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Phylogenetic systematics of the colorful, cyanide-producing millipedes of Appalachia (Polydesmida, Xystodesmidae, Apheloriini) using a total evidence Bayesian approach

Paul E. Marek *, Jason E. Bond

East Carolina University, Department of Biology, Howell Science Complex, N211A Greenville, NC 27858, USA

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Abstract

Here, we provide an exemplar-approach phylogeny of the xystodesmid millipede tribe Apheloriini with a focus on genus–group relationships—particularly of the genus *Brachoria*. Exemplars for the phylogenetic analysis were chosen to represent the maximum breadth of morphological diversity within all nominal genera in the tribe Apheloriini, and to broadly sample the genus *Brachoria*. In addition, three closely related tribes were used (Rhysodesmini, Nannariini, and Pachydesmini). Morphological and DNA sequence data were scored for Bayesian inference of phylogeny. Phylogenetic analysis resulted in polyphyletic genera *Brachoria* and *Sigmoria*, a monophyletic Apheloriini, and a "southern clade" that contains most of the tribal species diversity. We used this phylogeny to track morphological character histories and reconstruct ancestral states using stochastic character mapping. Based on the findings from the character mapping study, the diagnostic feature of the genus *Brachoria*, the cingulum, evolved independently in two lineages. We compared our phylogeny against prior classifications using Bayes factor hypothesis-testing and found that our phylogenetic hypothesis is inconsistent with the previous hypotheses underlying the most recent classification. With our preferred total-evidence phylogeny as a framework for taxonomic modifications, we describe a new genus, *Appalachioria*; supply phylogenetic diagnoses of monophyletic taxa; and provide a phylogeny-based classification for the tribe Apheloriini.
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1. Introduction

Arthropods are the most diverse group of animals on the planet, comprising about 80% of the 1.5 million presently described species (IUCN, 2004). Despite this overwhelming diversity, they remain among the poorest known. Current estimates predict that only one quarter of the arthropod species (conservatively) have been described. The millipede class, Diplopoda, encompasses a spectacular hidden diversity: about 8000 species have been described from a world-wide fauna estimated to be tenfold greater (Hoffman et al., 2002; Marek and Shelley, 2005). Several diplopodologists alone (e.g., Hoffman, Shear, and Shelley) have documented a substantial amount of alpha-level diversity among millipedes in North America, but many species remain to be described. This is especially the case in the United States where these workers have constructed a strong taxonomic foundation, but continue to describe a massive backlog of new species. In the Pacific Northwest, sampling in the winter has uncovered a previously unknown trove of diversity (Shear and Leonard, 2003, 2004; Shelley and Shear, 2005). In other areas, most notably in biodiversity hotspots like the mountains of southwest China, Polynesia–Micronesia, and Madagascar, the millipede fauna is practically unknown.

The relative scarcity of studies on Diplopoda belies the fundamental role they play in forest ecosystems. By decomposing leaves and aerating soils, millipedes recycle essential

* Corresponding author. Fax: +1 252 328 4178.
E-mail address: pm0623@ecu.edu (P.E. Marek).

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nutrients like nitrogen, carbon, and simple sugars. By mechanically fragmenting (chewing and excreting) leaf material and other detritus, millipedes reinforce finer-scale microbial decomposition by providing increased surface area for colonization by bacteria, mycelia, and algae (Hopkin and Read, 1992). Millipedes may also help break down tougher, more recalcitrant detritus like oak leaves, wood, and other items that earthworms cannot ingest (Hättenschwiler and Gasser, 2005).

An accurate and modern phylogenetic framework to accommodate the estimated planetary diversity of the Diplopoda is lacking. Although phylogenetic studies at or above the ordinal level are sufficient (Enghoff, 1984; Regier and Shultz, 2001; Regier et al., 2005; Sierwald et al., 2003), studies on lower-level groups are few (Bond and Sierwald, 2003; Enghoff, 1995; Tanabe, 2002). All of these previous studies have relied solely upon either molecular or morphological characters. To date, there have been no phylogenetic studies of millipedes using a total-evidence approach that combines both molecular and morphological data.

