by the length of the longer process (solenomere). The average of the two ratios is the R_p for that specimen. Each specimen was observed under a stereo microscope with attached digital camera. For each, both gonopods were photographed separately, with the two processes parallel to the microscope lens. From each image the lengths from the midpoint at the base of the two processes to the tip of each were measured (Figure 3.1b). Great care was taken to identify and exclude specimens with damaged gonopods. If only one gonopod was usable (14 occurrences), the ratio for the single gonopod was

used. This should be of no consequence as the difference between the left and right ratios of specimens with both present was negligible.

3.3 Results and Discussion

3.3.1 Literature survey

A total of 76 publications were found to contain new polydesmidan subspecies designations from 1950 to 1999. These included 244 subspecies designations representing 101 species. Of these designations, 114 were by original description at the subspecies level and 130 were full species reduced to subspecies rank. Of the latter, 99 were formally reduced and the remaining 31 were implied. Implied reductions can be inconvenient, for unless the new status of the nominate subspecies is formalized, it is unclear whether this species had been previously subdivided. The number of designations by decade (Figure 3.2, dark bars) declines over the time period, with about half as many designated in the 1990's as in the 1950's. However, this does not necessarily suggest a decreasing interest in subspecies, but rather a decrease in alpha-taxonomic research done on Polydesmida as evidenced by the number of newly described species during the same time interval (Figure 3.2, light bars).

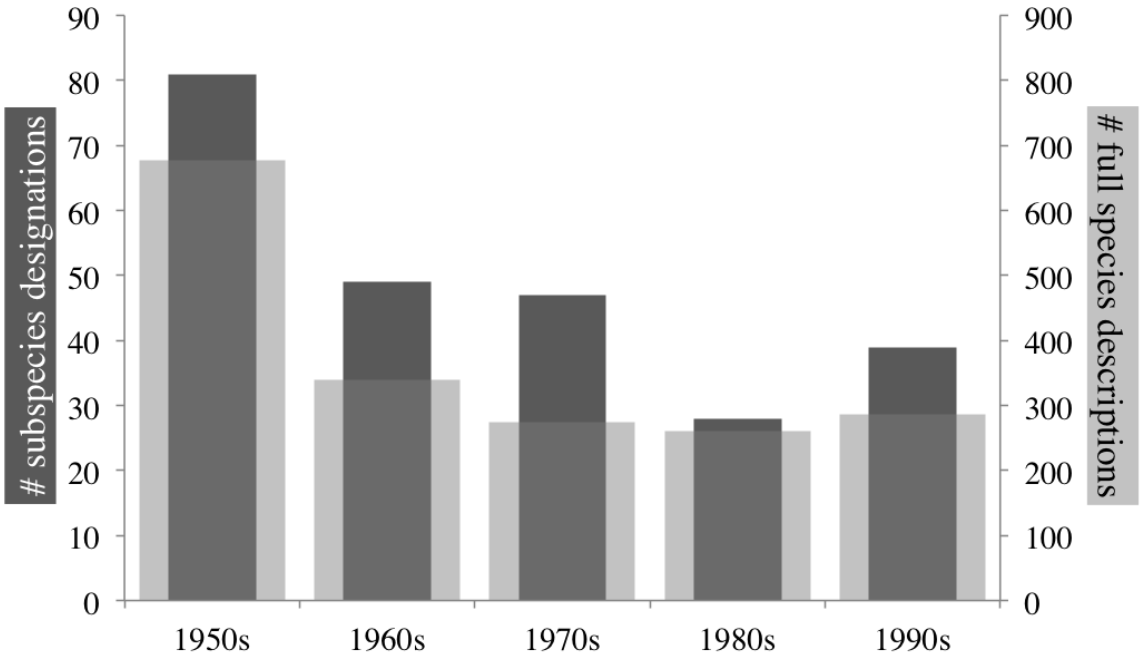

Figure 3.2. All subspecies designations (dark bars, left axis) found for this survey (total 224), separated by decade. Designations include original descriptions, formal rank reductions and implied rank reductions (see text). Estimated number of polydesmidan species described (light bars, right axis) over same time interval. (source: World Millipede Catalogue, Sierwald, unpub.)

The survey results clearly suggest the need to improve upon formal subspecies recognition. The most striking result is the lack of justification of the author's action. If there is any hope for a consensus on identifying what qualifies as a subspecies, both within a narrow field and across all zoology, authors must communicate their reasoning. Only 32% of the surveyed publications offered any justification for subspecies being chosen over full species. In many cases, the author did provide a clear explanation, but what they were actually justifying was the identification of a new form; a form that someone else could just as well consider a full species. This may suffice within some small communities of taxonomic specialists, but the reasoning must be clear for others as well, such as those who rely on these classifications in their ecological and evolutionary research. When appropriate justification is given, even if readers do not agree with it, they can at

least get a sense of how others weigh the concept. When this result is broken up by decade (Figure 3.3), it is evident that more recent works offer more frequent justification, but still lack it 50% of the time.

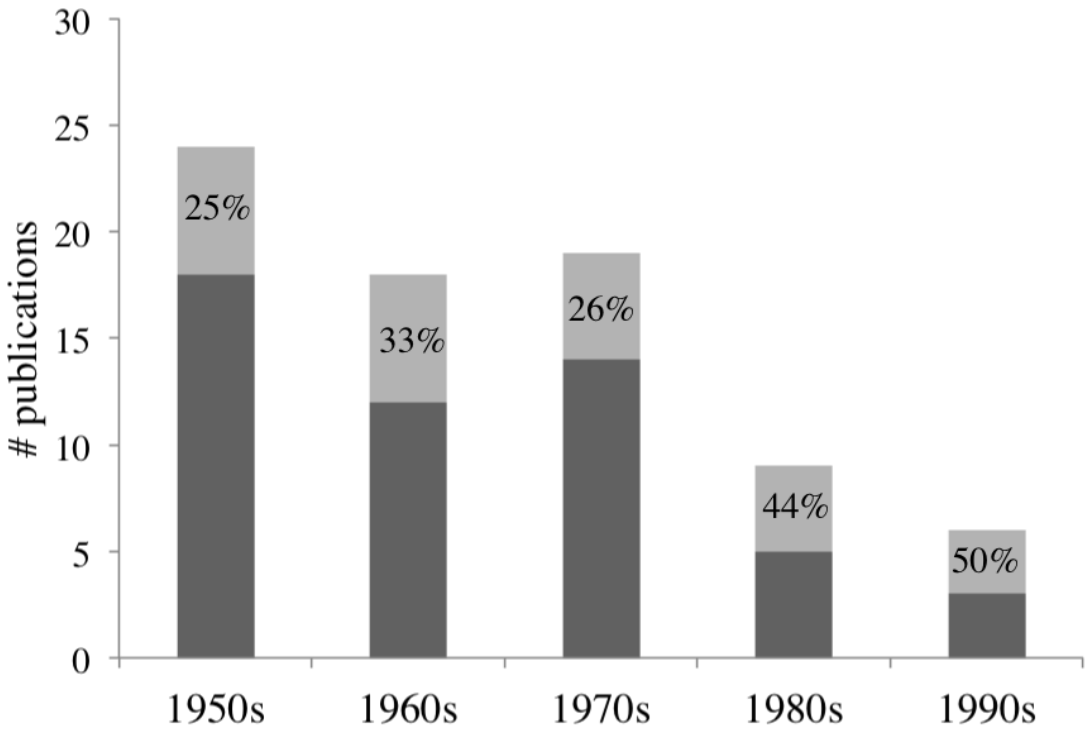

Figure 3.3. Number of publications which include designations of new polydesmidan subspecies. Light gray represents percentage (also shown) of publications that include justification for designating subspecies as opposed to full species.

Still, even with proper justification, designating a new subspecies can be unconvincing if data are lacking. Aberrant individuals are common in animal populations (Jocque, 2002), so designating a new taxon based on a single diagnosable specimen risks cluttering the literature with superfluous names. In this survey, 22% of the original descriptions were based on a single specimen and 14% on only two or three (Figure 3.4). These amounts could actually be even higher, as 21% did not specify the number. Besides the risk of being a mere aberration, one

specimen also provides little information on the distribution of the potential new taxon, thus the relevance of geography is disregarded. The survey revealed that, of the cases where a species was subdivided or added to, 36% provided information on the distributions of more than one subspecies. This is important because when a new subspecies is designated, the nature of the whole species is being modified. All information on the new subspecies distribution should be presented along with what is known of the existing subspecies, and ideally, how their distributions relate to one another. This information is valuable for justification of the new designation.

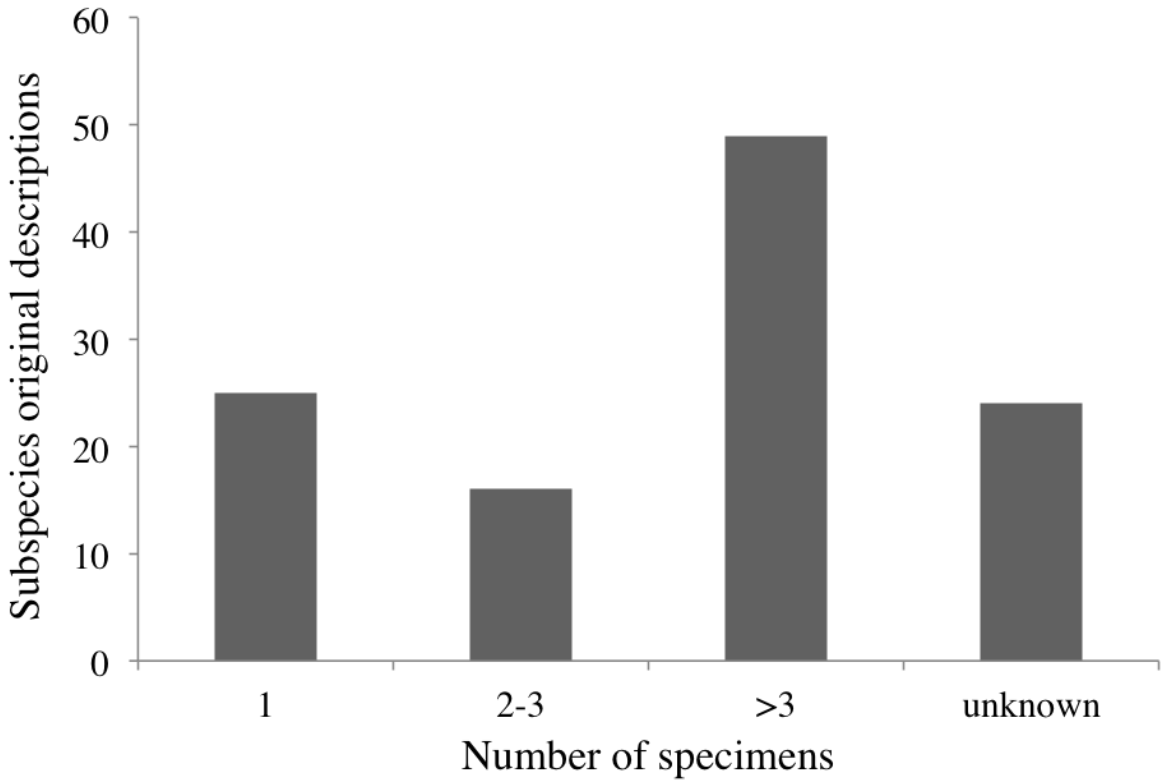

Figure 3.4. Number of specimens available for the original descriptions (114) of new subspecies.

The most common character used to diagnose new subspecies was gonopod anatomy, with 47 of the 101 species being subdivided based solely on this and 32 more with gonopod anatomy in combination with other characters (Figure 3.5). This is interesting because reproductive isolation can be reinforced by incompatible genitalia (Shapiro and Porter, 1989). From this viewpoint, the gonopod differences observed could hypothetically be evidence of full species status. However, it is hypothesized that the male genitalia of many different animals evolve disproportionately faster than the rest of the body (Eberhard, 1985b). Why they do is up for debate (Hosken and Stockley, 2004a), but it is therefore plausible to find genitalic variation within a species that is otherwise phenotypically homogenous. What is relevant to subspecies recognition is the consistency of any difference between geographic populations.

peripheral	size	color	gonopod	total
✓	-	-	-	4
-	✓	-	-	1
-	-	✓	-	4
-	-	-	✓	47
✓	✓	-	-	1
✓	-	✓	-	4
✓	-	-	✓	12
-	✓	✓	-	2
-	✓	-	✓	10
-	-	✓	✓	2
✓	✓	✓	-	2
✓	✓	-	✓	2
✓	-	✓	✓	0
-	✓	✓	✓	3
✓	✓	✓	✓	3
-	-	-	-	4
28	24	20	79	

Figure 3.5. Characters used in subspecies diagnoses of 101 species. Each row represents a different combination of the 4 characters. Each row's total is the number of species diagnosed by that exact combination. Column totals show the number of species with that character in its diagnosis whether alone or in combination.

3.3.2 Euryuridae subspecies

Euryurus leachii was subdivided by Hoffman in his seminal revision of genus *Euryurus* (Hoffman, 1978) due to an apparent north-south grade in the relative lengths of the two apical processes of the gonopod (Figure 3.1). Individuals from the eastern part of the range that appeared intermediate between the north and south forms were also mentioned. The name *E. leachii fraternus* Hoffman, 1978 was attributed to the southern population based on examination of ca. 80 male specimens representing the entire species' range. Hoffman did not provide a biological explanation for subdividing *E. leachii*, but suggested that this designation would "*compel future attention to their actual genetic status by someone having the opportunity to make the necessarily detailed field and laboratory studies*". He also mentioned the occasional discovery of an individual of one form within the range of the other, but did not interpret this as evidence against their subspecies status because he felt the difference in the *averages* of the two populations was relevant. Recently, Shelley *et al.* (2012) discovered *E. leachii* specimens from the western end of the range in Arkansas. Due to the intermediate form of the gonopods, however, the subspecific identification of these specimens was left undetermined.

As a result of the current study, the legitimacy of the division of *E. leachii* into subspecies is firmly challenged. The resulting distribution of R_p values (Figure 3.6d) is continuous and shows no discernable geographic pattern (Figure 3.6a). This is also true at a finer scale (Figure 3.6c). Overall, values ranged from 0.30 to 1.00 (Figure 3.1), with a mean of 0.68 ± 0.16 standard deviation. To test whether the average values of each subspecies differed, the data set was divided into two sets following the proposed boundary (Hoffman, 1978) (see dashed line in Figure 3.6). The average values were 0.65 for the northern specimens (*E. l. leachii*, n=47) and 0.70 for the southern specimens (*E. l. fraternus*, n=54). A t-test (equal variance, $F = 1.16$, $p <$

0.05) fails to reject that the populations are statistically the same ($p > 0.1$). This species has failed to meet the criteria that its subspecies represent morphologically and geographically distinct populations. At most, the differences observed in this species may be representative of a cline and formal recognition of subspecies should cease. *Euryurus leachii fraternus* is herewith synonymized with *Euryurus leachii leachii* and the subspecific epithets are dismissed.

The other euryurid genus, *Auturus* is represented in the extreme southeast United States by one subdivided species, *A. erythropygos*. Shelley (1982) synonymized two full species, *A. erythropygos* from the Carolinas and *A. becki* Chamberlin, 1951 from Florida into a single polytypic *A. erythropygos* and reduced each to subspecific status. His examination of specimens from multiple localities revealed an apparent north-south geographic trend in gonopod morphology. The apical margin of the gonopod in *A.e. becki* (Figure 3.7a) is significantly elongated. The same structure in *A.e. erythropygos* (Figure 3.7b) is not elongated and exhibits no variation across the range from the NC-VA border to the southern SC-GA border. However, the two taxa are separated by over 200 km, a substantial gap between supposed subspecies. Shelley recognized that he lacked solid evidence for synonymizing *A. erythropygos* and *A. becki* and reducing their status, but made the change regardless, anticipating future additional evidence. We propose that the two subspecies of *A. erythropygos* should each be returned to full species status. The morphological character difference is quite distinct and consistent within each species' range. No intergrade material (nor any *Auturus* specimen) has been found in the 200 km intervening area during three consecutive summer expeditions (pers. obs.). There is therefore no reason to suspect that these taxa represent anything but distinct species. *Auturus erythropygos*, and *Auturus becki* are hereby returned to full species status.

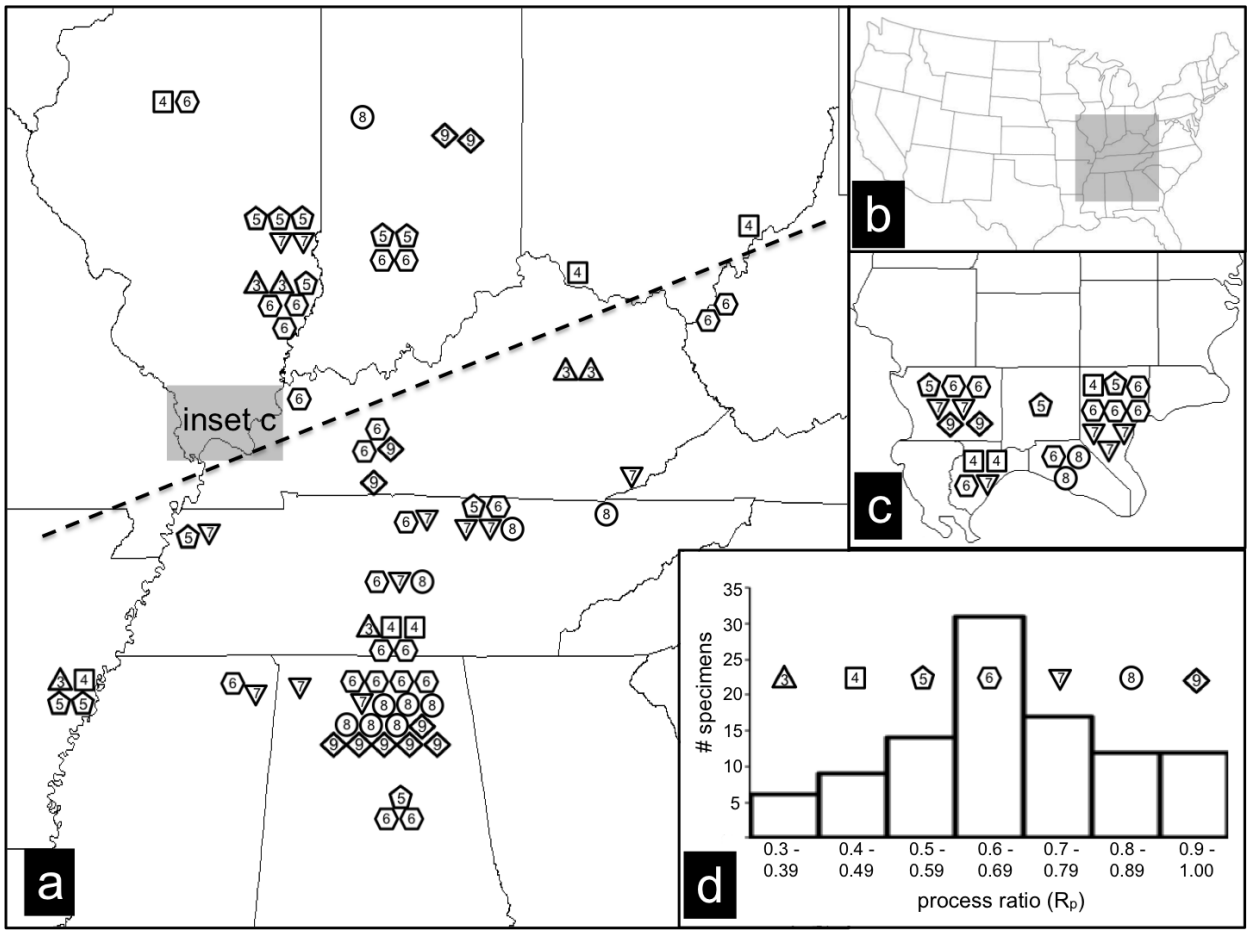

Figure 3.6. Results of *E. leachii* study showing *a*) geographic distribution of specimens used in study; *b*) location of range in *a*; *c*) smaller scale distribution of southern Illinois (inset c); *d*) statistical distribution of R_p values with key to markers in *a* and *c*. Markers were placed as close to the center of the collection county as possible, but avoiding overlapping. Thus, some clusters of markers may not be accurate at a fine scale. This does not affect the conclusions of this study. Dashed line shows border between former subspecies ranges.

The subspecies designations of *E. leachii* and *A. erythropygos* are examples of the too common practice of applying trinomials to populations when their true status is admittedly ambiguous. The motivation was to put a name to an apparent pattern of intraspecific variation and await future investigation. The problem with this practice is that if future research rejects the

proposed subspecies, the obsolete trinomials must still be carried forward in the taxonomic literature *ad infinitum*. Potentially significant morphological variation can and should be presented without risking the addition of excess names to the taxonomic literature.