The phylogenetic study presented here focuses on the tribe Apheloriini Hoffman, 1979 in the family Xystodesmidae Cook, 1895—one of 30 families in the most species-rich order Polydesmida Pocock, 1887. Xystodesmid millipedes occur in the Northern Hemisphere particularly in the broad-leaf deciduous forests of the Mediterranean region, northern Africa, eastern Asia, Russia, North America, and Mesoamerica (Marek and Shelley, 2005). The planetary center of diversity for this family is in the Appalachian Highlands of the eastern US, where over one-third of the approximately 300 described species occur. Foundational alpha-taxonomy is still lacking in this family—museum and recent field collections contain large numbers of undescribed species. For example, it has been estimated that the xystodesmid genus *Nannaria* Chamberlin, 1918a (endemic to the eastern US) comprises about 200 species (Hoffman, 1964), whereas only 25 of these are currently named (Hoffman, 1999).

Apheloriine millipedes (Fig. 1) occur predominately east of the Mississippi River (Fig. 2) in cool, moist broad-leaf forests—although a few species are specialists in other habitats such as cedar or pine forests. Both abundance and diversity are higher in areas with calcareous substrates such as karst beds or other limestone formations (Hoffman, 1990), perhaps because these millipedes integrate calcium carbonate into their cuticle (Hopkin and Read, 1992). Apheloriines are found beneath thin leaf layers in moist areas, including depressions or near streams, and prefer to feed on maple, oak, and tulip poplar leaves (Marek, pers. obs.). These millipedes compose a significant component of the diplopod fauna of the eastern US. Among large (>5 cm) millipedes in the Appalachian Highlands, they are some of the most commonly encountered. Indeed, species diversity of this tribe is highest in the Southern section of the Blue Ridge province in the southern Appalachians where they are distributed in mostly non-overlapping, parapatric ranges (Shelley and Whitehead, 1986). Apheloriines do not travel far; most species are narrow endemics with many known only from a single locality.

Brachoria Chamberlin, 1939, an apheloriine genus of 32 nominal species, occurs from southwestern Pennsylvania and southeastern Indiana to western North Carolina and eastern Louisiana (Fig. 2). Species diversity of this genus is highest in the Valley and Ridge province and the Cumberland Plateau section of the Appalachian Highlands. Individuals are aposematically colored, indicating to predators that they have defense secretions containing hydrogen cyanide (Eisner, 2004; Eisner et al., 1963, 2005; Eisner and Meinwald, 1966; Guldensteeden-Egeling, 1882; Whitehead and Shelley, 1992). Aposematic coloration in *Brachoria* is particularly interesting because it is highly variable within and between species and appears to mimic closely related sympatric genera, putatively representing mimicry rings (Marek, pers. obs.). As seen in Fig. 1, colors vary from yellow, orange, red, and violet. Low vagility and high potential for endemism, combined with extreme color variability, make apheloriines ideal models for addressing interesting questions about the evolution of aposematism and mimicry, speciation by population fragmentation (see Bond and Sierwald, 2002), and migration patterns in the Appalachian Mountains. Yet, investigations into mimicry and other evolutionary questions in this millipede group have been hindered by the absence of an accurate and updated taxonomic structure (for *Brachoria* and related millipedes in the tribe Apheloriini) and a robust phylogenetic framework necessary for comparative studies (Felsenstein, 1985).

This project uses ribosomal DNA sequences from the mitochondrial 16S, tRNA-Valine, and 12S genes and qualitative morphological characters in an exemplar approach to recover the evolutionary history of apheloriine millipedes. Whereas previous phylogenetic hypotheses have relied exclusively on male genitalic characters to construct them, the present analysis incorporates DNA sequences, as well as female genitalic characters and somatic characters. Here we provide an exemplar phylogeny of apheloriine millipedes with a concentration on generic relationships—particularly of the genus *Brachoria*. We used this phylogeny to: (1) reconstruct morphological character histories and ancestral states using stochastic character mapping; (2) compare prior classifications using Bayes factors; (3) describe a new genus *Appalachioria*; (4) supply phylogenetic diagnoses of monophyletic taxa; and (5) provide a phylogeny-based classification for the tribe Apheloriini.

1.1. Taxonomic history

The family that contains the tribe Apheloriini, Xystodesmidae, was a disparate collection of names until several authors summarily revised most of the genera (Hoffman, 1948, 1957, 1958, 1978a, 1979; Keeton, 1959, 1965; Shelley, 1980b, 1984c, 1995; Shelley and Whitehead, 1986). Classification in the tribe Apheloriini, however, is both unstable and

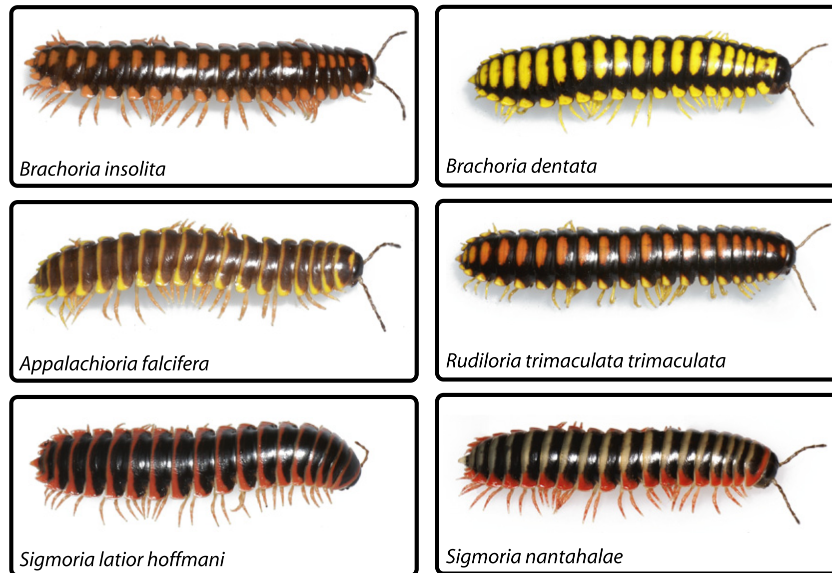

Fig. 1. Six examples of apheloriine millipedes. Bright coloration warns potential predators of defense secretions containing hydrogen cyanide.

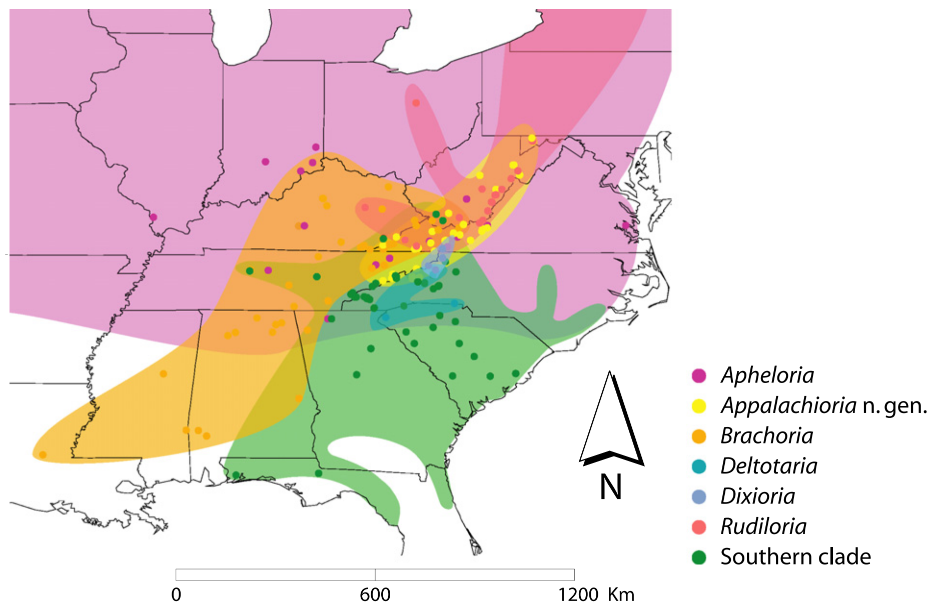

Fig. 2. Distribution of major apheloriine clades in the eastern United States. Dots indicate known specimen localities. Colored ranges are based on authors' collection data and that of (Shelley and Whitehead, 1986).

suffers from lack of explicit phylogenetic reasoning. Furthermore, the accepted relationships and character support that underlie the current classification *have never been tested*.