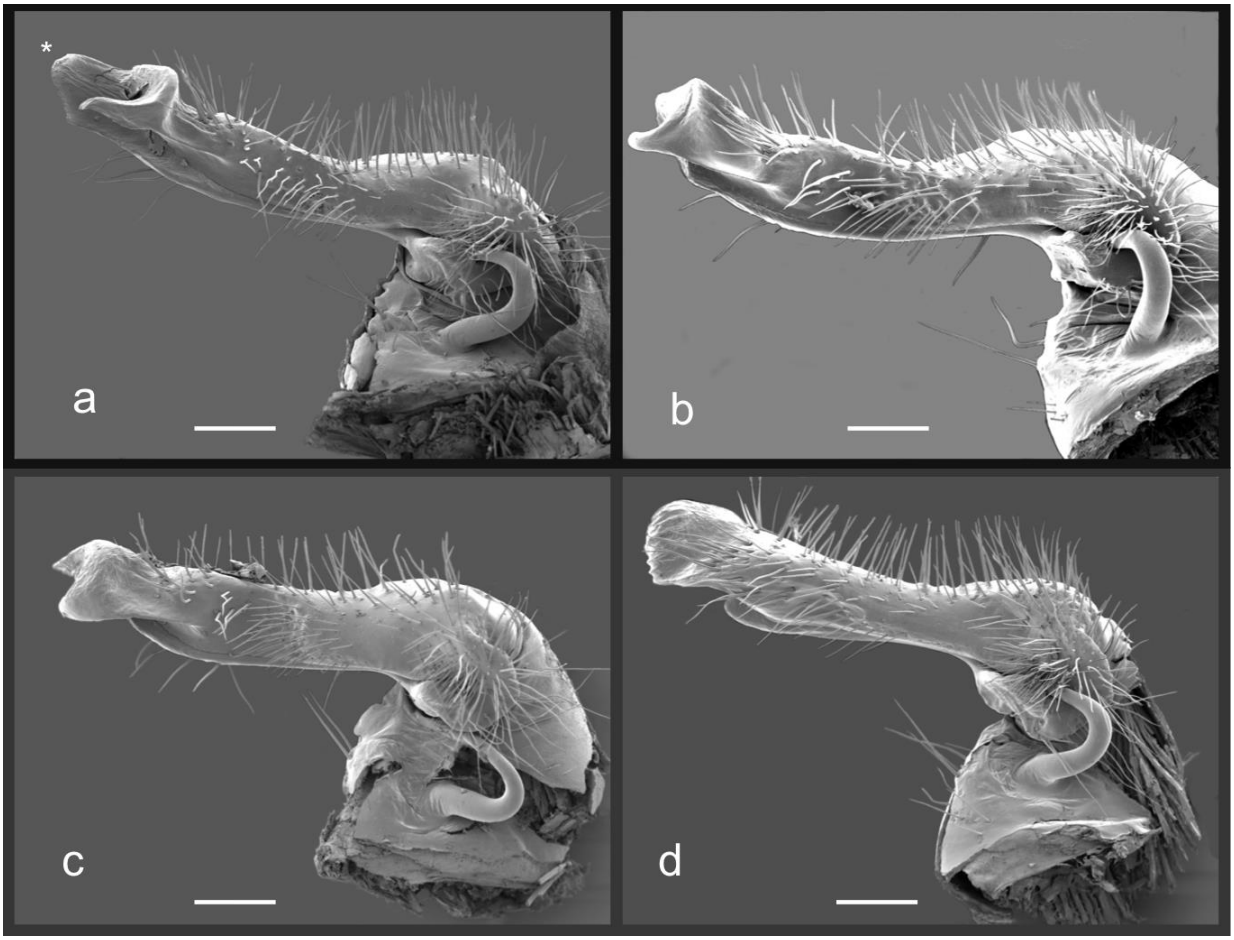

Figure 3.7. SEM images of *Auturus* subspecies gonopods, mesal view. a) *Auturus beckii*, b) *A. erythropygus*, c) *A. louisianus louisianus*, d) *A. louisianus phanus*. Bars = 200μm. *Tip of extended solenomere.

The third subdivided euryurid species, *Auturus louisianus* of Arkansas, Louisiana and Mississippi, was originally recognized as two full species, *A. louisianus* and *A. phanus* Chamberlin, 1942. Upon examination of multiple specimens from all across their ranges, Shelley (1982) determined that these were simply intraspecific geographic variants and formally reduced

them to subspecies. These subspecies differ by a slight, yet consistent, difference in the rotation of the distal portion of the gonopod (Figure 3.7c,d). The two forms have well-defined geographic ranges that are mostly separated by the Mississippi River, but come into contact at the southern edge of the range where morphological intergrades have been documented (Shelley, 1982c). Given the available information, Shelley's reduction of *A. louisianus* and *A. phanus* to subspecies status was well justified, and serves as a good illustration of the careful application of the subspecies designation.

3.3.3 Taxonomic revision summary

Family Euryuridae Pocock, 1909

Genus *Euryurus* Koch, 1847

Euryurus leachii (Gray), **new status**

Polydesmus leachii Gray, 1832 - *Euryurus leachii*: Hoffman & Browning, 1956 - *Euryurus leachii leachii*: Hoffman, 1978 - *Euryurus leachii fraternus* Hoffman, 1978

Genus *Auturus* Chamberlin, 1942

Auturus erythropygos (Brandt), **new status**

Polydesmus erythropygos Brandt, 1839 – *Euryurus erythropygus* [sic]: Peters, 1864 - *Eutheatus erythropygos*: Attems, 1938 - *Auturus georgianus* Chamberlin, 1942 - *Auturus erythropygos*: Hoffman, 1978 - *Auturus erythropygos erythropygos*: Shelley, 1982

Auturus becki Chamberlin, **new status**

Auturus becki Chamberlin, 1951 - *Auturus erythropygos becki*: Shelley, 1982

3.4 Conclusion

Subspecies recognition can be a useful reflection of stable geographic variation within a species. However, it should be avoided when there is insufficient data to support it. If subspecies is the most practical designation, it should be done attentively to avoid cluttering the literature and misleading ecological and evolutionary research. In cases where apparent intraspecific variation is not well understood, everything that is known can and should still be reported without designating a trinomial. We present the following suggestions when working with subspecies, regardless of one's philosophies on identifying and naming them. Some of these may seem obvious, but as the survey showed, they sometimes escape attention.

* Formal descriptions of subspecies should be based on a sufficient number of specimens. What qualifies as sufficient may vary among taxonomic specialties, but is most assuredly more than one.

* Subspecies designations, whether original descriptions or rank reductions, should include geographic range data for all known subspecies.

* Subspecies designations should include explicit rationale for recognizing taxa at the subspecific level. This is not only practical for its immediate purpose, but promotes the communication of scientific reasoning.

* Subspecies designations that result in the subsequent rank reduction of the nominate subspecies should include a formal declaration of the reduction.

* In conservation, ecological and evolutionary studies, existing subspecific names should be used cautiously. It is important to develop your own independent understanding of a species' variation.

4. A new species of *Euryurus* from southern Alabama and remarks on the status of *Illiniurus beattyi* Shear (Diplopoda: Polydesmida: Euryuridae).

4.1 Introduction

The diplopod family Euryuridae Pocock, 1909 is a polydesmidan group with a known distribution confined to the eastern United States. These millipedes are of moderate size (<35 mm) with a distinctive bright orange and black color pattern. They are characterized by a broad epiproct, the only North American polydesmidan family with this character.

Genus *Euryurus* Koch, 1847 has been the root for various taxonomic levels representing an inconsistent assortment of taxa, beginning as the subfamily Euryurinae Pocock, 1909 in the family Platyrhacidae Pocock, 1895 (1894c). Euryurinae contained three Latin American genera: *Amplinus* Attems, 1898, *Polylepiscus* Pocock, 1909, *Aphelidesmus* Brölemann, 1898, one Indonesian: *Polylepis* Bollman, 1893, and the North American *Euryurus*. By this time, over 20 species had been assigned to *Euryurus*, mostly South American, and Pocock essentially transferred all the Latin American species into *Aphelidesmus*. He recognized that the two North American species, *E. erythropygus* and *E. australis*, were not congeners of *Aphelidesmus* and left their status unchanged.

The subfamily was later reduced to tribal status by Brölemann (1916) along with Platyrhacini to make up the subfamily Platyrhacinae in Platyrhacidae. In this classification, Euryurini contained the genera *Euryurus, Amplinus, Polylepiscus, Polylepis* and the South American *Pycnotropis* Carl, 1914. *Aphelidesmus* was removed to the other platyrhacid subfamily Aphelidesminae.

The first use of Euryuridae as a family name was that of Chamberlin (1918) in his descriptions of *Aphelidesmus divergens* and *Polylepiscus boreri*. This use was merely a heading preceding the descriptions with no further explanation. His inclusion of *A. divergens* suggests he was working from Pococks's (1909) classification. Euryuridae was later synonymized with Platyrhacidae by Attems (1938), a move that was mentioned, but not accepted in later works (Hoffman, 1951, 1954). Hoffman (1954) then proposed that Euryuridae contain three subfamilies: Aphelidesminae, Amplininae and Euryurinae, the latter being the first exclusive familial grouping of euryurids in the present day context.

This classification remained unchanged until Hoffman (1975) reduced Euryuridae back to subfamily status within Platyrhacidae, with the three former subfamilies becoming tribes. Later, Hoffman (1998) reassessed the Platyrhacidae and concluded that the Euryurini actually have a closer affinity to xystodesmids than to platyrhacids and restored them to family status. As a result, Shelley (2002) formally grouped Euryuridae with Xystodesmidae, Eurymerodesmidae, Oxydesmidae and Gomphodesmidae in the superfamily Xystodesmoidea.

Currently, the family contains 12 species, three of which consist of subspecies pairs. The species are essentially identical in somatic characters, resulting in gonopod structure as the main species and genus delimiting character. The species are grouped into three genera: *Euryurus* Koch, 1847 (revised by Hoffman, 1978), *Auturus* Chamberlin, 1942 (revised by Shelley, 1982b) and *Illiniurus* Shear, 1968. These genera are defined by major character differences in gonopod structure, and species by variations within each generic model.

The gonopod structure of euryurids (Fig. 4.1) is simple, with most variation occurring at the acropodite. The prefemur is thick and hirsute, usually with a distinct concavity just basal to the acropodite. Additional processes are absent proximal to the prefemoral concavity and on the

coxae. Generic differences are as follows. *Euryurus* species have elongated acropodites with a narrow mucronate solenomere, often with an additional subterminal process resulting in a bifid appearance. Many species also have one or two additional processes adjacent to the prefemoral concavity, termed the femoral basal lamella and the distal prefemoral knob (Hoffman 1978). In contrast, *Auturus* species have very short acropodites with a flattened solenomere and additional processes coiling laterally to produce a blunt calyx shape. *Illiniurus* is described in detail below.

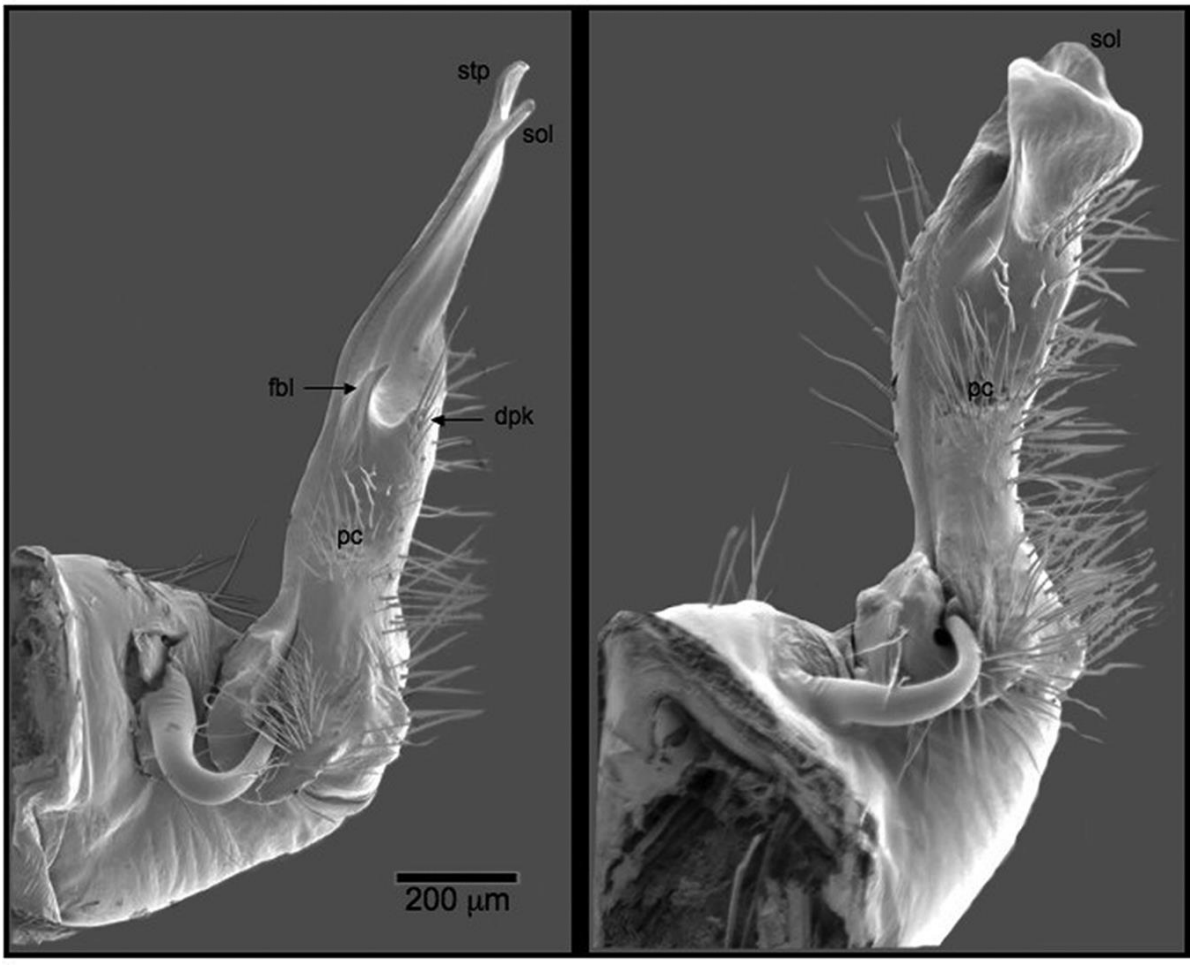

Figure 4.1. SEM images of left gonopod of *Euryurus leachii* (left) and *Auturus evides* (right), medial views. Solenomere (sol), sub-terminal process (stp), femoral basal lamella (fbl), distal prefemoral knob (dpk), prefemoral concavity (pc) are referred to in the text.

4.2 Materials and Methods

Specimens were examined with a Leica MZ8 dissecting microscope. Digital images were taken with a Microptics®-Imaging-System (based at the FMNH). Final images were assembled from 6-10 source images taken at different focal lengths using the software package Helicon Focus. Gonopods for SEM imaging were serially dehydrated in ethanol solutions, critical point dried and gold sputter coated. SEM images were taken with a JEOL 5600 LV scanning electron microscope (based at UIC) and retouched with Adobe Photoshop CS2.

Several different image types can be used to illustrate anatomical structures. Drawings, SEM and digital images are the most common and all have their own advantages and limitations. Drawings are ubiquitous in the diplopod literature and serve well, but they are interpretations of the illustrator and therefore subject to misinterpretation. When dealing with simple structures, such as euryurid gonopods, more objective SEM and digital images can convey as much, if not more, information as drawings. SEM images are informative due to their high resolution but are limited by only revealing the achromatic surface texture of the structure. These images can be supplemented with digital images which, although lacking in resolution, present the natural appearance of the structure. To maximize the amount of visual information given for this new species, all three image types are utilized in presenting the gonopod structure.

4.3 Results and Discussion

Illiniurus Shear, 1968. Proc. Biol. Soc. Wash. 81: 480.

Type species: *I. beattyi* Shear, by original designation.

Illiniurus beattyi Shear, 1968. Proc. Biol. Soc. Wash. 81: 480.

Type specimens: Male holotype, *vidi*, female paratype, *vidi* (MCZ) from Clear Springs, Union County, Illinois, collected 28 Oct. 1966 by J. M. Nelson.

Additional literature: Hoffman, 1978: 42-43. -- Hoffman, 1980: 164. – Shelley, 1982b: 3250. – Hoffman, 1999: 288 & 292.

Genus *Illiniurus* and its single species *I. beattyi* are based on a single male specimen from the southern tip of Illinois. It is characterized by gonopod structure, which is quite distinct from that of both *Auturus* and *Euryurus* (Fig. 4.2). The acropodite does not form the calyx characteristic of *Auturus*, is flattened, longitudinally folded and terminates with the solenomere. The solenomere itself is broad at its base, bordered on the lateral side by a small lamina, and a larger sub-terminal process on the medial side. In ventral view, this sub-terminal process and the solenomere form the bifid shape characteristic of *Euryurus* (Fig. 4.2). The distal prefemoral knob and prefemoral concavity are distinct; the femoral basal lamella is either highly modified or absent.

It is remarkable that the gonopod of this specimen has features of *Auturus* (short, blunt processes) and *Euryurus* (elongated, bifid telopodite), making it appear "transitional" between the two genera. This is even noted on the specimen's original label: "halfway between *Euryurus* and *Auturus*". The type locality of *I. beattyi* is within an area where two euryurid species,

Euryurus leachii Gray, 1832 and *Auturus evides* Bollman, 1887, live in sympatry and share the same habitat. The female paratype's cyphopod anatomy is indistinguishable from those of *E. leachii* specimens. Additionally, the vial containing the *I. beattyi* specimens at the time of description also contained a male *A. evides* (Shear 1968).

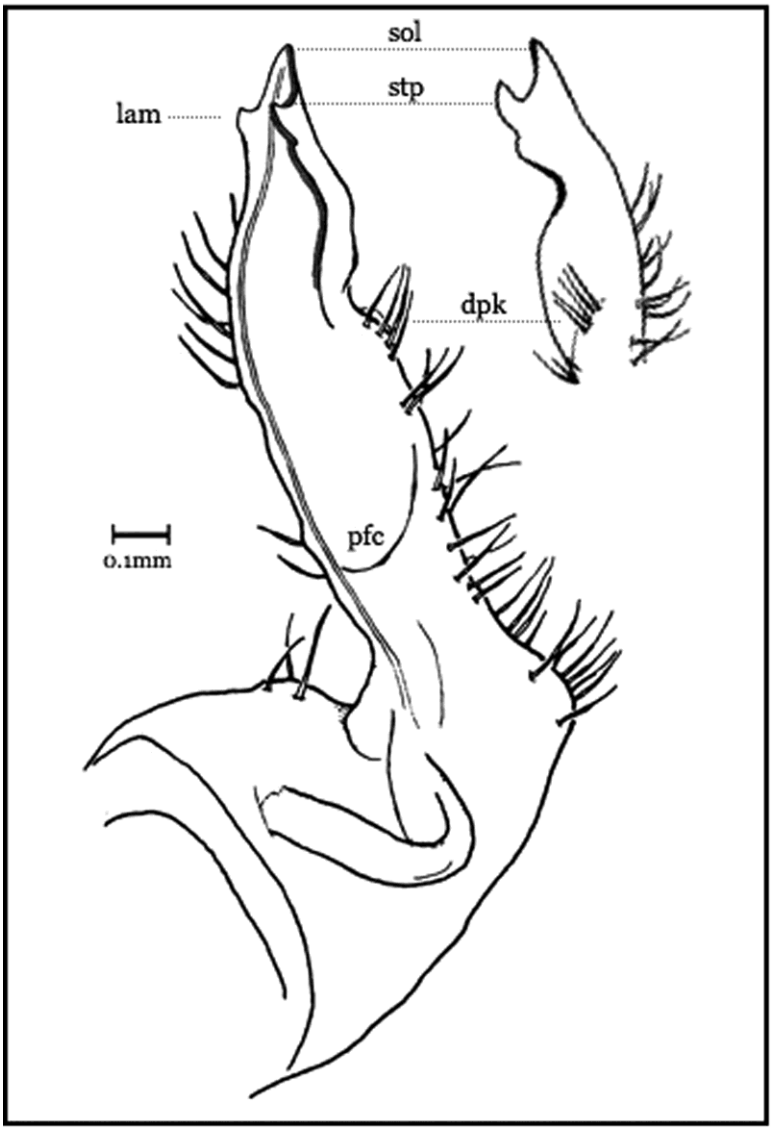

Figure 4.2. Left gonopod of *Illiniurus beattyi* holotype, medial view, and supplementary ventral view of acropodite. Small lamina mentioned in text (lam), other abbreviations as in Figure 4.1.

Expeditions by Field Museum personnel to the type locality in 2006 and 2007 failed to yield additional specimens of *I. beattyi*, yet numerous individuals of *E. leachii* and *A. evides* were found. The male *I. beattyi* specimen likely represents a case of gonopod deformity, a common phenomenon in diplopods, and if so, an aberrant individual of one of the other local euryurid species. Another possible scenario is that it is a hybrid of *E. leachii* and *A. evides* as evidenced by its gonopod appearing "transitional" between the two. Unless a population of this species is found, the status of genus *Illiniurus* and species *I. beattyi* as natural taxa should be held with reservation.

The type specimens are deposited at the Museum of Comparative Zoology. The original description states this (pg. 480), but also states deposition at the American Museum of Natural History (pg. 483). This discrepancy led Shelley (1982b) and Hoffman (1999) to assume the material was lost. Only the left gonopod was present in the vial at the time of this study.

Euryurus C. L. Koch 1847

System der Myriapoden, in *Kritische Revision der Insectenfauna Deutschlands*, ed. Herrich-Schäffer, 3, pg 138

Type species: *E. maculatus* Koch, 1847, by direct substitution (see Hoffman, 1999: 290).

8 species, eastern United States

Euryurus lecythanoictes, new species

Type specimens: Male holotype and paratypes (2 M, 1 F) from Escambia County, Alabama. Conecuh National Forest. ~2 miles north of AL 29 between mile markers 26 & 27; collected 2 Aug. 2008 by M. Jorgensen. Deposited Field Museum (FMNH-INS 043-944).

Material examined: Types specimens mentioned above, 1 male collected with the types (legs harvested for DNA), 1 male from type locality collected by M. Walker, 21 June 2007 (gonopods mounted for SEM, legs harvested for DNA). All specimens are adults.

Diagnosis: *Euryurus lecythanoictes* is distinguished from other *Euryurus* species by both the absence of a subterminal process and the presence of a distinct femoral lamella on the gonopod. Only one other known species, *E. mississippiensis* (Causey, 1955), is without a subterminal process. However, the femoral lamella is completely absent in *E. mississippiensis.*

Description (based on holotype; consistent with other males): Color in life: dorsally, very dark, almost black with bright orange paranota tips and mid-posterior portion of each metazonite. Yellowish speckling between orange areas. Ventrally, all yellowish except orange paranota tips. Dorsal surface smooth, moderately convex, with paranota extended laterally. Posterior segments with paranota angled acutely caudad. Collum width subequal to that of ensuing tergites. Head smooth with evident epicranial suture. Facial setae pattern: subantennal 1-1, frontal 1-1, genal 2-2, clypeal ca. 6-6, labral ca. 10-10. Antennae long (ca. 3 mm) with antennomeres 2-6 distally clavate and subequal in size and shape. Ozopores open laterally on segments 5, 7, 9-10, 12-13, 15-19. Hypoproct elliptical with 1 pair setae near caudal margin. Paraprocts with 2 pair setae, posterior-most pair closer to medial margin. Epiproct subquadrate and very broadly spatulate. Gonopods without subterminal process; solenomere flat and wide, distally mucronate and retrorse. Femoral lamella very distinct and broad, yet distally mucronate (Figs. 4.3-4.5). Female specimen: Somatic characters consistent with above. Cyphopods (Fig. 4.6) composed of

two ovoid valves shielded mesally by the receptacle and basally by the operculum (terminology *sensu* Hoffman, 1978). Mid-ventral margin of each valve extended into scleritized, 2-lobed ventral lamina. Distal portion of lamina forms partial covering of the reproductive opening. Long setae directed toward and covering ventral margins of valves.