Supra-specific classification has oscillated between two major hypotheses. Hoffman (1979) put forth a classification hypothesis comprising 12 genera based solely on genitalic characters; male apheloriines have the first leg pair on the seventh segment modified into gonopods, which are used as sperm transfer devices (see Figs. 5 and 8). Gonopods in millipedes, like aedeagi and other arthropod intromittent organs, are hypothesized to evolve rapidly and divergently and therefore be good for species-level taxonomy (Eberhard, 1985, 2004). Whitehead (in Shelley and Whitehead, 1986) presented two phylogenetic hypotheses of genus-group relationships: one inferred from genitalic morphology (Fig. 3A) and a second, revised hypothesis relying on morphology and on geographical proximity (Fig. 3B). Subsequently, Hoffman (1999), in a North American species check-list, overturned the prior classification (i.e., Fig. 3B) with an updated version of his 1979 hypothesis (Fig. 3C) that recognized 16 genera. In this, Hoffman (1999) suggested a few generic affiliations, but, unlike Whitehead (Fig. 3A and B), gave no "phylogenetic" hypothesis above the genus level. In a piecemeal fashion, Shelley (2000a,b, 2002) gradually overturned Hoffman's classification with

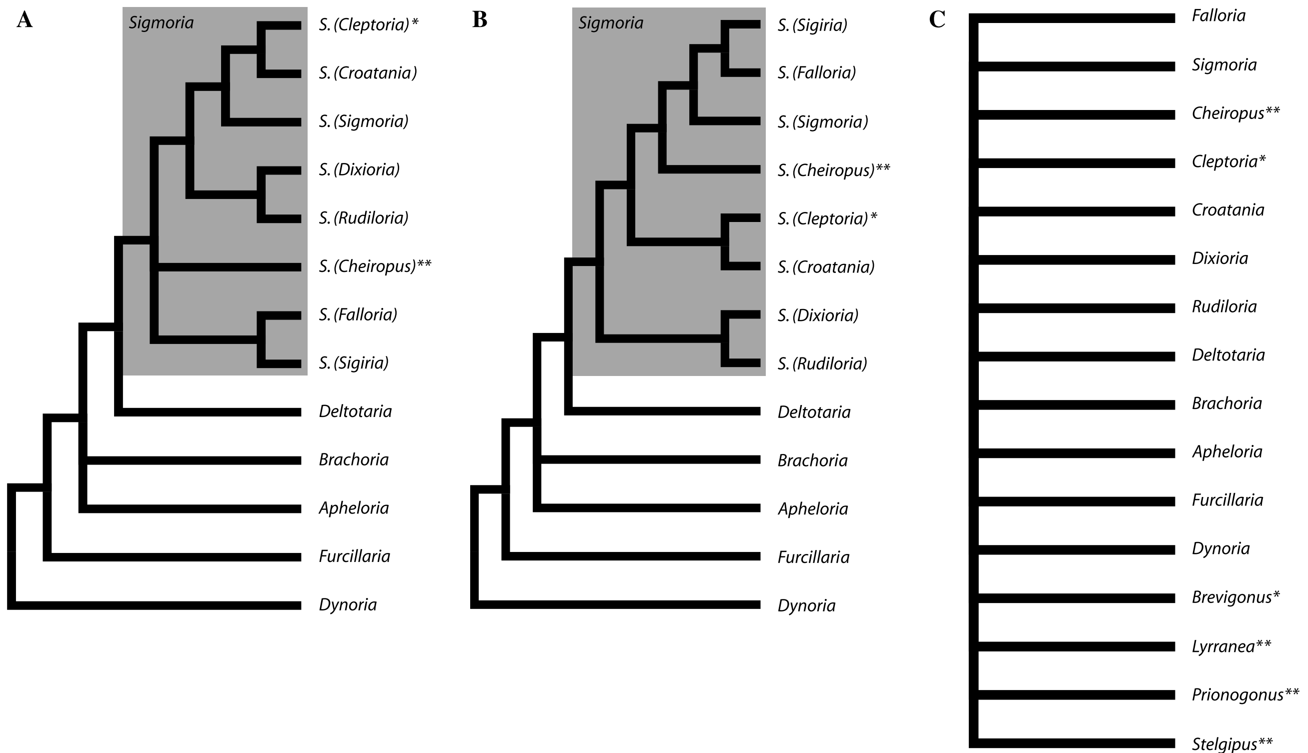

Fig. 3. Phylogenetic relationships underlying prior classification systems: (A). SW86, Whitehead (*in* Shelley and Whitehead, 1986), preliminary phylogenetic hypothesis, based on morphology; (B) SW86, second, revised hypothesis relying on morphology and on geographical proximity; (C) Hoffman (1999), phylogenetic hypothesis interpreted from classification, based on morphological apomorphies. *Genera in synonymy with subgen. *Cleptoria*, **genera in synonymy with subgen. *Cheiropus* (*sensu* SW86).

one based on Whitehead's second, revised hypothesis (Fig. 3B). This is the most current classification and comprises six genera, one of which, *Sigmoria* (hereafter referred to as "*Sigmoria s.l.*"), contains eight poorly-defined, geographically circumscribed subgenera.

Brachoria is the focal taxon of this study. Shortly after it was recognized in 1939, several additional species were described, including a closely-related genus, "*Tucoria*" Chamberlin, 1943a. In the only comprehensive revision to date, Keeton (1959) suggested that *Brachoria* may contain multiple independent lineages, but did not investigate this hypothesis further or provide substantive support for his ideas. Also since that time, additional material, including a large number of new species, has been collected making *Brachoria* ripe for reconsideration.

It is not surprising that apheloriine taxonomy and classification remain confused. They are based almost exclusively on gonopodal characters and geographic-distribution affinities. Taxonomy built almost entirely on these gonopodal characters and on the premise of rapid and divergent genitalic evolution, however, may be biased (Bond et al., 2003; Bond and Sierwald, 2003). Furthermore, geographic distribution should *never* be used as a criterion for delineating genera in any group of organisms; biogeographic distributions should be studied at the population–species level, or below, closest to where the actual pattern-shaping events occur. Resolving issues in apheloriine systematics requires additional, independent kinds of data and a modern phylogenetic approach.

1.2. Exemplar approach phylogenetics

Classification systems in millipedes and many other arthropod groups are built from type specimens upwards using overall similarity and a morphological diagnosibility criterion (Coyne, 1994; Cronquist, 1978; Sites and Marshall, 2004). These "bottom-up" techniques have traditionally been prescribed by custom, forming the methodological basis of the past 250 years of taxonomy, and have served as the foundation of higher level classification schemes. However, inferring the evolutionary history of a higher-level taxon containing a large number of subtaxa may be unfeasible. Instead of traditional methods, we chose to precede a taxonomic revision of the genus *Brachoria* with an exemplar approach (hereafter referred to as "EA") for reconstructing a phylogeny of Apheloriini.