All specimens: length ca. 25 mm, width at 10th segment 3.5 mm.

Etymology: *lecythanoictes*, noun in apposition. The name is derived from the Greek words *lecythos* (bottle) and *anoiktes* (one that opens) describing the "bottle opener" shape of the gonopod.

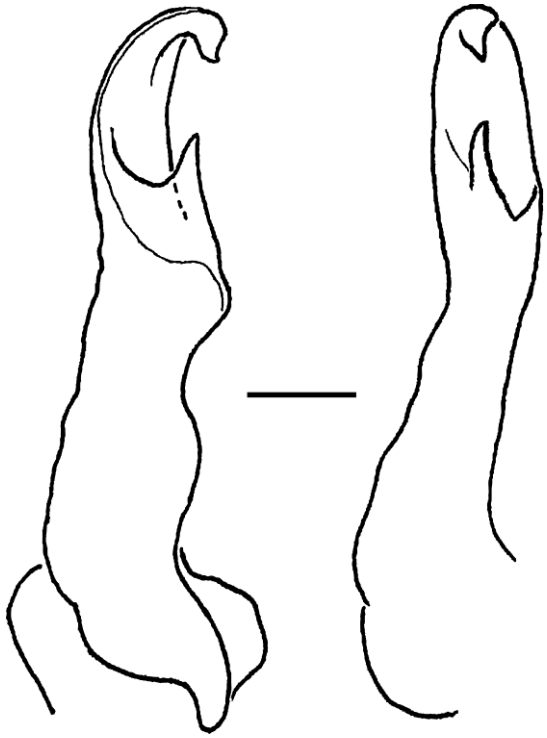

Figure 4.3. Right gonopod of *E. lecythanoictes* holotype, ventral view (left) and mesal view (right). Dense setae not depicted (see Figures 4.4 and 4.5). Bar = 0.2 mm.

Figure 4.4. Right gonopod of *E. lecythanoictes* holotype, ventro-lateral view. Bar = 0.2 mm.

Figure 4.5. SEM of *E. lecythanoictes* specimen; left gonopod, dorso-medial view (left); right gonopod, dorso-lateral view (right). Bar = 0.2 mm.

Geography and ecology: Known only from the type locality. This locality is over 100 km from the nearest known range of a congener (Fig. 4.7). An intensive survey of southern Alabama is necessary to determine the full distribution of *E. lecythanoictes* and its neighbors. All specimens were collected beneath the bark of dead hardwood logs in a hardwood-pine forest. All Euryuridae species are typically associated with decaying hardwood logs near water, found either beneath the bark or underneath the log (Shelley 1982b).

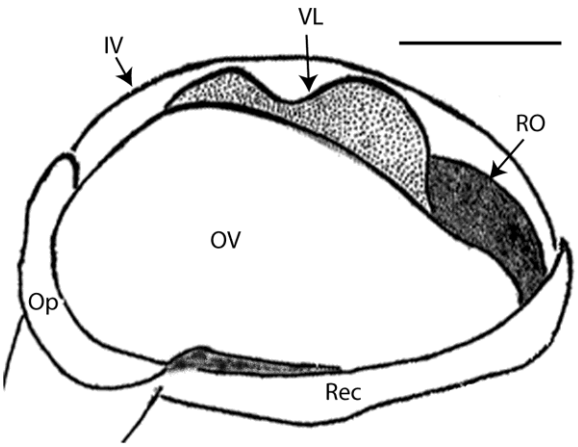

Figure 4.6. Right cyphopod of paratype specimen, ventro-caudal view. Setae not depicted. IV - inner valve; OV - outer valve; Op - operculum; Rec - receptacle; VL - ventral lamina; RO - reproductive opening. Bar = 0.2 mm.

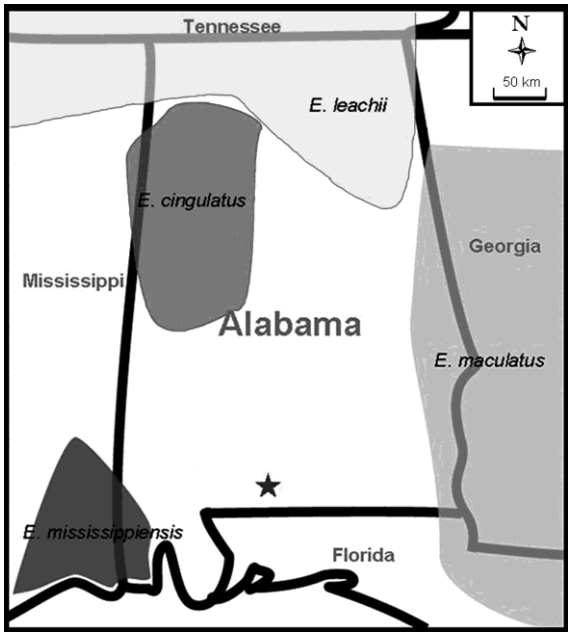

Figure 4.7. Type locality of *E. lecythanoictes* (star) and known distributions of neighboring Euryuridae species. Based on specimens listed in Hoffman 1978, Shelley 1982a, 1982b and collections by author.

The gonopod structure of *E. lecythanoictes* is quite different from all described euryurid species. This tempts one to designate a new genus to accommodate the species. However, this action would only serve to oversplit a species-poor family and add unnecessary complexity to millipede taxonomy. I therefore chose to assign it to *Euryurus* based on the elongation of the acropodite, the prominence of the femoral lamella, and the mucronation of the solenomere.

5. Phylogenetic study of the North American broad-tailed millipede group (Diplopoda, Polydesmida, Euryuridae) reveals remarkable discordances among mitochondrial, nuclear and morphological traits.

5.1 Introduction

The arthropod class Diplopoda (millipedes) is an extremely diverse, ecologically important, yet poorly understood group of animals. Millipedes inhabit the entire terrestrial biome, excluding the polar regions, and are significant participants in the detritification of many ecosystems. They have received considerable alpha-taxonomic attention resulting in ca.12,000 described species placed among 16 orders (Sierwald and Bond, 2007). However, the relationships among species and higher level taxa as delineated by traditional taxonomy is unlikely representative of the true phylogenetic structure of the group (Brewer et al., 2012a). In recent years, cladistic and molecular systematic methods have been applied to a handful of millipede groups at different taxonomic levels (Bond and Sierwald, 2003; Brewer et al., 2012b; Enghoff et al., 2011; Marek and Bond, 2006, 2007; Pitz and Sierwald, 2010; Sierwald et al., 2003; Walker et al., 2009a; Wesener et al., 2010). These studies are part of a new endeavor to revise millipede systematic research and develop an accurate phylogenetic framework that will bring millipede research up to speed with much of the animal world.

Here, we investigate the phylogenetic structure of Euryuridae Pocock, 1909, a family of polydesmidan millipedes endemic to the eastern United States (Figures 5.1, 5.2) and found primarily in association with rotting hardwood logs. Euryurids are characterized by their bright orange and black color pattern and their broad epiproct (caudal extension of last segment), which is a unique trait in North American polydesmidans. In fact, all euryurids are nearly identical in

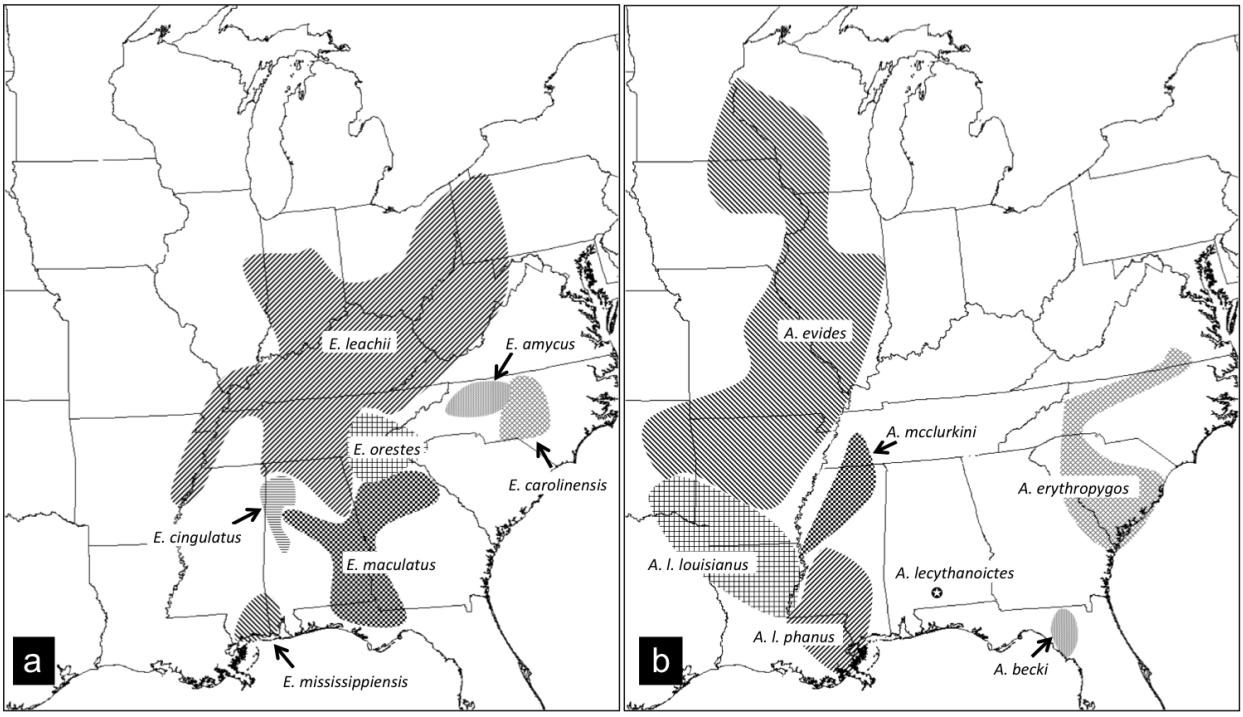

Figure 5.1. Distributions of Euryuridae species. a) genus *Euyurus*, b) genus *Auturus*. Shapes represent full extent of each species range based on collection records. Species do not necessarily inhabit entire range continuously. Collection sites are primarily old growth hardwood forests. Collection records from Hoffman, 1978; McAllister and Shelley, 2005; Shelley, 1982a, b, 1990; Shelley et al., 2012; and collections by MCJ.

appearance, differing only in the morphology of genital characters (Figure 5.3). The two genera, *Euryurus* Koch, 1847 and *Auturus* Chamberlin, 1942, have been traditionally diagnosed by a distinct difference in the male copulatory organs (gonopods), with *Euryurus* gonopods being apically elongated and acuminate and *Auturus* being short and blunt. However, Hoffman (1978) also noticed a seemingly consistent difference between the genera in the female organs (cyphopods) of the species he observed. This difference is hereby confirmed consistent with all known species of Euryuridae. The recently described species *E. lecythanoictes* Jorgensen, 2009 was placed in *Euryurus* based on the gonopod, although its shape was admittedly quite different from the typical congener. Based on the cyphopod, this species is better attributed to *Auturus* and

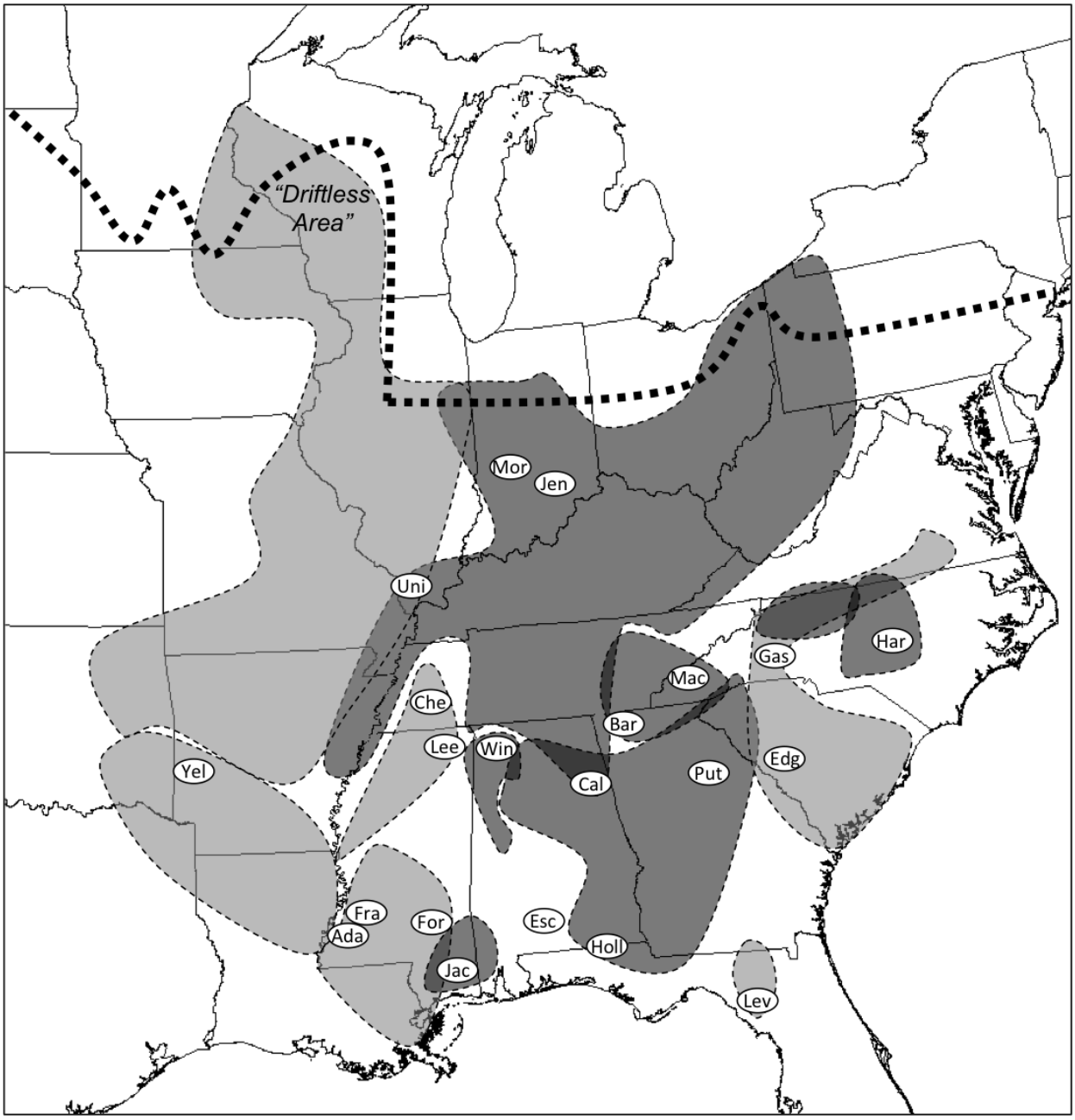

Figure 5.2. Distributions of all Euryuridae species emphasizing the geographic affinities between the two genera. *Euryurus* = dark shade, *Auturus* = light shade. Also shown are the collection sites of the voucher specimens. The three letter code for each site, which is used on the gene tree figures, corresponds to the first three letters of the county name (Table 5.2). Bold dashed line represents extent of last glacial maximum.

in this case, seems to be the more reliable character for genus designation. I (MCJ) believe I erred in the original designation and hereby formally recognize *Auturus lecythanoictes*, new combination. Currently, the Euryuridae consist of 14 nominal taxa (Table 5.1).

Euryuridae species
Auturus becki Chamberlin 1951
Auturus erythropygos (Brandt 1839)
Auturus evides (Bollman 1887)
Auturus lecythanoictes (Jorgensen 2009)
Auturus louisianus louisianus (Chamberlin 1918)
Auturus louisianus phanus Chamberlin 1942
Auturus mcclurkini Causey 1955
Euryurus amycus Hoffman 1978
Euryurus carolinensis (DeSaussure 1859)
Euryurus cingulatus Hoffman 1978
Euryurus leachii (Gray 1832)
Euryurus maculatus Koch 1847
Euryurus mississippiensis (Causey 1955)
Euryurus orestes Hoffman 1978

Table 5.1. Currently recognized species of the family Euryuridae.

Eururid taxonomic history has been tumultuous, at times including several Latin American groups no longer considered closely allied to present day euryurids (Hoffman, 1978; Hoffman, 1998; Jorgensen, 2009; Shelley, 1982b), and no phylogenetic hypothesis for Euryuridae has ever been proposed. Shelley (1982b) offered a hypothesis for the relationships among the *Auturus* species, although this was largely intuitive and not based on an analysis of characters. Euryuridae is currently part of the superfamily Xystodesmoidea Cook, 1895 along

with Eurymerodesmidae Causey, 1951 from North America, Gomphodesmidae Cook, 1896 from Africa, Oxydesmidae Cook, 1895 from Africa, and Xystodesmidae Cook, 1895 from North America and Eurasia (Shelley, 2002), although this classification has not been tested phylogenetically.

The nearly identical appearance of the different euryurid species suggests a recent radiation coupled with rapid evolution of genital morphology. Rapid genital evolution is a well documented phenomenon in animals (Eberhard, 1985a) and many hypotheses for the cause have been proposed (Hosken and Stockley, 2004b). Unfortunately, very little is known about how polydesmidan gonopods function (but see Tanabe and Sota, 2008) and therefore, what type of selective pressure could account for the extreme variability found in many millipede groups.

This phylogenetic study utilizes sequence data from three loci (16S, COI and ITS2) and gonopod/cyphopod morphology. Phylogenetic inference at this level requires genetic data from fast evolving regions. The 16S ribosomal subunit of the mitochondria has been successfully employed in lower level millipede research (Marek and Bond, 2006, 2007; Walker et al., 2009a) as well as in other groups. Another mitochondrial gene, cytochrome oxidase I (COI), has been utilized considerably across eukaryotes, both in lower level phylogenetic research and as a taxonomic barcode. The ITS2 region of the nuclear ribosomal DNA cluster has received heavy use in phylogenetic studies, especially at the species level (Alvarez and Wendel, 2003). It has also been proposed as a region for genetic barcoding of species (Yao et al., 2010), and analyses of its secondary structure has been successfully employed in higher level phylogenetic studies (Coleman, 2003).

5.2 Materials and Methods

5.2.1 Morphology

Euryurid species are nearly identical except for the genitalia; thus, the morphological data set consists only of gonopod and cyphopod characters. Gonopods and cyphopods from each species were serially dehydrated in ethanol solutions and critical point dried before mounting for scanning electron microscope (SEM) imaging. The left gonopod of each specimen was positioned for imaging in mesal aspect and the right for lateral aspect. Cyphopods were mounted with the 2nd sternite intact in ventral aspect. SEM images were taken with a JEOL 5600 LV scanning electron microscope (based at UIC). Characters for phylogenetic analysis (Figure 5.3, Table 5.2) were identified on SEM images and checked for consistency with 10-15 additional specimens under a dissecting microscope. The characters were scored in a matrix (Table 5.3) and analyzed under maximum parsimony in PAUP v.4.0b10 (Swofford, 1991). All characters were treated as equally weighted and unordered. The tree was outgroup rooted using a general *Brachoria* (Xystodesmidae) model derived from images in Marek and Bond, 2006 and Marek, 2010. The closest relative to Euryuridae is unknown so *Brachoria* was chosen due to its putative relationship with euryurids (both are in superfamily Xystodesmoidea) and to the availability of SEM images. Most of the characters were absent in *Brachoria*. Like Euryuridae, there is considerable variation in the solenomere shape of *Brachoria* species. All the solenomere states used could be attributed to one *Brachoria* species or another; therefore solenomere shape (character 6) was coded as missing.

Character list
1. Prefemoral concavity: (0) absent; (1) indistinct; (2) distinct.
2. Concavity position (ratio of length between gonopod apex and concavity and length between concavity and basal shield): (0) <1; (1) ≈1; (2) >1.
3. Distal prefemoral knob: (0) absent; (1) indistinct; (2) distinct.
4. Knob pilosity: (0) loose; (1) dense.
5. Femoral basal lamella: (0) absent; (1) pinch; (2) bump; (3) broad.
6. Solenomere shape: (0) broad; (1) intermediate; (2) acicular.
7. Subterminal process: (0) absent; (1) acicular; (2) flat.
8. Lamellar process: (0) absent; (1) pointed; (2) dull.
9. Coxa shape: (0) ovoid; (1) subtriangular; (2) triangular.
10. Cyphopod ventral lamina: (0) absent; (1) present.

Table 5.2. Character set for morphological phylogenetic analysis.

Species	Character									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Brachoria	**0**	**-**	**0**	**-**	**0**	**-**	**0**	**0**	**0**	**0**
A. becki	**1**	**1**	**2**	**1**	**1**	**0**	**0**	**1**	**1**	**1**
A. erythropygos	**1**	**0**	**2**	**1**	**1**	**0**	**0**	**1**	**2**	**1**
A. evides	**2**	**1**	**1**	**0**	**1**	**0**	**0**	**2**	**2**	**1**
A. lecythanoictes	**2**	**0**	**2**	**1**	**3**	**1**	**0**	**0**	**2**	**1**
A. l. louisianus	**2**	**0**	**1**	**0**	**1**	**0**	**0**	**2**	**0**	**1**
A. l. phanus	**1**	**1**	**0**	**-**	**1**	**0**	**0**	**2**	**0**	**1**
A. mcclurkini	**2**	**0**	**2**	**1**	**1**	**0**	**0**	**2**	**2**	**1**
E. amycus	**2**	**1**	**0**	**-**	**2**	**2**	**1**	**0**	**1**	**0**
E. carolinensis	**2**	**1**	**1**	**0**	**2**	**2**	**1**	**0**	**1**	**0**
E. cingulatus	**0**	**-**	**0**	**-**	**0**	**2**	**1**	**0**	**2**	**0**
E. leachii	**2**	**1**	**2**	**0**	**3**	**2**	**2**	**0**	**2**	**0**
E. maculatus	**2**	**1**	**2**	**0**	**0**	**2**	**2**	**0**	**2**	**0**
E. mississippiensis	**1**	**1**	**0**	**-**	**0**	**2**	**0**	**0**	**0**	**0**
E.orestes	**2**	**1**	**1**	**0**	**3**	**2**	**2**	**0**	**2**	**0**

Table 5.3. Morphological data matrix

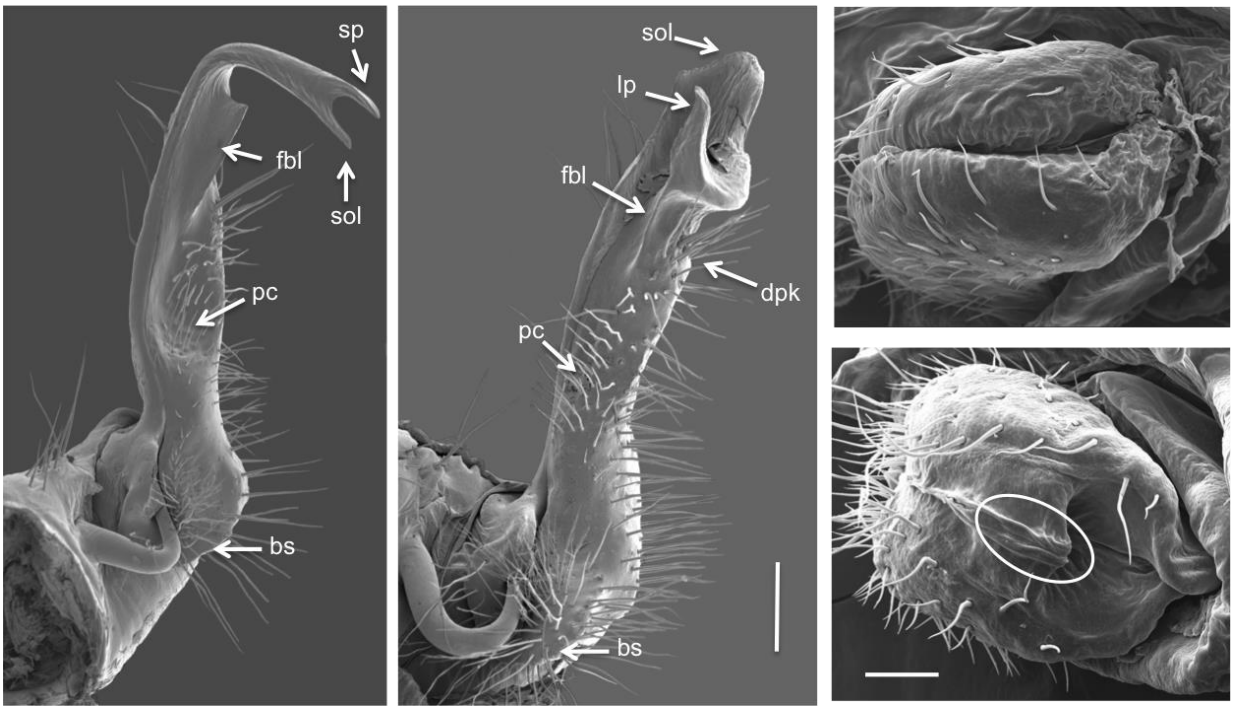

Figure 5.3. Landmarks used in morphological character dataset. Left: left gonopod of *E. orestes*. Middle: left gonopod of *A. becki*. Right top: right cyphopod of *E. maculatus*. Right bottom: right cyphopod of *A. becki*, ventral lamina circled. sp – subterminal process; sol – solenomere; fbl – femoral basal lamella; pc – prefemoral concavity; bs – basal shield; lp – lamellular process; dpk – distal prefemoral knob. Scale bars: gonopod - 200µm; cyphopod - 50µm.

5.2.2 Sequencing

Live specimens (Table 5.4) were collected by hand and held in 95% ethanol until DNA extraction. Species identification was confirmed by examination of gonopods under a dissecting microscope. Females were identified as the same species as males from the same locality. DNA was extracted from either ca. 8 legs or from one sternite including all four legs from the posterior end. Extraction was done with the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol.

The following reagent stocks were used for all PCR and cycle sequencing reactions: dNTPs - 2mM (0.5mM A,C,G and T); primers - 25µM; $MgCl_2$ - 25mM; PCR buffer - 100mM

Tris-HCl, pH 8.3, 500mM KCl; Taq polymerase - 5u/µl (Sigma) or 1u/µl (FMNH – generated at Field Museum). Recipes are given for 25µl reactions. Products were cleaned with either Exo-SAP (USB - 1 unit shrimp alkaline phosphatase and 5 units exonuclease) or GELase™ (Epicentre), then sequenced using the Big Dye v.3.1 cycle sequencing kit (Applied Biosystems) and run on an ABI 377 or ABI 3730.

species	#/sex*	collection site
A. becki	1M, 1F	Levy County, Florida
A. erythropygos	1M, 1J	Gaston County, North Carolina
	1F	Edgefield County, South Carolina
A. evides	2M	Union County, Illinois
A. lecythanoictes	2M	Escambia County, Alabama
A. louisianus louisianus	1M, 1F	Yell County, Arkansas
A. louisianus phanus	1M	Adams County, Mississippi
	1M	Franklin County, Mississippi
A. mcclurkini	1F	Lee County, Mississippi
	1F	Chester County, Tennessee
E. carolinensis	1F, 1J	Harnett County, North Carolina
E. cingulatus	1M, 1F	Winston County, Alabama
E. leachii	2F	Union County, Illinois
	1M, 1F	Jennings County, Indiana
	1F	Morgan County, Indiana
	1M	Calhoun County, Alabama
E. maculatus	2M	Putnam County, Georgia
	1M	Holmes County, Florida
E. mississippiensis	1M, 1F	Jackson County, Mississippi
E. orestes	1M, 1F	Macon County, North Carolina
	1F	Bartow County, Georgia
Auturus species undetermined	1F	Forrest County, Mississippi

Table 5.4. Voucher specimen information. *M-male, F-female, J-juvenile.

PCR amplification of the 16S mitochondrial rRNA sequence followed Marek and Bond (2006). The PCR reaction contained 10.875µl purified H_20, 2.5µl 10X PCR buffer, 2.5µl dNTPs, 2.5µl $MgCl_2$, 2.5µl of each primer, 0.5µl DMSO, 0.5µl BSA, 0.125µl Taq polymerase (Sigma), and 0.5µl DNA extraction. Primers for amplification were LR-J-12887dip2 and LR-N-EURY1 (Table 5.5). Thermocycler program consisted of an initial denaturing at 95ºC for 2 minutes then 30 cycles of 1) denaturing at 94ºC for 30 seconds, 2) annealing at 58ºC for 30 seconds, 3) extension at 72ºC for 60 seconds, and concluded with a final extension at 72ºC for 2 minutes. Each product was sequenced with above PCR primers and the internal bridging primer, LR-J-APHE1 (Table 5.5).

PCR amplification of the cytochrome oxidase I (COI) mitochondrial gene followed Hebert et al. (2003). The PCR reaction contained 14.8µl purified H_20, 2.5µl 10X PCR buffer, 2.5µl dNTPs, 3µl $MgCl_2$, 0.3µl each primer, 0.1µl Taq polymerase (Sigma) and 1.5µl DNA extraction. Primers used in amplification and cycle sequencing were HCO2198 and LCO1490 (Table 5.5). Thermocycler program consisted of an initial denaturing at 94ºC for 60 seconds then 5 cycles of 1) denaturing at 94ºC for 60 seconds, 2) annealing at 45ºC for 90 seconds, 3) extension at 72ºC for 90 seconds, then 35 cycles of 1) denaturing at 94ºC for 60 seconds, 2) annealing at 50ºC for 90 seconds, 3) extension at 72ºC for 60 seconds, concluding with a final extension at 72ºC for 5 minutes.

PCR amplification of the second internal transcribed spacer (ITS2) followed Ji et al. (2003). The PCR reaction contained 17.5µl purified H_20, 2.5µl 10X PCR buffer (with 1.5mM $MgCl_2$), 2.5µl dNTPs, 0.25µl each primer, 1µl Taq polymerase (FMNH) and 1µl DNA extraction. Primers used in amplification and cycle sequencing were CAS5p8sFc and CAS5p8sB1d (Table 5.5). Thermocycler program consisted of an initial denaturing at 94ºC for 4

minutes then 35 cycles of 1) denaturing at 95ºC for 20 seconds, 2) annealing at 62ºC for 40 seconds, 3) extension at 72ºC for 20 seconds, and concluded with a final extension at 72ºC for 2 minutes.

primer	sequence	source
16S forward (LR-N-EURY1)	5'-GTATAGAGAGTGAAAATTGAGG-3'	this study
16S reverse (LR-J-12887dip2)	5'-CCGGTCTGAACTCAGATCATGT-3'	Marek and Bond 2006
16S bridge (LR-J-APHE1)	5'- GTTTCACCTTCATACCAGC-3'	Marek and Bond 2006
COI forward (LCO1490)	5'-GGTCAACAAATCATAAAGATATTGG-3'	Hebert et al. 2003
COI reverse (HCO2198)	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	Hebert et al. 2003
ITS2 forward (CAS5p8sFc)	5'-TGAACATCGACATTTYGAACGCACAT-3'	Ji et al. 2003
ITS2 reverse (CAS5p8sB1d)	5'-TTCTTTTCCTCCSCTTAYTRATATGCTTAA-3'	Ji et al. 2003

Table 5.5. Primers used for DNA amplification and sequencing.

5.2.3 Molecular analysis

Sequences were edited and assembled with Sequencher® version 4.1 sequence analysis software (Gene Codes Corporation, Ann Arbor, MI USA http://www.genecodes.com). Multiple sequences were aligned with ClustalW 2.0 (Larkin et al., 2007) in Mesquite (Maddison and Maddison, 2011) followed by manual adjustment. Gene trees were estimated by maximum likelihood analysis implemented in Garli v.2.0 (Zwickl, 2006) (https://code.google.com/p/garli/). Twenty replicates were run and the best tree used. Model selection for each gene was selected by the Akaike Information Criterion with MrModeltest v. 2.2 (Nylander, 2004). The 16S and COI genes were concatenated and analyzed together under GTR+I and HKY+I+G models,

respectively. The ITS2 gene was analyzed separately under the GTR+I+G model. One 6-13 base pair region of ITS2 was excluded from the analysis due to questionable alignment. Nodal support values are percentage of 1000 bootstrap replicates generated in Garli under the same models.

Outgroup selection for tree rooting was based on Hoffman's (1998) hypothesis of close relatedness to the xystodesmidan genus *Melaphe*, a Eurasian taxon. Local xystodesmidan genera, *Dicellarius* and *Pachydesmus* were also used. Unfortunately, all outgroups tested were on extremely long branches, making the root position questionable. We therefore chose an alternative strategy to estimate the root position: the ingroup datasets were analyzed by MCMC implemented in MrBayes (Ronquist et al., 2012) under a relaxed clock model. This model makes the assumption that evolutionary rates among lineages are very similar (which is reasonable given the close relatedness the group) but allows for each lineage's rate to change over time independently (a conservative approach). The consensus tree generated from the posterior distribution is automatically rooted, and a posterior probability for the root position can be calculated (Huelsenbeck et al., 2002). Bayesian analyses were performed with the same substitution models used in the likelihood analyses. Each analysis consisted of two runs of four chains each, three heated and one cold, run for 1 million generations. The first 25% of saved trees were discarded as burnin. The final average standard deviations of split frequencies were <0.01.

5.3 Results

The tree generated from the morphological analysis (Figure 5.4, score=29, CI=0.62) contains two major in-group clades: one consisting of all *Auturus* species plus *E. mississippiensis* and the other with the remaining *Euryurus*. Within the former, *E. mississippiensis* diverges first,

rendering *Auturus* monophyletic. *Auturus becki* and *A. erythropygos*, the only two *Auturus* species found in the eastern euryurid range, are sister species and form a polytomy with *A. lecythanoictes* and *A. mcclurkini*, which are the closest geographically though still quite distant. The widespread *E. leachii* forms a sister group with *E. orestes* of the southern Appalachian region, which together group with *E. maculatus* to the south. These three form a sister clade to *E. carolinensis* and *E. amycus*, sister species with small parapatric ranges in North Carolina.

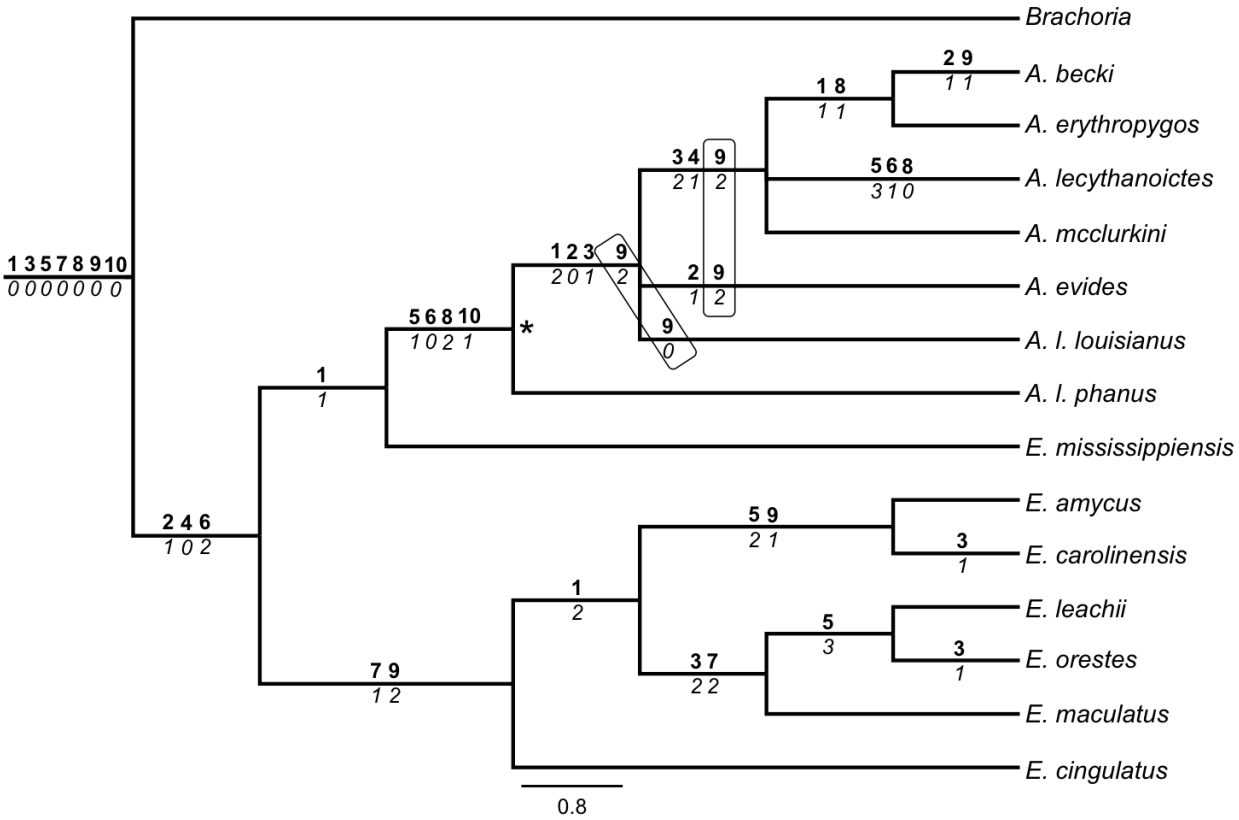

Figure 5.4. Strict concensus MP tree of morphological data. Score = 29. Consistency index = 0.62. Character state changes: character number above branch, corresponding state below branch (Table 5.4). Boxed character state changes are equally parsimonious, mutually exclusive alternatives. All nodes have Bremer support of 1, except * of 2.

The 16S and COI sequences were 931-1000 bases pairs (bp) and 506-535 bp (116 and 75 parsimony informative), respectively. Most of the length variation was due to the quality of the sequence ends, not to indels. One *E. mississippiensis* COI sequence was a half sequence (292 bp), but this did not affect the results. Estimated model parameters are shown in Table 5.6. The mitochondrial gene tree (Figure 5.5) is well supported and reveals the genera as polyphyletic. The estimated root position is highly supported (posterior probability = 0.99). Three deep clades are resolved, the first composed solely of *A. lecythanoictes* with the remaining species forming a sister group. This group forms "east" and "west" clades (Figure 5.5), which have a geographic pattern, but are only partially congruent with traditional taxonomy. *Auturus erythropygos* and *A. becki*, the only *Auturus* species found in the eastern part of the range, are grouped with the majority of the *Euryurus* species. The other major clade consists of the remaining *Auturus* species and *E. cingulatus*. *Euryurus leachii* was recovered as paraphyletic, with *A. erythropygos* nested within it. *Euryurus maculatus* and *A. l. phanus* were recovered as polyphyletic.

gene	substitution rates	state frequencies (A, C, G, T)	alpha	proportion invariant
16S	(AC) 1.10; (AG) 8.46; (AT) 0.50; (CG) 0.28; (CT) 4.95; (GT) 1.00	0.428, 0.227, 0.070, 0.275	-	0.731
COI	(tr/tv) 18.04	0.442, 0.233, 0.131, 0.195	0.782	0.700
ITS2	(AC) 1.13; (AG) 1.66; (AT) 1.95; (CG) 0.34; (CT) 3.01; (GT) 1.00	0.137, 0.305, 0.359, 0.199	0.549	0.519

Table 5.6. Model parameters of best maximum likelihood trees estimated by Garli.

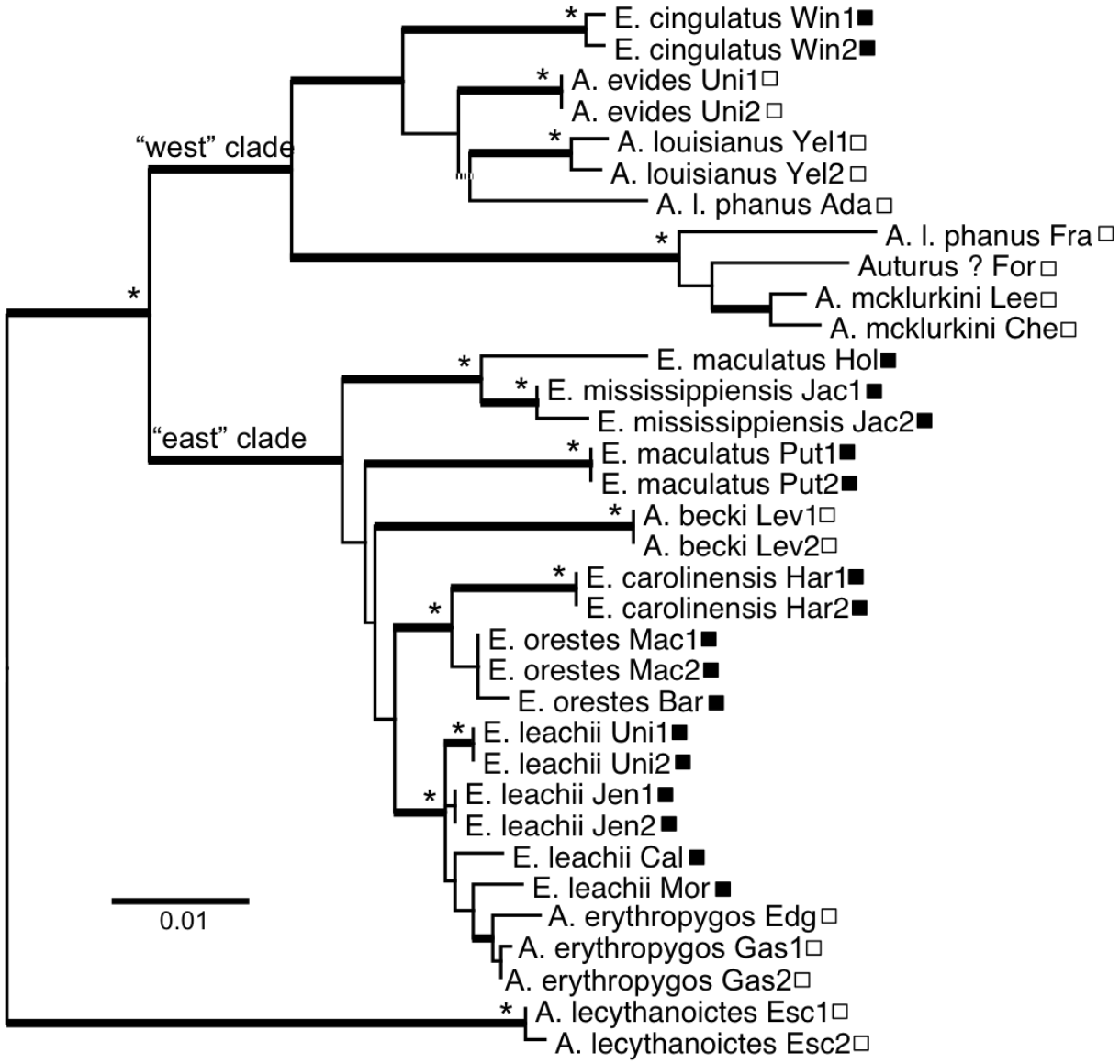

Figure 5.5. ML tree of mitochondrial gene sequences. Bootstrap support (1000 replicates): <50: broken lines; 50-75: thin lines; >75: thick lines; >95: asterisk. Species names followed by collection site code (first three letters of collection county, see Table 5.2), specimen number and genus identifier (open = *Auturus*, closed = *Euryurus*).