In EA phylogenetics, "exemplar" species are used as terminals to represent higher-level taxa. The same specimen—representing a species—is scored for both morphological and molecular characters (Ideally, the specimen is deposited at a museum with a specimen code, which is cited in the study.) The more species used as exemplars to represent higher-level taxa the better (Wheeler, 1992; Wiens, 1998). However, fewer species can be used if they more fully represent the maximum breadth of morphological diversity within a focal higher-level taxon (Prendini, 2001). This criterion provides a trade-off between minimizing taxa and maximizing the severity of the test for the

preexisting monophyly hypothesis, while simultaneously providing enough exemplars to resolve the evolutionary history within the higher-level taxon of interest. The EA to phylogenetic inference is highly testable and expandable due to its explicit methods and use of individual species to represent supra-specific taxa. Numerous studies (Bond and Opell, 2002; Christoffersen, 1989; Flynn et al., 2005; Griswold et al., 1998; Miller, 1991; Neves and Watson, 2004; Prendini, 2000) have effectively approached higher-level relationships using EA. By using this method for inferring evolutionary history, one confers more scientific rigor in taxonomy, allowing hypotheses of relationship to be tested and updated. It also makes a daunting and potentially impractical task, reconstructing higher-level phylogenies from a large number of species, more tractable and pragmatic. Furthermore, because this approach incorporates reproducible methods for phylogeny reconstruction and uses a reduced set of all the species contained in a higher-level group as terminal taxa, it benefits from expandability. By incorporating more exemplar species when they become available, the phylogeny and the classification structure can be continuously updated with great ease. Likewise, as new species are discovered, they can be described with reference to their location within the preexisting phylogeny. In a simulation study investigating methods of coding and sampling higher-level taxa in phylogenetics (Wiens, 1998), the use of multiple species as terminal taxa (EA or "species-as-terminals" method) outperformed alternative methods (such as representing a taxon by inferring its ancestral states). EA is ideal for a group like Apheloriini where there is a large set of species and a pre-existing bottom-up classification, but where supra-specific relationships are unclear.

2. Materials and methods

2.1. Taxon sampling

2.1.1. Molecular partition

DNA sequence data came from specimens collected in the field specifically for this study. No museum specimens were used in the molecular partition. Molecular data were collected for 53 taxa (Appendix A). Of 13 nominal genera and subgenera in the tribe Apheloriini, 12 were represented in the phylogenetic analysis. Additionally, 10 species from two tribes hypothesized to be closely related sister-groups (Hoffman, 1958; Shelley, 1980a; Shelley and Whitehead, 1986) were also included: six species from Rhysodesmini Brölemann, 1916 and four species from Pachydesmini Hoffman, 1979. Of the Pachydesmini, a hypothetically primitive species, *Dicellarius atlanta* (Chamberlin, 1946), was used to root the tree. In *Brachoria*, of 32 nominal species, 14 were included—four of them undescribed species.

At least two nominal species (one male and one female specimen per species) were chosen per genus–group name according to (1) availability of fresh material for DNA extraction and (2) the maximum morphological-diversity criterion (Prendini, 2000; Prendini et al., 2003). The latter criterion aims to falsify a preexisting hypothesis of monophyly by using two species within a higher-level taxon that are maximally distinct morphologically such that they have the lowest probability of being related. We define "maximally distinct" species as the two species with the greatest number of morphological character-state differences between them. Because species are identified by male genitalia, DNA sequence data were always scored from male specimens except in the case of one species; a female specimen from Cumberland Mountain State Park in Tennessee was determined to be *Pachydesmus crassicutis laticollis* (Attems, 1899) because it is known to be the only species present there. Six unknown female specimens in Apheloriini were also included in an attempt to identify them.

2.1.2. Morphological partition

All DNA voucher specimens used in the molecular data set (Appendix A) are the same specimens used in the morphological data set except for the female *Pachydesmus crassicutis laticollis* specimen, which was replaced by a male and female specimen of *Pachydesmus clarus* (Chamberlin, 1918b), the only chimeric taxon used in the analysis. The use of a chimera was justified because pachydesmine-species relationships were not a large objective of the study, and mainly functioned to root the Apheloriini (see Malia et al., 2003). Also, more taxa were added to the morphological data set because preserved museum specimens were more readily available—eight more *Brachoria* species, and two species of: *Croatania* Shelley, 1977 (Apheloriini); *Rhysodesmus* Cook, 1895 (Rhysodesmini); *Nannaria* (Nannariini); *Thrinaxoria* Chamberlin and Hoffman, 1950 (Pachydesmini); and one more *Dicellarius* Chamberlin and Hoffman, 1950 species were included. Male and female specimens of the same species were treated as one terminal.