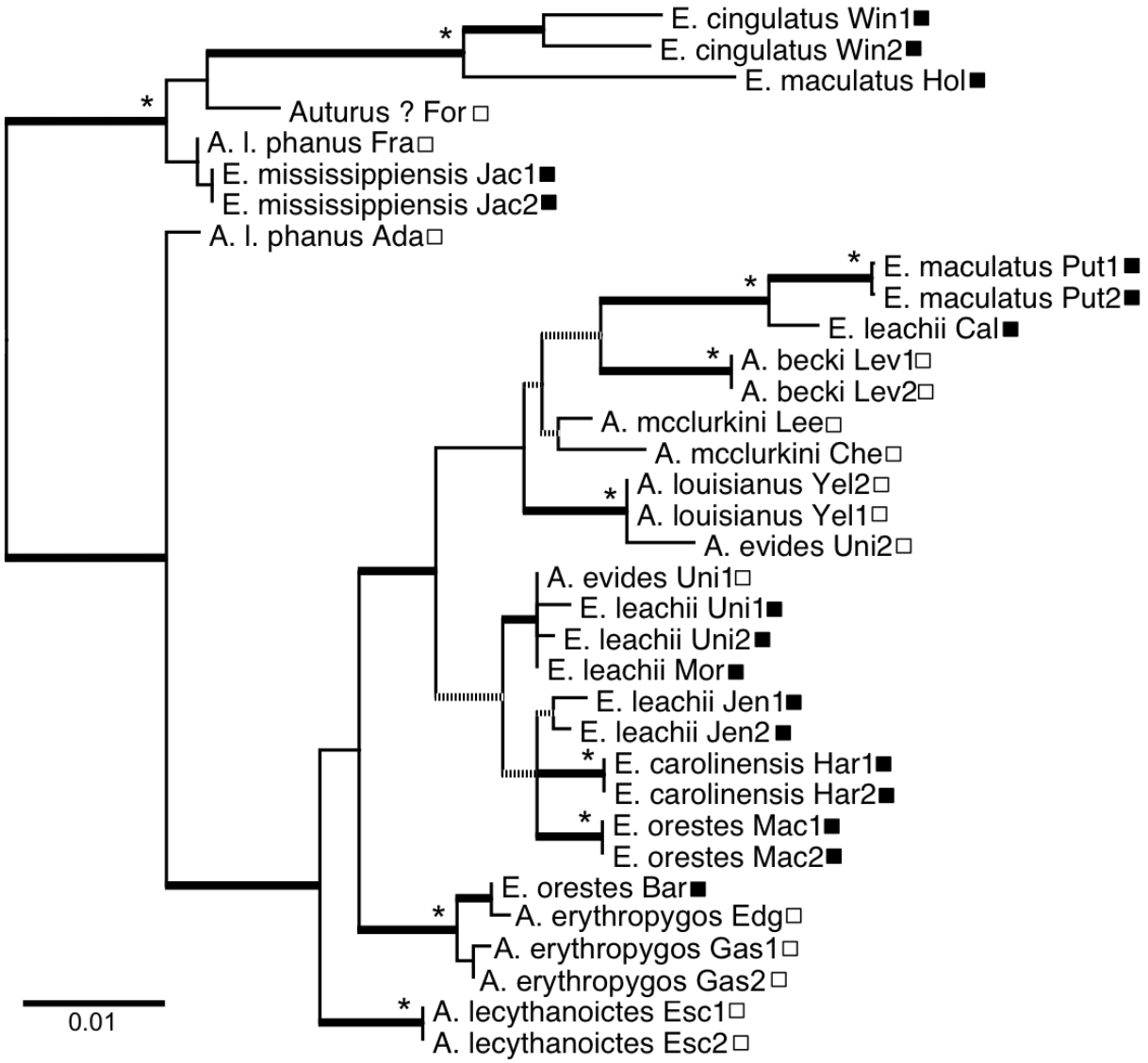

Figure 5.6. ML tree of ITS2 gene sequences. Bootstrap support (1000 replicates): <50: broken lines; 50-75: thin lines; >75: thick lines; >95: asterisk. Species names followed by collection site code (first three letters of collection county, see Table 5.2), specimen number and genus identifier (open = *Auturus*, closed = *Euryurus*).

The ITS2 sequences were 799-869 bp with 90 being parsimony informative. Estimated model parameters are shown in Table 5.6. Estimated root position is unsupported (posterior probability = 0.52). The ITS2 gene tree (Figure 5.6) exhibits greater conflict with traditional taxonomy and little congruence with the morphological or mitochondrial trees. Few of the relationships uncovered in the mitochondrial tree appear in the ITS2 tree, and the estimated root positions of the two trees are entirely inconsistent with each other. The only pattern shared with the mitochondrial tree is the polyphyly of *E. maculatus* and *A. l. phanus*, but the polyphyly of *E. orestes*, *A. evides* and *E. leachii* are unique to this tree. This is especially interesting with *A. evides*, as both specimens are from the same collection locality.

5.4 Discussion

5.4.1 Phylogeny

This study illustrates extensive phylogenetic discordance among three different data sets. We consider this millipede group to be of relatively recent origin because 1) all species are identical in morphology with the exception of genitalia, 2) all live in the same microhabitat (within decaying hardwood logs) and 3) genetic distances among species are small relative to putative outgroups. The differences in genital morphology that define species are likely the result of intense sexual selection occurring over a relatively short period of time. Reproductive isolation is not necessarily complete, either mechanically or physiologically. Despite the variation in gonopod shape, cyphopod anatomy appears to have changed little within each genus. Unfortunately, so little is known about the interactions between the gonopods and cyphopods of millipedes that we cannot determine with confidence whether the key characters that define the

genera are real synapomorphies or homoplasies caused by selection on some sexual function. Assuming they are synapomorphies, and given the recent radiation of the group, the discordance of data sets seen here is best explained by incomplete lineage sorting and/or introgressive hybridization, which have left a genetic signal that distorts the true species relationships. Such discordances among datasets is not uncommon; recent studies in other arthropod groups have uncovered conflicting results among different datasets (Gillespie et al., 2013; Havermans et al., 2010; Herrera et al., 2010; Lapoint et al., 2011; Pollard et al., 2006; Rintelen et al., 2007; Vera et al., 2012). These authors most commonly attributed their conflicting results to incomplete lineage sorting and introgressive hybridization although convergence of morphological traits and cryptic species were also proposed.

The phylogenetic tree generated from the morphological data (Figure 5.4), although not strongly supported and including two polytomies, is somewhat representative of the traditional view of this family's taxonomic structure. The morphological characters that define these two genera are generally very clear. The cyphopod anatomy provides a distinct dichotomy. Gonopod anatomy is less obvious, with certain species (e.g., *E. cingulatus*, *E. mississippiensis*, *A. lecythanoictes*) deviating considerably from the typical generic forms. We expect, due to the genus-defining characteristics, that each genus is monophyletic, or at least paraphyletic. This is what we observe; *Auturus* is monophyletic and *Euryurus* paraphyletic with only the position of *E. mississippiensis* challenging monophyly of the genus. Two patterns in the shallower relationships stand out as significant as well. The two *Auturus* species that are geographically distant from congeners (*A. erythropygos* and *A. becki*, Figure 5.1b) form a sister pair. Given the geographic affinity, this is probably the true relationship. Also, the sister relationship of *E. amycus* and *E. carolinensis* is most likely the true relationship, given the parapatry of their small

ranges (Figure 5.1a). This conclusion remains to be examined further as no genetic data are yet available for *E. amycus*.

The mitochondrial gene tree (Figure 5.5) is well resolved and highly supported at most nodes. With the relaxed-clock estimated root position, *A. lecythanoictes* forms its own clade and diverges first. If the position of the root is moved to the branch leading to the majority of the *Euryurus* species (the "east" clade), the tree is more similar to the morphological tree. However, if the mitochondrial gene tree reflects the true species relationships, there would have to be an incredible convergence of both male and female *Auturus* characteristics in *A. erythropygos* and *A. becki*. Likewise, *E. cingulatus* would exhibit convergence of the *Euryurus* form, although this scenario is more plausible given this species' seeming superficial similarity to congeners. Although neither genus is monophyletic on the mitochondrial tree, the two inner clades correspond to an east-west geographic split. This points to past hybridization events resulting in the introgression of mitochondrial haplotypes into neighboring species genomes (Funk and Omland, 2003). This appears to be the case with *A. erythropygos*, which is nested within the clade of *E. leachii*, and given the short branch lengths, it happened relatively recently. Any introgression involving *A. becki* and *E. cingulatus* would have been more ancient, as evidenced by their longer branches and sister relationships to multiple taxa.

The ITS gene tree (Figure 5.6) is considerably more ambiguous than the morphological and mitochondrial trees. This is mainly due to the several unsupported nodes and to the profound separation of conspecific specimens on the tree (*E. maculatus, A. l. phanus, E. leachii, A. evides* and *E. orestes*). If some process can split conspecifics up to such a degree, any insight into species relationships should be viewed with reservation. Since the ITS2 gene forms repetitive arrays with hundreds of copies, the possibility of paralogous copies cannot be ignored. Although

the uniformity of the different copies is largely maintained through concerted evolution (Hillis and Dixon, 1991), divergent paralogs have been reported in other studies (Harris and Crandall, 2000; Hugall et al., 1999; Vogler and DeSalle, 1994; Wesson et al., 1992). If multiple paralogs were recovered during PCR, we would expect multiple ambiguous sites due to the simultaneous sequencing of the different copies, but there were ambiguities in only two specimens: one *E. cingulatus* with five and one *E. orestes* with seven. Like the mitochondrial tree, the structure of the ITS tree is probably best explained by incomplete lineage sorting and/or introgression.

Collectively, the trees provide both interesting and confounding insight into the phylogenetic history of this group. For example, the *E. leachii* specimen from Calhoun County, AL unsurprisingly groups near the *E. leachii* specimens from the other three collection sites on the mitochondrial tree, but it forms a strongly supported sister group with the *E. maculatus* specimens from Putnam County, GA on the ITS tree. One possible explanation for the discordance of this specimen is that ITS2 haplotypes have failed to completely sort within *E. leachii* since the split with *E. maculatus*. However, this split would have occurred deep in the phylogeny, at a node leading to eight species. Even more extreme, the split between *E. maculatus* specimens from its two sites would trace to the root of the tree. This magnitude of incomplete lineage sorting is highly unlikely.

An alternative explanation is the capture of *E. maculatus* ITS haplotypes by *E. leachii* through hybridization. This scenario is further supported by geographic affinities. Calhoun, AL is much closer to Putnam, GA than to the other *E. leachii* localities of this study; in fact, the Calhoun site is nearly within the known *E. maculatus* range (Figures 5.1, 5.2). The polyphyly of the *E. maculatus* specimens could also be caused by hybridization events with neighboring species. The specimen from Holmes County, FL forms a mitochondrial clade with *E.*

mississippiensis and an ITS clade with *E. cingulatus.* Overall, the potential of gene exchange between species, like the evidence for incomplete lineage sorting, necessitates discretion of any phylogenetic interpretation.

Another interesting pattern concerns the placement of the *A. l. phanus* specimens. The positively identified specimens are from Adams and Franklin Counties in Mississippi and were collected less than 50km apart. A third specimen from Forrest County, MS could only be identified as an *Auturus* female, but was collected within the known range. On the mitochondrial tree, the Franklin and Forrest specimens group with *A. mcclurkini* on a comparatively long branch. The Adams specimen forms a clade with *A. l. louisianus*, which then groups with *A. evides* and *E. cingulatus.* On the ITS tree, the Forrest specimen is sister to the clade containing *E. cingulatus* and the *E. maculatus* from Holmes, FL. Sister to this clade is the Franklin specimen paired with *E. mississippiensis*, a close neighboring species. The Adams specimen resolves as sister to all the remaining specimens.

Ideally, comparing gene trees against a background topology of true species relationships can identify and tease apart phenomena such as incomplete lineage sorting and introgression. As the initial goal of this study was to determine the species relationships, we do not have a foundation on which to pursue such details. We have three phylogenetic trees based on three different datasets and choosing one as representing the true phylogeny is pure conjecture. It is tempting to give the mitochondrial tree more credibility over the ITS tree due to the overall higher nodal support, less discordance between members of the same species, the distinct geographic pattern and its better congruence with the traditional taxonomic breakdown. When all three datasets were combined and analyzed together (not shown), the resulting topology corresponded to that derived from the mitochondrial data alone. The morphological tree is also appealing, especially

due to the monophyly of *Auturus*, but this is based on too few characters to accept with confidence.

Given all the evidence from morphology, genetic sequences and geography, the following conclusions of euryurid relationships can be inferred. *Euryurus amycus* and *E. carolinensis* are sister species, based on their geographic and morphological affinities. They are part of a clade that also includes *E. leachii* and *E. orestes,* which collectively make up the northern most distributed *Euryurus*. With the exception of the position of *A. erythropygos* on the mitochondrial tree and the discordant specimens of *A. evides*, E. *leachii* and *E. orestes* on the ITS tree, *E. carolinensis, E. leachii* and *E. orestes* consistently form a monophyletic group on the gene trees. On the morphological tree, only the presence of *E. maculatus* in the clade challenges this grouping. *Euryurus maculatus* falls outside this clade on both gene trees; its exact position is unclear due to discordance of the different specimens, but it is most likely sister to the north clade. *Euryurus mississippiensis* and *E. cingulatus*, the two most morphologically divergent *Euryurus* species, fall outside this clade, though their positions cannot be determined*; E. cingulatus* falls out first on the mitochondrial tree, *E. mississippiensis* is first on the morphological tree and they fall out together on the ITS tree.

Inferring the relationships among *Auturus* species is even more challenging, given the available information. *Auturus evides* and *A. l. louisianus* appear to be closely allied on both gene trees, although one *A. evides* ITS specimen groups with the sympatric *E. leachii* specimens, probably a result of hybridization. We would expect *A. l. phanus* to group with them, due to its geographic proximity and similar morphology, but as explained earlier, these specimens are highly incongruent both within and between the gene trees. This study also does not support the subspecies status of *A. louisianus*, but any taxonomic adjustment should wait for more thorough

population level data. *Auturus erythropygos* and *A. beckii* are likely sister species as they appear on the morphological tree, additionally supported by their geographic affinity, but they are consistently separated on the gene trees. The remaining *Auturus* species exhibit no consistent patterns among the trees.

5.4.2 Biogeography

Euryurids are endemic to the eastern United States (Figures 5.1, 5.2) and have not been recorded outside this range. They are found almost exclusively in hardwood forests associated with rotting logs (Shelley, 1982b), although they have been encountered in pine forest (Shelley et al., 2012) and caves (McDaniel and Smith, 1976; Shelley, 1982b). Individual species ranges are largely allo- or parapatric. Ranges occasionally overlap, but species rarely co-occur locally (e.g. *A. erythropygos*, *E. carolinensi* and *E. amycus*). Southern Illinois is the only location where two species (*E. leachii* and *A. evides*) are known to be sympatric. Their ranges are also the two largest and the only ones to extend north of the southern borders of Missouri and Kentucky. The ranges and habitat preference of euryurids are largely congruent with the millipede tribe Apheloriini, a hyper-diverse group of the family Xystodesmidae with 17 genera and 106 species (Marek and Bond, 2006, 2007). However, apheloriines generally inhabit leaf litter as opposed to rotting wood.

Many species' ranges are bounded by major rivers. For example, *Auturus l. louisianus* is bounded by the Arkansas River to the north and the Mississippi River to the east. The Mississippi also forms the southern border of *A. l. phanus*, the western border of *A. mcclurkini* and the eastern border of *A. evides* south of where the Ohio River joins. However, there are also several species ranges that span major rivers: *A. evides* and the Mississippi north of the Ohio, *E.*

leachii and the Ohio, and *E. maculatus* and the Appalachiola River are a few marked examples. The Appalachian Mountain range also serves as a significant geographic barrier. Although *E. orestes* is established at the southern end of the mountain range, most of the Appalachians are unoccupied at higher elevations. The western face of the mountains marks the eastern extent of the *E. leachii* range.

Interpreting the significance of euryurid distributions in a historical context requires a sense of the age of the group and the timing of speciation events; unfortunately, fossil information is lacking for this group. We can nevertheless get a very broad estimate if we compare evolutionary rates with similar organisms of known age. The universal mitochondrial arthropod clock of 2.3% per million years (My^{-1}) pairwise divergence (Brower, 1994) has been frequently used in estimating dates of many arthropod taxa, including millipedes (e.g., Bond and Sierwald, 2002; Brewer et al., 2012b), but other studies have revealed a wider range of rates, from 1.5% My^{-1} (Farrell, 2001) to 3.54% My^{-1} (Papadopoulou et al., 2010), both based on COI sequences of beetles. The minimum interspecific uncorrected pairwise distance of COI sequences in this study (ignoring the hypothesized introgression of *A. erythropygos* and *E. leachii*) is 1.3% between *E. carolinensis* and *E. orestes*. If we conservatively estimate a time frame for their split by applying the extreme rates, we get a range of 370–870 thousand years ago (kya). The highest interspecific distance is 8.0%, between *A. lecythanoictes* and *A. mcclurkini,* putting the time frame of their split at 2.3-5.3 million years ago. From this we can conclude with some confidence that the euryurids have existed for at least a few million years, and that the speciation events responsible for the 14 extant species occurred over 370 kya.

One pattern of euryurid distributions that stands out is the extensive northern expansion of *A. evides* and *E. leachii*. At the height of the last glaciation, all euryurid species were likely

confined to small southern refugia, as was true for many temperate life forms (Hewitt, 2004; Soltis et al., 2006). As the ice sheet retreated and northern areas became hospitable, *E. leachii* and *A. evides* were first to colonize these areas, leaving the other species confined to the southern range. Also, the northern-most extent of *A. evides* corresponds with the well-known Driftless Area (Figure 5.2), which remained glacier free during the last glacial maximum. This phenomenon may have reduced the extent by which *A. evides* had to retreat and/or may have allowed an earlier recolonization of the north.

The curiosity of rivers variably forming boundaries may be easily explained. The actual barrier to dispersal may not be the river itself, but the other euryurids established on the opposite bank. The crossing of major rivers may not be uncommon in this group. Flooding events could conceivably float logs containing euryurids across rivers with relative frequency. However, colonizers could be potentially out-competed by established species or possibly even assimilated into the population. The areas where rivers form hard boundaries tend to be in the south. The ability of *A. evides* to establish across the Mississippi and *E. leachii* across the Ohio may simply be due to the absence of other euryurids at the time of their range expansion. Sympatry of different euryurid species is known only in southern Illinois where *E. leachii* and *A. evides* are both found. This may be a recent event coinciding with their post-glacial expansions, and exclusion of one species has yet to result.

Several studies utilizing more extensive genetic data have revealed the significance of recent glaciations on the genetic structure of different organisms in the eastern United States (Church et al., 2003; Heilveil and Berlocher, 2006; Howes et al., 2006; Walker et al., 2009a). The last two glaciations in North America were the Wisconsin Episode, which lasted from 60 to 12.5 kya, and the Illinois Episode, which lasted from 190 to 130 kya (Curry et al., 2011). The

Pre-Illinoian period (1,800 – 190 kya) is less understood, but had at least two major glaciation episodes (Curry et al., 2011). Advancing ices sheets forced populations into small southern refugia, often resulting in bottlenecks. Retreating ice sheets opened up new areas for colonization, while the melt water filled drainage basins, fragmenting populations and forming barriers to gene flow. Although speciation of euryurids predates at least the last two glaciations, there have been numerous advances and retreats in the group's several million year history. The current distributions and genetic structures of extant euryurids have been largely influenced by the complex dynamics of a minimum of four glacial cycles. Any attempt to determine how more ancient geological events contributed to their radiation would require application of a more precise molecular clock developed specifically for this group.

5.4.3 Conclusions

The most significant result of this study is the evidence for ongoing, or at the least, recent gene exchange among euryurid species. The Euryuridae is a group with distinct, geographically stable morphologies, and yet may still be in an incipient stage of speciation. While we think hybridization between current neighboring species is plausible, the hybridization events evident here could have occurred at a time when morphological differences were less severe. Additional research is required if a robust phylogenetic hypothesis of the Euryuridae is to be proposed. Faster evolving molecular markers such as microsatellites may be better suited if hybridization events are ancient enough.

6. The millipedes of the North American family Euryuridae (Polydesmida): Taxonomic history, morphological atlas and species compendium.

6.1 Introduction

The arthropod order Diplopoda is a richly diverse (ca. 12,000 described species), ecologically important group of terrestrial invertebrates. Unfortunately, it is also one of the least understood animal groups in terms of natural history, systematics and ecology. Taxonomic problems are especially abundant. The current classification scheme seems oversplit, with 68% of genera containing only 1 or 2 species, and many species have received no taxonomic attention beyond their original description (Sierwald and Bond, 2007). The family Euryuridae Pocock, (1909), is one of the better studied groups of millipedes, with its two genera, *Auturus* Chamberlin, (1942) and *Euryurus* Koch, (1847a) having been revised by Shelley (1982b) and Hoffman (1978), respectively. Additional significant work was added later by Hoffman (1998) and Jorgensen (2009). Here, we build on the previous work by collecting together for the first time a complete taxonomic history, a morphological study including SEM images of all species, and complete citations and diagnoses for each species.

6.2 Taxonomic history / Literature review

A complete account of the taxonomic history is not only interesting, but also quite relevant, as taxonomic placement implies hypotheses of relationships. The taxonomic history of the family Euryuridae, and the species that would find their way into it, was quite unstable in the beginning. Classifications changed frequently and were given little justification. Often,

justification consisted of merely citing ambiguous affinities without any specific character information.

Much of the history of Euryuridae has been covered in the past (Hoffman, 1954; Hoffman, 1978; Jorgensen, 2009; Shelley, 1982b), but never in its entirety in one place. The following is an attempt to gather all pertinent information from the literature on Euryuridae, including the ca. 30 non-euryurids that were at some point assigned to *Euryurus*. For the most part, information is presented chronologically and, to avoid confusion, all species currently in the Euryuridae are underlined in this section. The current classifications of the formerly associated species are presented in Table 6.1.

Family **Euryuridae** Pocock

Euryurinae Pocock, 1909: 147.

Euryurini Brolemann, 1916: 584 -- Hoffman, 1980: 164.

Euryuridae Chamberlin, 1918: 249 -- Euryuridae Hoffman, 1998: 136

Genus ***Euryurus*** Koch

Euryurus Koch, 1847: 38. Type species: *E. maculatus* Koch, by direct substitution and synonymy (Hoffman, 1978).

Eutheatus Attems, 1938: 294

Singuliurus Causey, 1955: 23. Type species: *S. mississippiensis* Causey, by original designation.

Genus ***Auturus*** Chamberlin

Auturus Chamberlin, 1942: 7. Type species: *A. phanus* Chamberlin, by original designation.

In 1832, T. E. Gray described the species *Polydesmus leachii* from an unspecified locality. The description consisted of three simple drawings (the entire specimen in dorsal aspect, the head, and the gonopods) and the words "Grey, with yellow spots" next to the index entry. Brandt (1839) very briefly described *Polydesmus erythropygos*, with the type locality given only as North America. Koch (1847a) created the new genus *Euryurus* to accommodate his new species, *E. maculatus*, *E. margaritaceus* and *E. squamatus*, of which he cited unknown localities. He proposed short, posteriorly pointed paranota, a broad epiproct, and ozopore position as genus defining characters. A more detailed description of *E. maculatus*, including drawings, was later published (Koch, 1863: 7, plate 3, figures 8a, 8b). *Polydesmus carolinensis* from South Carolina was described by DeSaussure (1859b) and included in his new subgenus *Paradesmus*.

Peters (1864b) reduced *Euryurus* to subgenus within *Polydesmus*, transferring part of *Paradesmus* to it. He described the new species *P. erythropus* of unknown locality, *P. ater* of Venezuela, *P. tricuspidatus* of Guinea and *P. flavomarginatus* of unknown locality in *Euryurus* and also placed *P. erythropygus* (sic)[1], *P. dealbatus* Gervais, 1847 of Colombia, *P. polygonatus* Gervais, 1847 of Colombia, *P. klugii* Brandt, 1839 of Mexico and *P. erichsoni* Brandt, 1839 of Mexico in the subgenus. *Euryurus maculatus* and *P. carolinensis* were synonymized with P. *erythropygus*. Later, Peters (1864a) described several more species of *Euryurus*: *P albocarinatus, P. fumigatus, P. tripunctatus, P. uncinatus, P. semicinctus, P. areatus, P. hybridus* and *P. taenia*, all from South America. He also renamed *P. erythropus* as *P. callipus*, as the former name was preoccupied by *P. erythropus* Lucas, 1858 (Chelodesmidae).

Humbert and DeSaussure (1869) moved *P. tricuspidatus* and *P. flavomarginatus* to a new subgenus *Oxydesmus* and *E. margaritaceus, E. squamatus* and *P. klugii* to a new subgenus

[1] This spelling of would occur more commonly in the literature until Hoffman (1978).

Pachyurus. DeSaussure and Humbert's (1872) catalogue listed 12 species under subgenus *Euryurus: erythropygus, dealbatus, albocarinatus, fumigatus, tripunctatus, uncinatus, semicinctus, areatus, hybridus, polygonatus, taenia* and *callipus*. *Polydesmus ater* and *P. erichsoni* were moved to subgenus *Pachyurus*. All of these subgenera were subsequently elevated to full genus level by Latzel (1884). Meanwhile, *Euryurus pallipes* from Japan was described by Koch (1877).

Bollman (1887a) described *Paradesmus evides* from Minnesota, comparing it with *P. erythropygus*, although it is not clear whether the "*P*" stands for *Paradesmus* or *Polydesmus*. Regardless, he soon listed the latter species as *Euryurus erythropygus* (Bollman, 1887b), the first time the species was associated with the generic name at full rank, and later he transferred *P. evides* to *Euryurus* as well (Bollman, 1888c). Bollman (1888b) also described *Euryurus erythropygus australis* of Georgia, United States, whose specific/subspecific status would mysteriously fluctuate in subsequent publications. *Euryurus flavocarinatus* Daday, 1889 of Mexico, *E, devillei* Silvestri, 1897 of Ecuador, *E. flavocarinatus* (again) Silvestri, 1898 of Colombia, *E. melanostigma* Silvestri, 1898 of Colombia and *E. atratus* Pocock, 1900 of British Guiana were the last species described in *Euryurus* in the 19th century.

In his System der Polydesmiden II, Attems (1899) provided the first comprehensive treatment of the genus *Euryurus*. In it, he described *E. aterrimus* of Venezuela and *E. glaphyros* of Costa Rica and listed the 12 species from DeSaussure and Humbert's (1872) catalogue plus the new species of Koch, Daday and Silvestri. He listed Bollman's *E. evides* but not *E. erythropygus australis*. *Euryurus maculatus* and *P. carolinensis* were still listed as synonyms of *erythropygus*. Of the 20 species attributed to *Euryurus* by Attems, only *erythropygus* and *evides* are euryurids by current definition. Brölemann (1904) described the Brazilian species, *Euryurus*

elongatus and *E. octocentrus.* Curiously, both actually belonged in *Aphelidesmus*, a genus Brölemann himself erected six years prior (1898a). He even went so far as to compare them with the type species, *Aphelidesmus hermaphroditus*, which he referred to as "*Eury. hermaphroditus*".

Pocock (1909) divided the polydesmidans, which up to this point were a single family, into several different families. He placed all *Euryurus* species into their own subfamily Euryurinae, assigned to the family Platyrhacidae. Within Euryurinae, all South American *Euryurus* species were transferred to *Aphelidesmus* (*E. dealbatus* was already transferred by Brölemann), and *Amplinus* and *Polylepiscus* were also included in the subfamily. This left only *E. pallipes* of Japan, Daday's *E. flavocarinatus* of Mexico and the three United States species in the genus *Euryurus*. Pocock made no mention of *E. pallipes* or *flavocarinatus*; indeed, these species appear to have received no attention since their descriptions and are *incertae sedis.* Of the United States species, Pocock mentioned *E. erythropygus* and *E. australis* (but not *E. evides*) and claimed these were the only two species that belonged in this genus, making *Euryurus*, for the first time, exclusive in the modern sense. Carl (1914) summarized more clearly the changes made by Pocock (1909) by listing most of the South American species individually under either *Aphelidesmus* or his new genus *Pycnotropis*. He reiterated the status of *Euryurus*, naming *E. erythropygus* and *E. evides*, but did not mention *australis.* No mention was made of *albocarinatus, atratus*, or *callipus*, but these three would end up formally assigned to Aphelidesmidae.

The subfamily Euryurinae was later reduced to tribal status by Brölemann (1916) and then appeared at full family level in Chamberlin (1918a). That same year, Chamberlin (1918b) described *E. louisiana* from Louisiana, United States.

In his voluminous monograph on Polydesmida, Attems (1938) proposed changing *Euryurus* to *Eutheatus,* thinking *Euryurus* was preoccupied by *Euryurus* Rafinesque, 1815 (Annelida). This change was not always recognized by subsequent authors and was formally reversed by Hoffman (1954) when he determined that *Euryurus* Rafinesque was a *nomen nudum*. Additionally, for whatever reason, Attems omitted much of the aforementioned revisionary work. *Eutheatus* was listed under the family Platyrhacidae and, although he correctly listed all the recognized species of the time (*eyrthropygus*, *australis*, *evides* and *louisiana*), he also listed *E. maculatus*, *E. margaritaceus*, *E. squamatus* and *E. pallipes*. However, *E. maculatus* was still listed as a synonym of *E. erythropygus*, and *E. margaritaceus* and *E. squamatus* had been placed in *Pachyurus* seventy years prior (Humbert and De Saussure, 1869).

(The rest of this narrative will only mention true euryurids, so underlining of species names has ceased). Chamberlin (1942) proposed the genus *Auturus* under Euryuridae to accommodate his new species from the southern United States, *A. phanus* from Louisiana, *A. mimetes* from Missouri, *A. dixianus* from Louisiana, *A georgianus* from Georgia and *A. scotius* from Louisiana, and the older species, *E. evides* and *E. louisiana*. He remarked on their relatedness to *Euryurus*, citing similarities in paranota, tergite texture, ozopore position and epiproct shape as shared characters. He distinguished *Auturus* from *Euryurus* by the form of the gonopods. Loomis (1943) described *Euryurus falcipes* from Florida and formally elevated *E. australis* to full species status. The recognition of *E. falcipes* was due to a misinterpretation of Bollman's *E. australis* description (1888b). Bollman described the "upper" branch of the gonopod as five-times longer than the "lower", but due to the different position presented by Loomis (1943, fig. 15), the lower branch is the longer. This misunderstanding was later discovered by Hoffman and the names were synonymized (Hoffman, 1951). Five new euryurid species were described in the 1950's:

Auturus florus Causey, 1950 from Arkansas, *Auturus becki* Chamberlin, 1951 from Florida, *Eutheatus aculeatus* Causey, 1952 from Illinois, *Auturus mcclurkini* Causey, 1955 from Tennessee and *Singuliurus mississippiensis* Causey, 1955 from Mississippi. For the latter, Causey erected a new genus due to the simple nature of the gonopods. She (Causey, 1955) also listed *A. florus* as a synonym of *A. evides*.

In 1954, Hoffman proposed that Euryuridae contain three subfamilies: Aphelidesminae, Amplininae and Euryurinae, the latter being the first exclusive familial grouping of euryurids by todays definition. Later, the first euryurid to be described, *Polydesmus leachii* Gray, 1832, which had been largely neglected for 125 years, was revived by Hoffman and Browning (1956), who examined the holotype, placed it properly in *Euryurus* and noted that *E. aculeatus* actually refers to this species. In 1958, Chamberlin and Hoffman published their *Checklist of the Millipeds of North America,* which included a complete listing of the species of Euryurinae. *Euryurus* included three species: *australis, erythropygus* and *leachii*; *Auturus* included ten species: *becki, dixianus, evides, florus* (they evidently overlooked Causey's synonymization), *georgianus, louisianus, mcclurkini, mimetes, phanus* and *scotius*; and the monotypic *Singuliurus* included *mississippiensis*.

A fourth euryurid genus, *Illiniurus*, was created by Shear (1968) to accommodate his new species *I. beattyi* from southern Illinois. However, this genus and species are likely based on an aberrant specimen and are now considered invalid (Jorgensen, 2009).

In 1975, Hoffman proposed reducing Euryuridae back to subfamily level within Platyrhacidae, with the three former subfamilies becoming tribes. He continued this classification in his seminal revision of the genus Euryurus (Hoffman, 1978). Here, he referred *E. erythropygos* (properly spelled) to *Auturus* and synonymized *A. georgianus* with it. *Euryurus*

maculatus and *E. carolinensis* were resurrected from over 100 years of synonymy with *A. erythropygos* and each restored to full species status. *Euryurus australis* was synonymized with *E. maculatus*, and *E. falcipes* was also listed as a new synonymy, even though this had already been synonymized with *E. australis* (by Hoffman, 1951). He also described three new species: *E. orestes* from North Carolina, *E. cingulatus* from Alabama and *E. amycus* from North Carolina. *Singuliurus* was synonymized with *Euryurus*, and *E. leachii* was divided into subspecies: *E. leachii leachii* and *E. leachii fraternus*.

In 1982, Shelley followed suit with an excellent revision of the genus *Auturus*. In it, Shelley recognized four species: *A. erythropygos, A. evides, A. louisianus* and A. *mcclurkini*. *Auturus becki* was reduced to a subspecies of *A. erythropygos*, and *A. phanus* to a subspecies of *A. louisianus*. *Auturus mimetes* was synonymized with *A. evides*, and *A. dixianus* and *A. scotius* were synonymized with *A. louisianus phanus*.

Later, Hoffman (1998) reassessed the Platyrhacidae and concluded that the Euryurini actually have a closer affinity to the polydesmidan family Xystodesmidae, in particular, the Mediterranean genus *Melaphe* Cook, 1904. He reinstated the family Euryuridae, now containing exclusively the genera *Auturus*, *Euryurus* and *Illiniurus* of the eastern United States. Later, Shelley (2002), grouped Euryuridae, Xystodesmidae, Oxydesmidae, Gomphodesmidae and Eurymerodesmidae together as the superfamily Xystodesmoidea.

A new species, *E. lecythanoictes* from Alabama, was described by Jorgensen (2009) and later transferred to *Auturus* (in press). *Euryurus leachii fraternus* was synonymized with *E. l. leachii*, and *A. louisianus louisianus* and *A. l. phanus* were restored to full species status (Jorgensen et al., 2013). Currently, the Euryuridae consists of 13 species: 7 *Euryurus* and 6 *Auturus* (1 sub-divided).

species	locale	author	original genus	current genus
albocarinatus	SA	Peters 1864a	*Polydesmus (*sub *Euryurus)*	*Aphelidesmus*
areatus	SA	Peters 1864a	*Polydesmus (*sub *Euryurus)*	*Aphelidesmus*
ater	SA	Peters 1864b	*Polydesmus (*sub *Euryurus)*	*Polydesmus*
aterrimus	SA	Attems 1899	*Euryurus*	*Aphelidesmus*
atratus	SA	Pocock 1899	*Euryurus*	*Aphelidesmus*
callipus	?	Peters 1864a	*Polydesmus (*sub *Euryurus)*	*Haematotropis*
dealbatus[1]	SA	Gervais 1847	*Polydesmus*	*Aphelidesmus*
devillei	SA	Silvestri 1897	*Euryurus*	*Pycnotropis*
elongatus	SA	Brolemann	*Euryurus*	*Aphelidesmus*
erichsoni[1]	Mexico	Brandt 1839	*Polydesmus*	*Amplinus*
erythropus	?	Peters 1864b	*Polydesmus (*sub *Euryurus)*	ex *E. callipus*
flavocarinatus	Mexico	Daday 1889	*Euryurus*	*incertae sedis*
flavocarinatus	SA	Silvestri 1898	*Euryurus*	*Pycnotropis*
flavomarginatus	?	Peters 1864b	*Polydesmus (*sub *Euryurus)*	*Coromus*
fumigatus	SA	Peters 1864a	*Polydesmus (*sub *Euryurus)*	*Aphelidesmus*
glaphyros	C. Rica	Attems 1899	*Euryurus*	*Aphelidesmus*
hermaphroditus[3]	SA	Brolemann	*Aphelidesmus*	*Aphelidesmus*
hybridus	SA	Peters 1864a	*Polydesmus (*sub *Euryurus)*	*Aphelidesmus*
klugii[1]	Mexico	Brandt 1839	*Polydesmus*	*Amplinus*
margaritaceus	?	Koch 1847	*Euryurus*	*Pachyurus*
melanostigma	SA	Silvestri 1898	*Euryurus*	*Pycnotropis*
octocentrus	SA	Brolemann	*Euryurus*	*Haematotropis*
pallipes	Japan	Koch 1877	*Euryurus*	*incertae sedis*
polygonatus[1]	SA	Gervais 1847	*Polydesmus*	*Colomborus*
semicinctus	SA	Peters 1864a	*Polydesmus (*sub *Euryurus)*	*Aphelidesmus*
squamatus	?	Koch 1847	*Euryurus*	*Pachyurus*
taenia	SA	Peters 1864a	*Polydesmus (*sub *Euryurus)*	*Pycnotropis*
thomsonii[2]	Africa	Lucas 1858	*Polydesmus*	*Coromus*
tricuspidatus	Africa	Peters 1864b	*Polydesmus (*sub *Euryurus)*	*Coromus*
tripunctatus	SA	Peters 1864a	*Polydesmus (*sub *Euryurus)*	*Aphelidesmus*
uncinatus	SA	Peters 1864a	*Polydesmus (*sub *Euryurus)*	*Aphelidesmus*

Table 6.1. All species formerly attributed to *Euryurus.* SA = South America. [1]Assigned to subgenus *Euryurus* by Peters 1864b. [2]Referred to *Euryurus* by Karsch 1879. [3]Mentioned as "*Eury. hermaphroditus*" in Brolemann 1904b

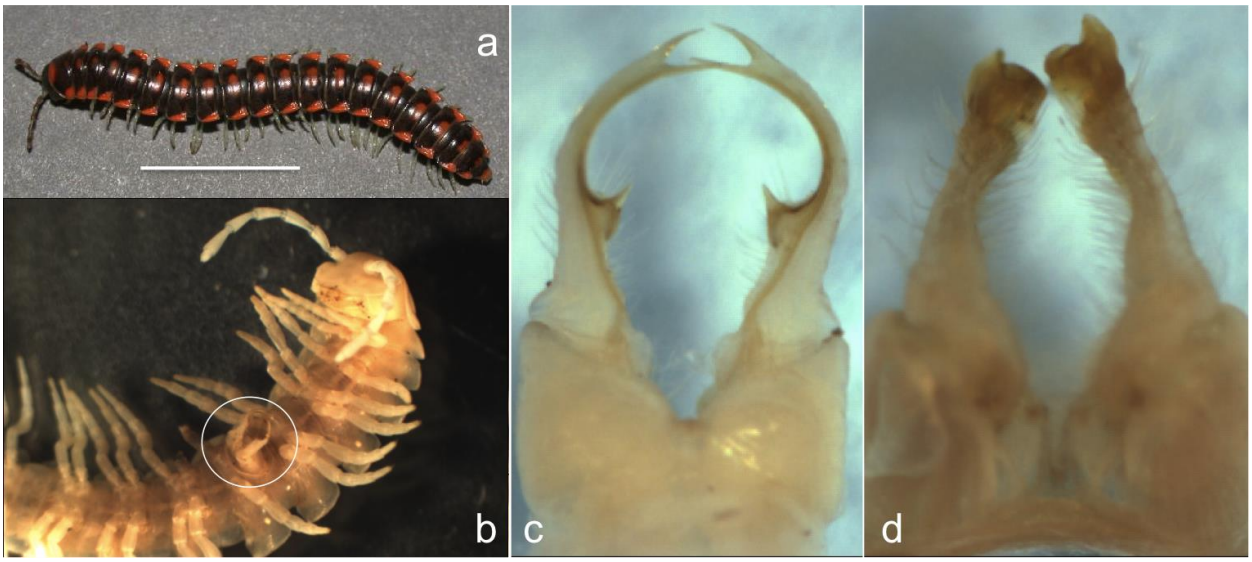

Figure 6.1. a) Live specimen of *Euryurus leachii*. Scale bar = 1cm. b) ventral aspect of preserved *E. leachii* specimen, gonopods circled. c) gonopods of *E. leachii*. d) gonopods of *Auturus evides*. *E. leachii* photograph (a) courtesy of Dr. Paul Marek.

6.3 Characters of Euryuridae

All Euryuridae species are largely indistinguishable in somatic characteristics. The following descriptive section applies to the non-sexual traits of all species and recounts much of what has already been reported by Hoffman (1978) and Shelley (1982b). Euryurids are composed of 20 body rings plus the head. In life, the dorsal surface is very dark with bright orange on the tips of the paranota and mid-posterior of each metazonite (Figure 6.1a). Yellowish speckling occurs on much of the darker areas. The ventral surface is yellowish except for the orange paranota tips. Old, alcohol preserved specimens are entirely yellowed (Figure 6.1b). The dorsal surface is smooth, moderately convex, with paranota extending distinctly laterally (Figure 6.2a). Paranota at posterior end are angled acutely caudad. Ozopores open laterally at the tip of

the paranotum (Figure 6.2b) in the usual polydesmidan pore formula (rings 5, 7, 9-10, 12-13, 15-19). Sterna (Figure 6.2c) and legs (Figure 6.2d) are simple with no uncommon structures.

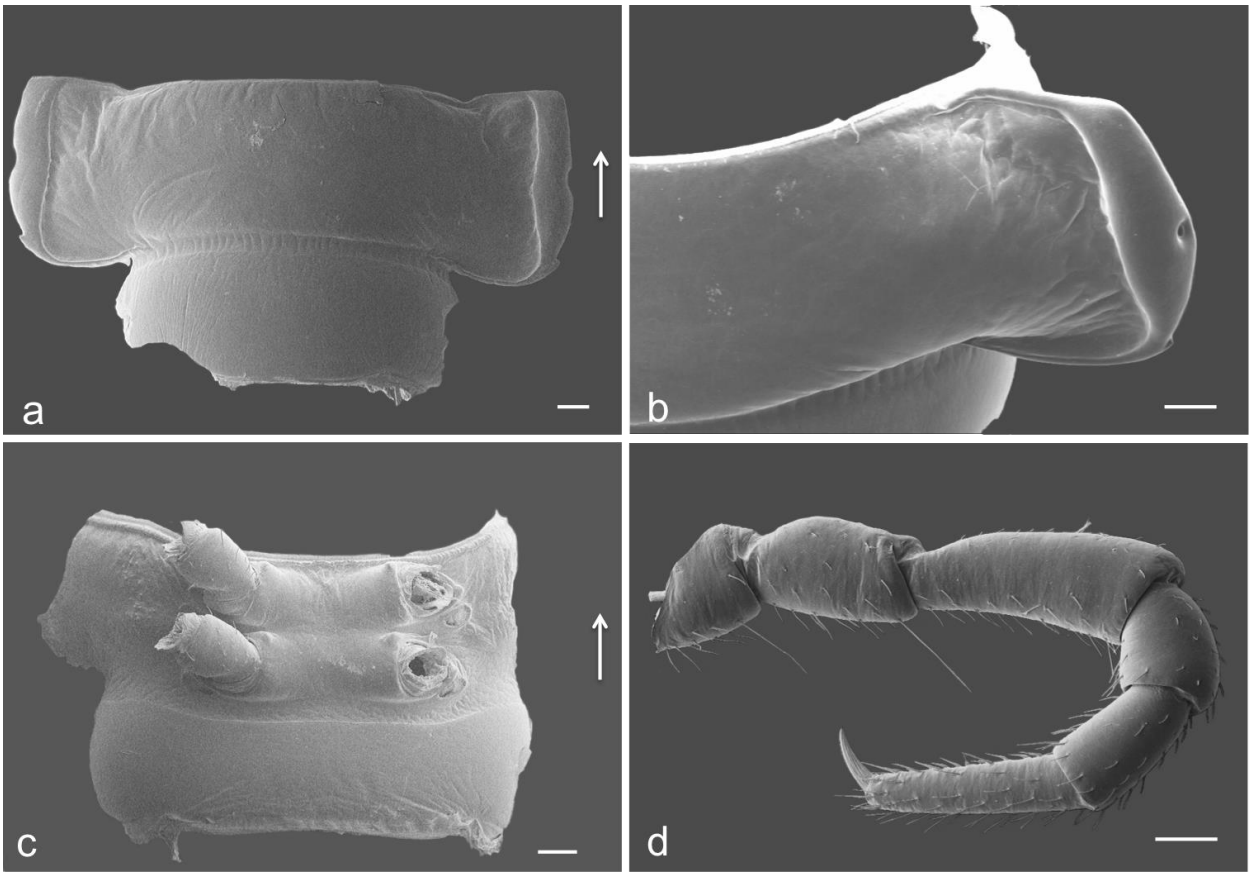

Figure 6.2. *Auturus evides*. a) dorsal view of 10th segment. b) right paranotum of 10th segment showing position of ozopore. c) ventral view of 10th segment. d) leg of 10th segment. Scale bars = 200μm. Arrows indicate anterior direction.