2.1.3. Total evidence

Because all DNA voucher specimens used in the molecular data set are the same specimens used in the morphological data set, an intersection of the two sets was straightforward. The combined data set was constructed from those taxa with complete molecular and morphological data (the six unidentified female specimens were also included). A second data set was constructed from a union of all available taxa and characters. That is, the morphology-only taxa were united with those having both morphology and sequence data. For the specimens with incomplete data, characters were scored as missing.

2.2. Molecular protocols

Live specimens collected in the field were brought back to the laboratory for processing. Left legs on segments 8–19 were dissected, immersed in RNAlater (Qiagen Inc., Valencia, CA), initially stored at 10 °C for 24 h, and archived in a −80 °C freezer. Genomic DNA was isolated using the

DNeasy (Qiagen Inc., Valencia, CA) Tissue Kit from 3–5 legs (depending on size of the millipede). A region of the mitochondrion spanning the ribosomal 16S, 12S, and the intervening tRNA-Valine genes (mean length = 1352 bp) was amplified using the polymerase chain reaction (PCR) from purified genomic DNA using the primer pair LR-J-12887dip2 (5′-CCGGTCTGAACTCAGATCATGT-3′) and SR-N-145XXdip2 (5′-GGACGTCAAGTCAAGG TGCAG-3′). Cycle sequencing reactions consisted of the two PCR primers and the internal bridging primer, LR-J-APHE1: GTTTCACCTTCATACCAGC. DNA amplification, purification, and sequencing follow standard procedures described by Hendrixson and Bond (2005).

2.3. Sequence alignment and phylogenetic inference

The computer program Prank (Probabilistic Alignment Kit) ver. 1508b (available for download at http://www.ebi.ac.uk/goldman/prank) was used to align the nucleotide data. Prank employs the approach outlined by Löytynoja and Goldman (2005). The default gap opening and extension probabilities with the correction for insertion sites and the allowance that gaps may be closed were used. Alignments were considered based on the two models of substitution processes available in the software package, JC (Jukes and Cantor, 1969) and HKY85 (Hasegawa et al., 1985). First-iteration alignments were based on a guide tree taken from ClustalX (Thompson et al., 1997) using a pairwise gap opening and extension cost of 15/6. Sequences were aligned by the computer program instead of manually, to preserve repeatability and objective criteria (DeSalle et al., 1994; Gatesy et al., 1993; Giribet and Wheeler, 1999). The aligned data set was outputted as a Nexus file (Maddison et al., 1997) and is available for download at www.treebase.org matrix Accession No. M2738.

Aligned sequences were divided into three partitions based on comparison with published mitochondrial genomes of various arthropods: 16S, tRNA-Valine, and 12S. Partitions were confirmed by folding the middle tRNA-Valine region into its secondary structure (Mathews et al., 1999; Zuker, 2003). Inferred apheloriine tRNA-Val secondary structure is the standard cloverleaf form with D, anticodon, variable, and TΨC loops. DNA site substitution models for each of the character partitions were evaluated using the hierarchical likelihood ratio test (hLRT) in MrModeltest 2.2 (Nylander, 2004). The hLRT was used over the Akaike information criterion (AIC) to err on the side of an overparameterized model (Huelsenbeck and Rannala, 2004; Lemmon and Moriarty, 2004).