Collum is relatively large, though extending no wider than ensuing tergites, with lateral ends depressing downward. Head surface is smooth with evident epicranial suture (Figure 6.3a). Facial setae pattern: subantennal 1-1, frontal 1-1, genal 2-2, clypeal ca. 6-6, labral ca. 10-10. Antennae are long (ca. 3 mm) with antennomeres 2-6 distally clavate and subequal in size and

shape (Figure 6.3b). Gnathochilarium (Figure 6.3c) and mandibles (Figure 6.3d) are typical polydesmoid form. Telson (Figure 6.4a): hypoproct is elliptical with 1 pair of setae near its caudal margin. Paraprocts have 2 pair of setae, the posterior-most pair closer to the medial margin. Epiproct is subquadrate and very broadly spatulate. Gonopods extend through a large ovoid aperture (Figure 6.4b) on seventh segment. Gonopores open at distal end of small tubular processes (gonapophyses) on the coxa of the second leg pair (Figure 6.4c). Adult body length is typically 25-30mm and width 3-5mm. Size is somewhat variable intraspecifically, and larger specimens tend to be female.

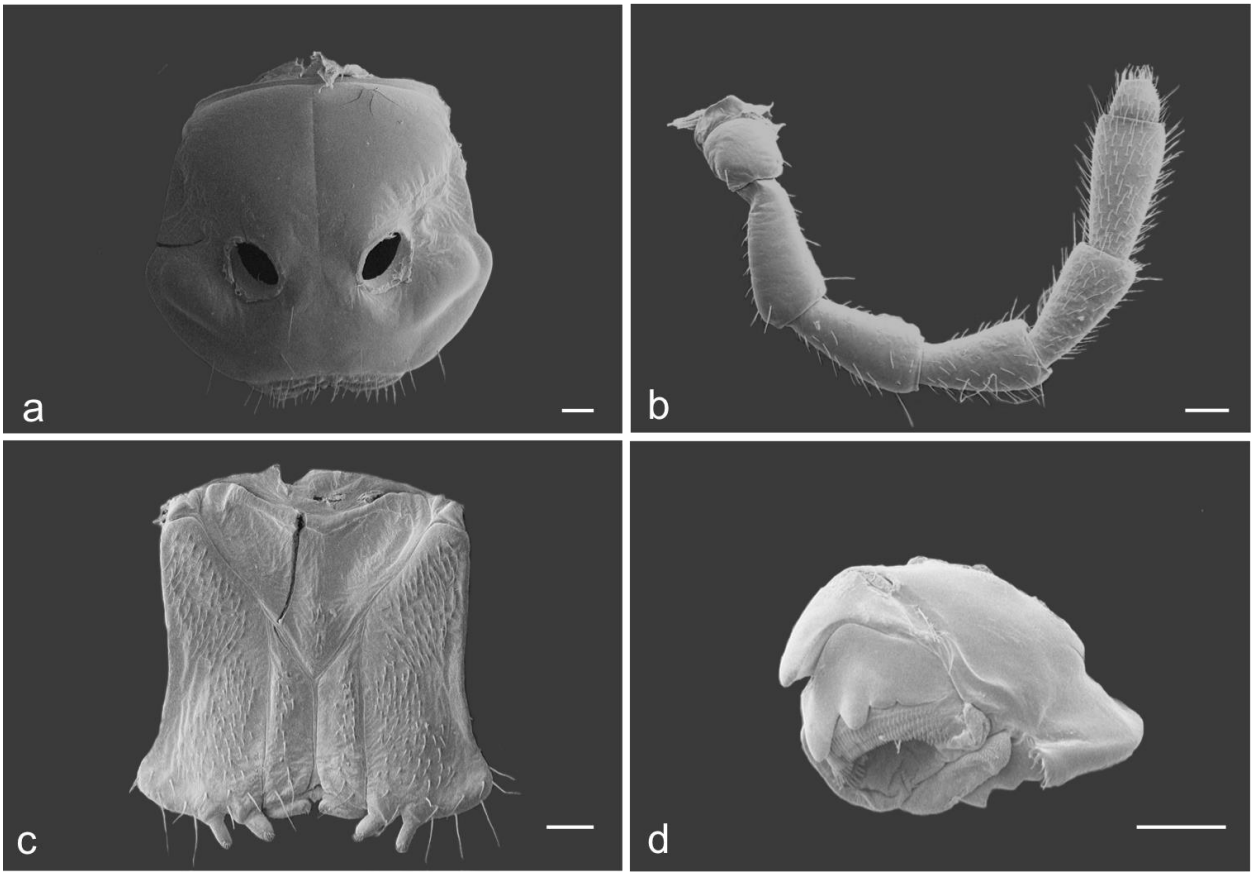

Figure 6.3. *Euryurus leachii* (a,c) and *Auturus evides* (b,d). a) dorsal head plate. b) antenna. c) gnathochilarium. d) mandible. Scale bars = 200μm.

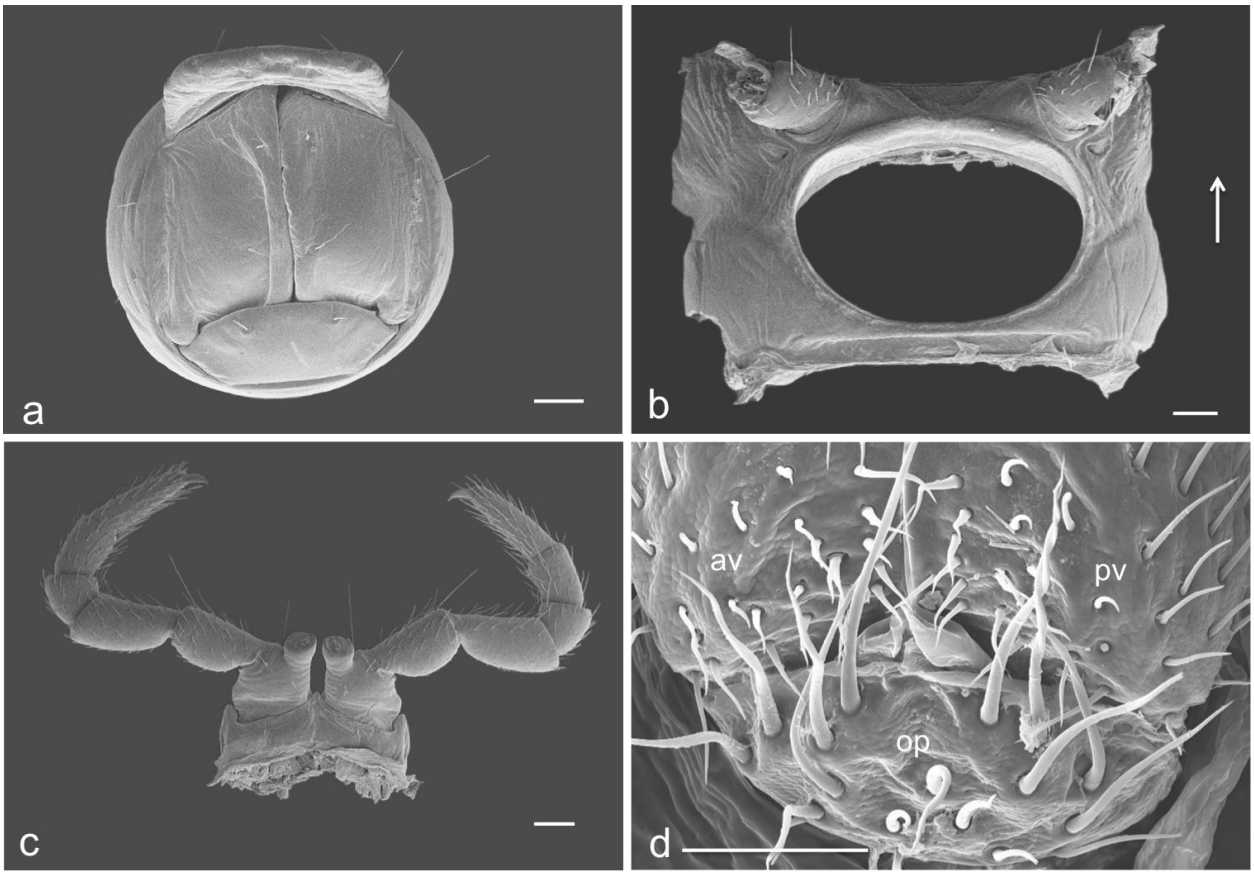

Figure 6.4. *Auturus evides*. a) telson. b) ventral view of 7th segment showing gonopod aperture. c) *Euryurus leachii*. second leg pair showing gonapophyses, ventro-caudal aspect. d) *A. becki.* base of cyphopod showing position of operculum (op). av - anterior valve, pv - posterior valve. Scale bars = 200μm.

6.3.1 Generic distinction / cyphopod anatomy

Auturus and *Euryurus* have traditionally been distinguished from each other based on gonopod anatomy. Species of *Euryurus* have gonopods with significantly elongated, acicular solenomeres, and *Auturus* solemomeres are short and broad (Figure 6.1c-d). However, this distinction was recently challenged by the discovery of the new species *A. lecythanoictes.* This species' gonopods fit neither description, being elongated, yet broad and acuminate. Originally the species was assigned to *Euryurus* based mainly on the elongated solenomere. However, after

a SEM survey of the cyphopods of all species, it was confirmed that there is a distinct dichotomy in cyphopod anatomy, as first proposed by Hoffman (1978). The cyphopods of *lecythanoictes* were of the *Auturus* type and the species was promptly transferred. At this time, the cyphopod distinction is the only definitive character for genus delimitation, although gonopod anatomy, in most cases, is reliable. Further study with multiple specimens and precise measurements may reveal interspecific differences in cyphopod characteristics, as hypothesized by Hoffman (1978).

As in all polydesmidans, the cyphopods (Figures 6.4c and 6.5) are just located posterior to the second leg pair and are extended through a small aperture during use. Each cyphopod is composed of four sclerites, the basal operculum, a pair of valves and the mesal receptacle (terminology, sensu Hoffman 1978). The paired valves form the core of the structure, with the reproductive opening at the distal end. The operculum is firmly attached to the paired valves at the base (Figure 6.4c). The receptacle is loosely positioned mesally to the paired valves and is articulated to cover the reproductive opening. The valves and operculum are quite hirsute, with especially long setae of the operculum

The difference between the genera lies in the ventral surface where the valves meet. In *Euryurus* species, the valves are not fused, and a rift runs between them along the entire length of the ventral surface (Figure 6.5d). *Auturus* valves are fused for most of the length, except distally where a large orifice containing the reproductive opening is positioned. The distal margin of the paired valves forms a lamina that partly covers the orifice (Figure 6.5b).

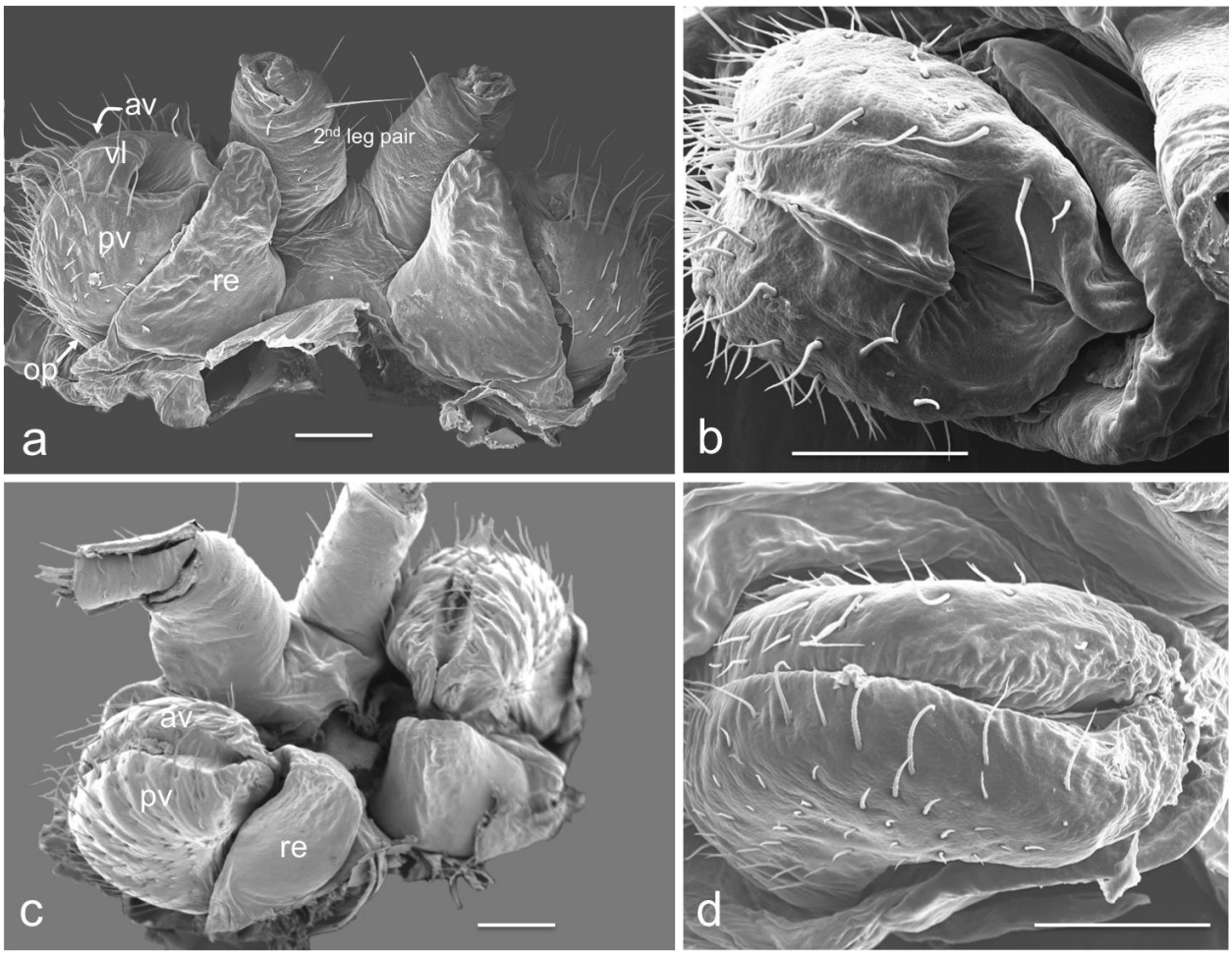

Figure 6.5. a) *Auturus becki*, 2nd sternum with cyphopods, ventro-caudal aspect. b) *A. becki*, right cyphopod, ventro-lateral aspect. c) *Euryurus leachii*, 2nd sternum with cyphopods, ventro-caudal aspect. d) *E. maculatus*, right cyphopod, ventro-lateral aspect. av – anterior valve, pv – posterior valve, vl – ventral lamina, op – operculum, re – receptacle. Scale bars = 200μm.showing position of operculum (op). av - anterior valve, pv - posterior valve. Scale bars = 200μm.

6.3.2 Gonopod morphology

The base of the femoral margin is raised into a semi-circular ridge that partially surrounds the cannular opening (Figure 6.6a). The cannular opening closes distally to form the origin of the prostatic groove (Figure 6.6a), which runs mesially along the entire length of the telopodite and terminates at the very tip of the solenomere in *Euryurus* (Figure 6.6c) and just short of the margin of the solenomere in *Auturus* (Figure 6.6b). The proximate half of the prefemur is robust and tubular then abruptly flattens on the mesal side to form the prefemoral concavity in several species (Figure 6.6a). The border between the prefemur and the acropodite (a.k.a. femur) runs somewhat longitudinal at varying degrees depending on species. This border is very clear due to the dark, highly sclerotized nature of the acropodite and the pilosity of the prefemur (Figure 6.1c-d). At the distal end of the prefemur, a variably hirsute protrusion, the distal prefemoral knob, is present in several species (Figure 6.6b-c). Adjacent to this knob in several species is another structure called the femoral basal lamella (Figure 6.6b-c), which Hoffman (1978) termed with reference to *Euryurus* species only.

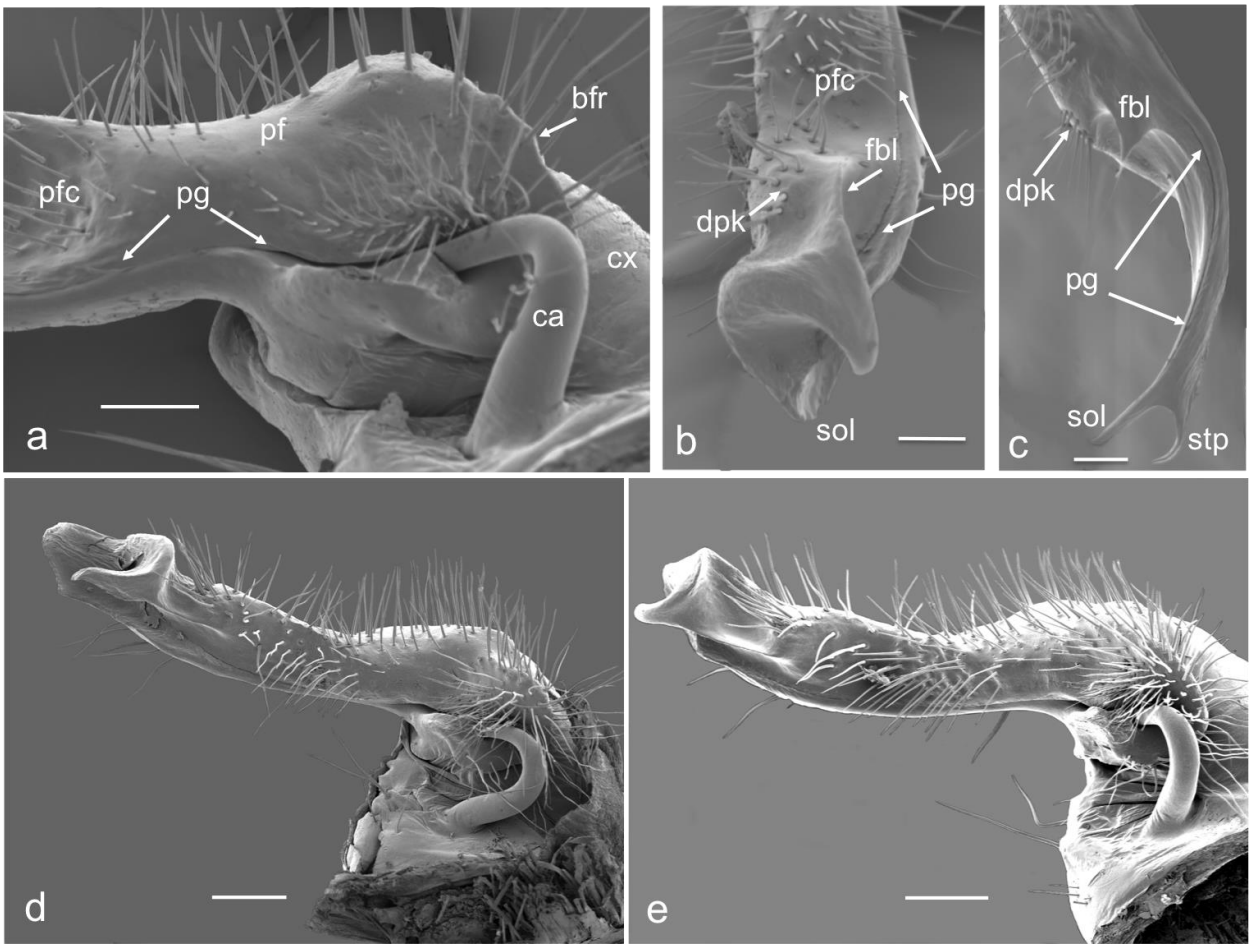

Figure 6.6. a) basal characters of the euryurid gonopod (*E. orestes*). b) distal character of *Auturus* gonopod (*A. evides*). c) distal characters of *Euryurus* gonopod (*E. leachii*).
d) *A. becki*, left gonopod, mesal aspect. e) *A. erythropygos*, left gonopod, mesal aspect. pfc - prefemoral concavity, pg - prostatic groove, pf - prefemur, ca - cannula, bfr - basal femoral ridge. Scale bars a, b, c = 100μm, d, e = 200μm.

Here, homology is proposed with a structure in *Auturus* based on morphological similarity and location. In Auturus, the lamella is flattened at the base and forms a distal digitiform process (Figure 6.6b), here termed the lamellar process (the tibiotarsus of Shelley 1982). The extremely different acropodites between *Euryurus* and *Auturus* make this homology obscure, but it becomes clear with SEM images in the proper orientation. In *Euryurus*, the

acropodite is significantly elongated, and the solenomere is acicular, with most species also possessing an adjacent subterminal process, either acicular or slightly spatulate (Figure 6.6c). *Auturus* acropodites are very short, and the solenomeres are broad and flattened which form a calyx with the lamellar process (Figure 6.6b). The position of the calyx "opening" between the solenomere and lamellar process varies among species. Details on specific differences are given in the diagnoses of each species below.

The phenomenon of such extreme variability in gonopod anatomy among species otherwise identical in appearance warrants some discussion. Sexual selection has been hypothesized to lead to speciation events through several mechanisms (Panhuis et al., 2001). An interesting result of this can be the apparent rapid and divergent evolution of male reproductive organs. Eberhard (1985a) attributed this to cryptic female choice and male-male genitalic competition. Another popular hypothesis for genital diversity is the "coevolutionary arms race" between sexes, prompted by competing reproductive interests (Arnqvist and Rowe, 2002). These ideas are based on the assertion that, in these particular cases, the male genitalia function as more than just gamete dispensers, but contribute to the reproductive success of the male through secondary roles such as female stimulation and hindrance of fertilization by other males. Intense selection on these secondary functions can result in relatively rapid change if individual survival is not at risk. Removal of rival sperm from previous matings with scoop-like processes is one secondary function that has been well studied, especially in Odonata (Córdoba-Aguilar et al., 2003), and has also been demonstrated in millipedes of the order Spirostreptida (Barnett and Telford, 1996; Barnett et al., 1993). The shapes of the subterminal and lamellar processes of some euryurids hint at this function, but this possibility has yet to be explored. Millipedes of the polydesmid genus *Parafontaria* have been observed inserting their gonopods into the female before charging them with sperm (Tanabe and Sota, 2008). However, this behavior was attributed to conspecific mate recognition or female

stimulation and not to sperm removal. The especially long solenomere of some euryurids may also be advantageous by placing sperm deeper within the spermathecae. Since fertilization does not occur until the egg is oviposited, sperm deposited deeper may have first contact with the egg and enhance reproductive success of longer males.

6.4 Species of Euryuridae

The following list includes complete citations, type specimen information and diagnoses of gonopod anatomy. Much of the citation, type specimen and distribution information comes from Chamberlin and Hoffman (1958), Hoffman (1978; 1999) and Shelley (1982a, b). The diagnoses use new terminology based on the findings of the SEM survey and are meant to compliment those of Hoffman (1978) and Shelley (1982b). Depository institution abbreviations: AMNH - American Museum of Natural History, New York; ANSP - Academy of Natural Sciences, Philadelphia; BMNH - British Museum (The Natural History Museum), London; FMNH - Field Museum of Natural History, Chicago; MCZ - Museum of Comparative Zoology, Harvard University; MHNG - Muséum d'Histoire Naturalle, Genève; USNM - United States National Museum, Washington; VMNH - Virginia Museum of Natural History, Martinsville; ZMB - Zoologisches Museum der Humboldt-Universität, Berlin.

Auturus becki Chamberlin

Auturus becki Chamberlin, 1951: 29, fig. 2 (O.D.), syntypes, 3 male, 1 female (USNM) from along Suwanee River in Florida.

Auturus erythropygos becki: Shelley, 1982: 3262, figs. 23-24 (new status) – Hoffman, 1999: 289 -- McAllister and Shelley, 2005: 187 – Jorgensen et al., 2013 (returned to full species).

Distribution: Northern Florida: Columbia, Hamilton, Suwanee and Levy Counties.

Diagnosis: Distal margin of solenomere significantly elongated. Prefemoral concavity indistinct. Distal prefemoral knob distinct. Lamellar process pointed and distally oriented.

Auturus erythropygos (Brandt)

Polydesmus erythropygos Brandt, 1839: 313 (O.D.), male lectotype (ZMB) from "America boreali", probably in the vicinity of Georgetown, South Carolina (Shelley, 1982b) – Brandt, 1841: 134 – Gervais, 1847: 106 – DeSaussure, 1860: 38.

*Polydesmus (*sub *Euryurus) erythropygus* [*sic*]: Peters, 1864b: 541.

Euryurus maculatus: Peters, 1864b: 541 (synonymized).

Polydesmus carolinensis: Peters, 1864b: 541 (synonymized).

Auturus georgianus Chamberlin, 1942: 8, pl. 3 fig. 22 (O.D.), male holotype (USNM) from Chatham County, Georgia – Chamberlin. 1951: 29 -- Hoffman, 1951: 238 – Shelley, 1978: 61, figs. 57-59 – Hoffman, 1978: 41 (synonymized).

Auturus erythropygos: Hoffman, 1978: 41 (new comb.).

Auturus erythropygos erythropygos: Shelley, 1982b: 3261, figs. 18-22 (new status) – Shelley, 1990: 61, fig. 1 -- Hoffman, 1999: 288 -- McAllister and Shelley, 2005: 187 – Jorgensen et al., 2013 (returned to full species).

Distribution: Southeastern Virginia, North Carolina, South Carolina, eastern Georgia.

Diagnosis: Solenomere extending no farther than lamellar process. Prefemoral concavity indistinct. Distal prefemoral knob distinct. Lamellar process pointed, curved toward solenomere.

Auturus evides (Bollman)

Paradesmus evides Bollman, 1887a: 229 (O.D.), male holotype (USNM, presumed lost) from Winona County, Minnesota.

Euryurus evides: Bollman, 1888b: 2 (new comb.) – Attems, 1899: 280.

Eutheatus evides: Attems, 1938: 295 (genus name change)

Auturus mimetes Chamberlin, 1942: 8, pl. 3 fig. 21 (O.D.), male holotype (USNM) from Jefferson County, Missouri – Chamberlin, 1947: 34 -- Causey, 1950: 37 -- Hoffman, 1951: 238 – Shelley, 1982: 3253 (synonymized).

Auturus evides: Chamberlin, 1942: 7 (new comb.) -- Hoffman, 1951: 238 -- Hoffman, 1999: 289 -- McAllister and Shelley, 2005: 187.

Auturus florus Causey, 1950: 37, figs. 1-2 (O.D.), male holotype (ANSP) from Newton County, Arkansas – Causey, 1955: 22 (synonimized).

Distribution: Eastern Oklahoma, northern Arkansas, Missouri, Illinois, eastern Iowa, southeastern Minnesota, western Wisconsin.

Diagnosis: Prefemoral concavity distinct. Distal prefemoral knob indistinct. Lamellar process dull, even with solenomere, oriented laterally.

Auturus lecythanoictes (Jorgensen)

Euryurus lecythanoictes Jorgensen, 2009, male holotype and 2 male, 1 female paratypes (FMNH) from Escambia County, Alabama

Auturus lecythanoictes: Jorgensen et al, 2013 (new comb.)

Distribution: Known only from type locality.

Diagnosis: Solenomere elongated, distally mucronate and retrorse. Prefemoral concavity and distal prefemoral knob both distinct. Femoral basal lamella very broad and *Euryurus*-like.

Auturus louisianus louisianus (Chamberlin)

Euryurus louisiana Chamberlin, 1918a: 371 (O.D.), male holotype (MCZ) from Natchitoches Parish, Louisiana

Eutheatus louisianae: Attems, 1938: 557 (genus name change).

Auturus Louisiana: Chamberlin, 1942: 7 (new comb.) – Causey, 1955: 22.

Auturus louisianus: Hoffman, 1951: 238 -- Chamberlin and Hoffman, 1958: 58.

Auturus louisianus louisianus: Shelley, 1982: 3257, figs. 7-10 (new status) -- Hoffman, 1999: 289 -- McAllister and Shelley, 2005: 187.

Distribution: Northern Louisiana, southern Arkansas, northeastern Texas, eastern Oklahoma.

Diagnosis: Prefemoral concavity distinct. Distal prefemoral knob indistinct. Lamellar process dull, curved and oriented laterally.

Auturus louisianus phanus Chamberlin

Auturus phanus Chamberlin, 1942: 7, pl. 2 fig. 20 (O.D.), male holotype (USNM) from Saint Helena Parish, Louisiana -- Hoffman, 1951: 238.

Auturus dixianus Chamberlin, 1942: 8 (O.D.), female holotype (USNM) from Saint Tammany Parish, Louisiana – Hoffman, 1951: 238 – Shelley, 1982: 3258 (synonymized).

Auturus scotius Chamberlin, 1942: 9, pl. 3 fig. 23 (O.D.), male holotype (USNM) from Saint Helena Parish, Louisiana -- Hoffman, 1951: 238 – Shelley, 1982: 3258 (synonymized).

Auturus louisianus phanus: Shelley, 1982: 3258, figs. 11-12 (new status) -- Hoffman, 1999: 290 -- McAllister and Shelley, 2005: 187.

Distribution: Southeastern Louisiana, southern Mississippi.

Diagnosis: Solenomere shares distal margin with lamellar process. Prefemoral concavity indistinct. Distal prefemoral knob absent. Lamellar process dull.

Auturus mcclurkini Causey

Auturus mcclurkini Causey, 1955: 23, fig. 1 (O.D.), male holotype (AMNH) from Madison County, Tennessee -- Hoffman, 1999: 290 -- McAllister and Shelley, 2005: 187.

Distribution: Western Tennessee, northern Mississippi

Diagnosis: Solenomere elongated and robust. Prefemoral concavity and distal prefemoral knob both distinct. Lamellar process dull. Distinct curve in acropodite.

Euryurus amycus Hoffman

Euryurus amycus Hoffman, 1978: 65, figs. 5, 13, 16 (O.D.), male holotype (VMNH) from Wilkes County, North Carolina -- Hoffman, 1999: 290.

Distribution: North Carolina: Wilkes and Surry Counties.

Diagnosis: Prefemoral concavity distinct. Distal prefemoral knob absent. Femoral basal lamella small. Subterminal process acicular, shorter than solenomere.

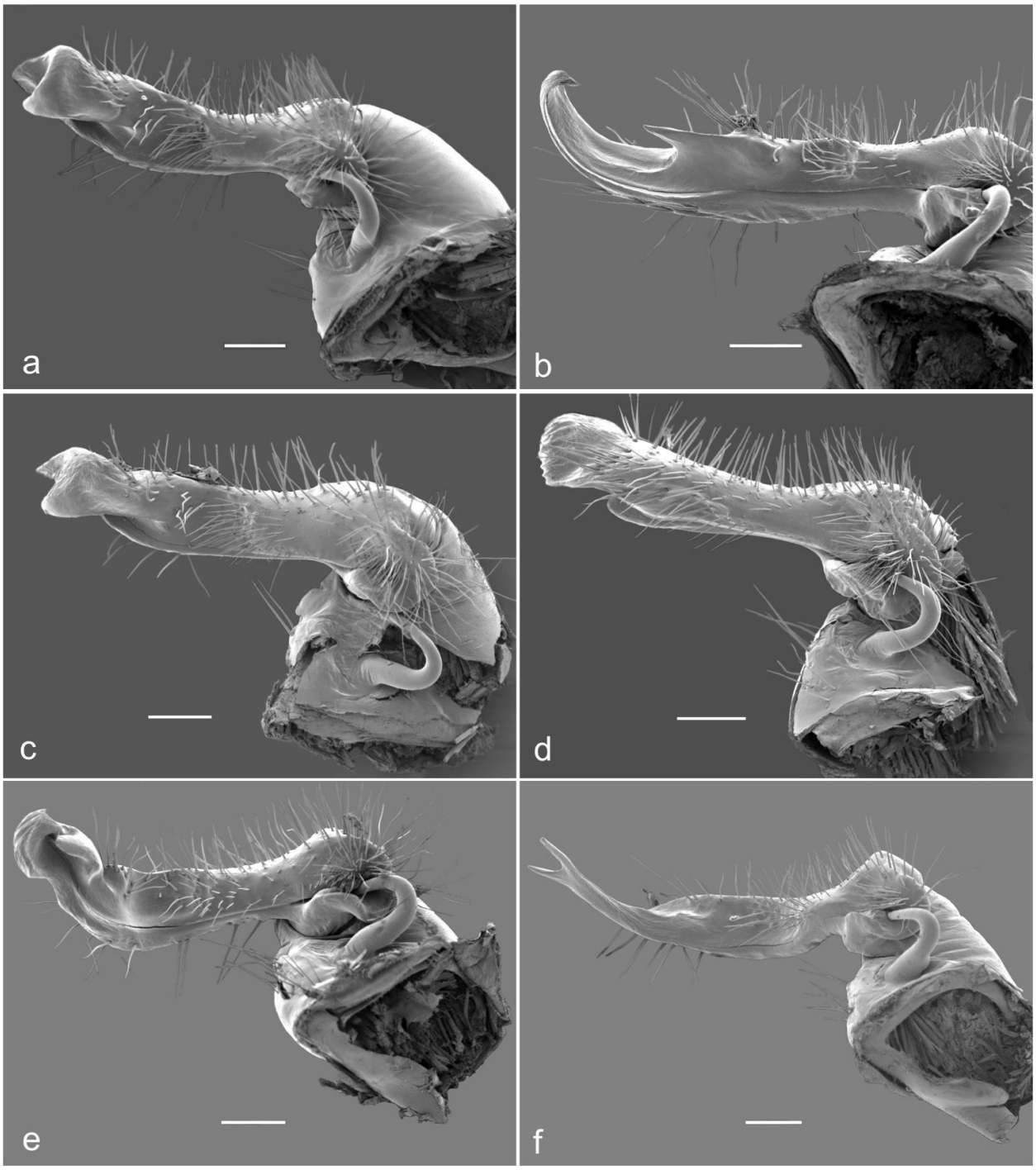

Figure 6.7. Left gonopod, mesal aspect. a) *Auturus evides*. b) *A. lecythanoictes*. c) *A. louisianus*. d) *A. louisianus phanus*. e) *A. mcclurkini*. f) *Euryurus amycus*. Scales bars = 200μm.

Euryurus carolinensis (DeSaussure)

Polydesmus (sub *Paradesmus*) *carolinensis* DeSaussure, 1859: 325 (O.D.), male holotype (MHNG) from South Carolina, precise locality unknown – DeSaussure, 1860: 37, pl. 1 figs. 3-3d -- Peters, 1864b: 541.

Euryurus carolinensis: Hoffman, 1978: 61, figs. 6, 9, 12 (new comb.) – Shelley, 1978: 61, figs. 56, 60-61 -- Hoffman, 1999: 291.

Distribution: Central North Carolina

Diagnosis: Prefemoral concavity distinct. Distal prefemoral knob indistinct. Femoral basal lamella small. Subterminal process acicular, shorter than solenomere.

Euryurus cingulatus Hoffman

Euryurus cingulatus Hoffman, 1978: 64, figs. 14, 15 (O.D.), male holotype (USNM) from Walker County, Alabama – Shelley, 1982: 259, fig. 9 -- Hoffman, 1999: 291.

Distribution: Northwestern Alabama.

Diagnosis: Prefemoral concavity, distal prefemoral knob and femoral basal lamella all absent. Cingulum present at prefemur/acropodite boundary. Acropodite flattened. Subterminal process acicular and subequal with solenomere.

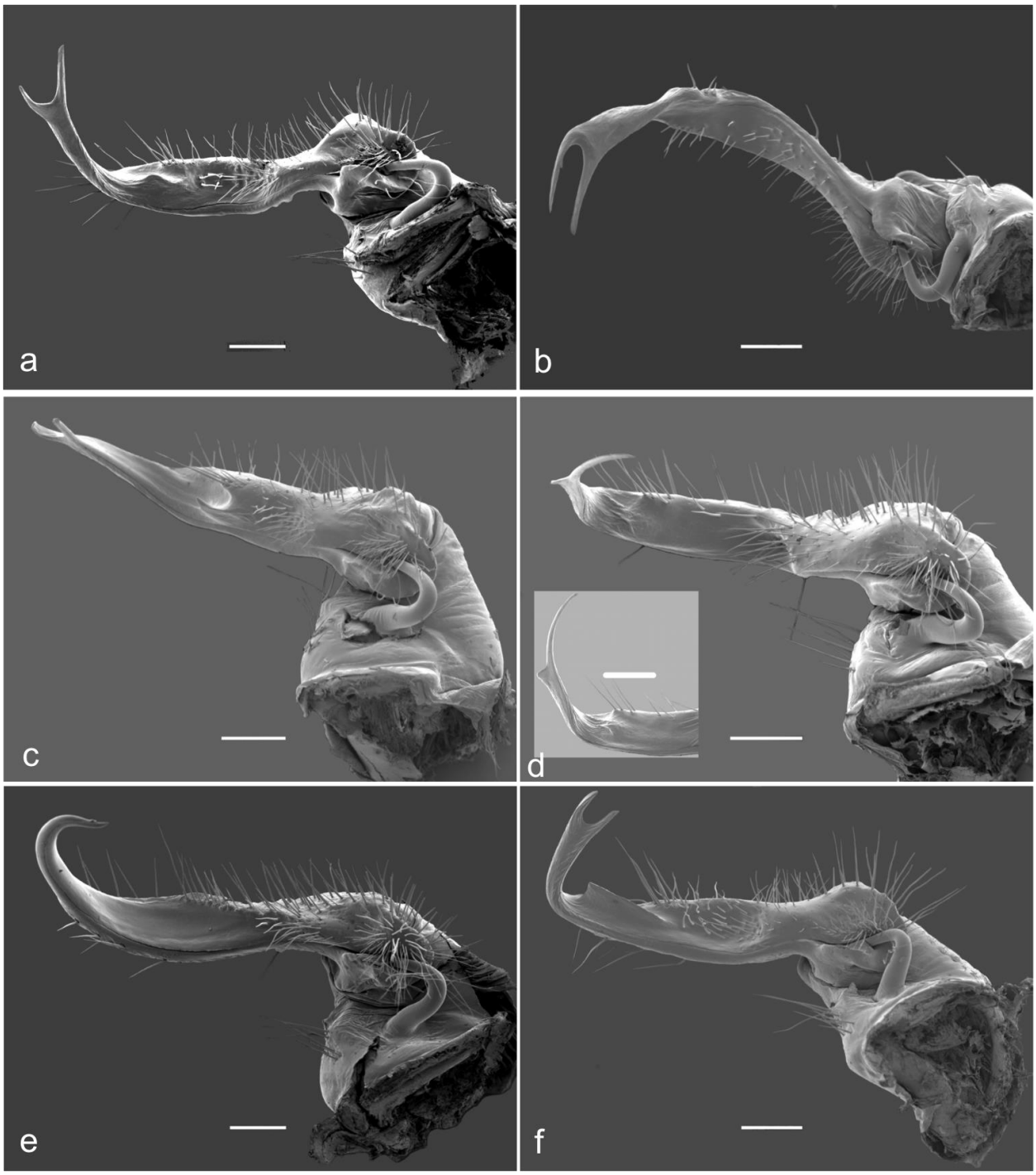

Figure 6.8. Left gonopod, mesal aspect. a) *Euryurus carolinensis*. b) *E. cingulatus*. c) *E. leachii*. d) *E. maculatus* (inset, acropodite at dorsal aspect). e) *E. mississippiensis*. f) *E. orestes*. Scale bars = 200μm.

Euryurus leachii (Gray)

Polydesmus leachii Gray, 1832: pl. 135 fig. 3 (O.D.), male holotype (BMNH) from unknown locality – Brandt, 1841: 134 – Gervais, 1847: 105.

Eutheatus aculeatus Causey, 1952: 9, fig. 8 (O.D.), male holotype (AMNH) from Madison County, Illinois – Hoffman and Browning, 1956: 186.

Euryurus aculeatus: Causey, 1955: 23.

Euryurus leachii: Hoffman and Browning, 1956: 186, fig. 1 (new comb.) – Shelley, 1990: 61, fig. 1 -- Shelley et al., 2012: 1-4.

Euryurus leachii leachii: Hoffman, 1978: 55, figs. 4, 7, 10, 13 (new status) -- Hoffman, 1999: 291 – Jorgensen et al., 2013 (returned to full species).

Euryurus leachii fraternus Hoffman, 1978: 58, figs. 11, 13 (O.D.), male holotype from Warren County, Tennessee – Shelley, 1982: 261, fig. 9 -- Hoffman, 1999: 291 –Jorgensen et al., 2013 (synonymized).

Distribution: Central Illinois to western Pennsylvania. South to northern Mississippi and Alabama. Eastern Arkansas.

Diagnosis: Prefemoral concavity and distal prefemoral knob both distinct. Femoral basal lamella broad. Subterminal process spatulate with varying relative length.

Euryurus maculatus Koch

Euryurus maculatus Koch, 1847: 138 (O.D.), no type specimen designated (see Hoffman, 1978) – Koch, 1863: 7, pl. 3 fig. 8 – Peters, 1864b: 541 (synonymized with *E. erythropygus*) -- Shelley, 1982a: 259, figs. 2, 7-9.

Euryurus erythropygus australis Bollman, 1888: 345 (O.D.), male holotype (USNM) from Butts County, Georgia -- Hoffman, 1978: 49 (synonymized).

Eutheatus australis: Attems, 1938: 295 (new status) – Chamberlin and Hoffman, 1958: 57.

Euryurus falcipes Loomis, 1943: 403, fig. 15 (O.D.), male holotype (MCZ) from Liberty County, Florida – Loomis, 1944: 175 -- Hoffman, 1951: 238 (syn. with *australis*) – Hoffman, 1978: 50 (synonymized).

Euryurus australis: Loomis, 1943: 403 (new status) -- Hoffman, 1951: 238.

Distribution: Alabama to Georgia. Florida panhandle.

Diagnosis: Solenomere extremely longer than in other species. Prefemoral concavity and distal prefemural knob both distinct. Femoral basal lamella absent. Subterminal process flattened and very short.

Euryurus mississippiensis (Causey)

Singuliurus mississippiensis Causey, 1955: 23, fig. 2 (O.D.), male holotype (AMNH) from Jackson County, Mississippi.

Euryurus mississippiensis: Hoffman, 1978: 65, fig. 12 (new comb.) – Shelley, 1982a: 254, figs. 1, 3-6, 9 -- Hoffman, 1999: 291.

Distribution: Extreme southeast Mississippi and southwest Alabama.

Diagnosis: Prefemoral concavity indistinct. Distal prefemoral knob and femoral basal lamella both absent. This is the only *Euryurus* species without a subterminal process.

Euryurus orestes Hoffman

Euryurus orestes Hoffman, 1978: 62, figs. 3, 17, 18 (O.D.), male holotype (VMNH) from Macon County, North Carolina -- Hoffman, 1999: 292.

Distribution: Southern Appalachian area of Tennessee, Georgia and North Carolina.

Diagnosis: Prefemoral concavity distinct. Distal prefemoral knob indistinct. Femoral basal lamella very broad. Subterminal process spatulate and subequal with solenomere.

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