Sequences were characterized for homogeneity using χ^2 tests of stationarity and nucleotide composition in the program PAUP* 4.0b10 (Swofford, 2002). After constant sites were removed, base composition bias was calculated according to Irwin et al. (1991) using the formula: $2/3(|A-0.25|+|C-0.25|+|G-0.25|+|T-0.25|)$. Bayesian phylogenetic inference under MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) was used to evaluate tree topologies and models of nucleotide substitution for the aligned dataset. Partitions (16S, tRNA-Valine, 12S) were unlinked for nuisance parameters and linked for branch length and topology. Two simultaneous analyses of four Markov Chain Monte Carlo (MCMC) chains (one heated and three cold) were run initially for 1,000,000 generations while sampling one tree per 100 generations. Convergence between simultaneous runs was reached when the average standard deviation of split frequencies fell below 0.01 (Ronquist et al., 2005). When runs failed to converge, the number of generations was increased by increments of 1M. Overlay plots of the generation versus parameter values for the two simultaneous runs were examined in the program Tracer 1.3 (Rambaut and Drummond, 2003) to assess stabilization and mixing of likelihood and parameter values. Following the burn-in phase, parameter values were averaged using the "sump" command, and posterior clade probabilities were calculated and the likelihood scores for all the topologies averaged using the "sumt" command in MrBayes.

2.4. Morphological character coding, scoring, and phylogenetic analysis

New morphological characters were combined with characters derived from the literature. Using a Leica 12.5 stereomicroscope, qualitative morphologic structures of the male and female exoskeleton and genitalia were assessed to develop primary hypotheses of character homology (Appendix B). Specimens examined using scanning electron microscopy (FEI Quanta 200 Environmental Scanning Electron Microscope) were air-dried and sputter coated with gold prior to viewing. Specimens were scored for binary and multi-state characters using DELTA 1.04 (Dallwitz, 1980; Dallwitz et al., 1999). DELTA was then used to generate a Nexus file and to store the morphological-character set for taxonomic diagnoses, descriptions, keys, etc.

Phylogenies were reconstructed from the morphological data set using Bayesian inference. Missing characters were treated as unknown. PAUP* 4.0b10 was used to evaluate parsimony informative sites. MrBayes was used to evaluate tree topologies and a model of character-state change for the data set. The Markov k (Mk) model (Lewis, 2001), was applied to the data both with and without Γ-distributed rates of character change. For both the Mk and Mk+Γ models, two simultaneous analyses of three MCMC chains (one heated and two cold) were run initially for 1,000,000 generations while sampling one tree per 300 generations. Convergence between simultaneous runs, parameter stabilization, mixing, burnin, posterior clade probabilities, and likelihood values were evaluated as described above. Likelihood values for the separate models (Mk and Mk+Γ) were compared using Bayes factors (B_{10} = Harmonic Mean Ln Likelihood Mk − Harmonic Mean Ln Likelihood Mk+Γ) and a subsequent interpretation of $2\ln B_{10}$ according to the table described by Kass and Raftery, 1995, p. 177 (Brandley et al., 2005; Nylander et al., 2004; Wiens et al., 2005).

2.5. Total evidence

MrBayes was used to evaluate tree topologies and models of character change for the combined data sets of morphology and molecular characters. Partitions (16S, tRNA-Valine, 12S, and morphology) were unlinked for parameters as described above. For the intersection data set (comprising taxa where molecular and morphology data are both known), two simultaneous analyses of four MCMC chains (one heated and three cold) were run initially for 1,000,000 generations while sampling one tree per 100 generations; whereas for the union data set (comprising taxa where either molecular or morphology data is missing), one tree was sampled per 300 generations. Convergence between simultaneous runs, parameter value stabilization, mixing, burnin, posterior clade probabilities, and likelihood values were evaluated as described above.

2.6. Bayes factor comparisons of alternative phylogenetic hypotheses

Alternative phylogenetic hypotheses were evaluated using Bayes factors. This method differs from traditional hypothesis-testing because it does not offer a criterion for absolute rejection of a null hypothesis, but instead an evaluation of the evidence in favor of the null hypothesis (Kass and Raftery, 1995). The phylogeny inferred from the molecular data set was constrained to current hypotheses (Whitehead in Shelley and Whitehead, 1986—hereafter referred to as "SW86" in Fig. 3A or B) of genus–group relationships in the tribe Apheloriini (Fig. 3A and B). The classification by Hoffman (1999) (Fig. 3C) was not compared because it lacked phylogenetic resolution above the genus level and would have resulted in a spurious and artificially high Bayes factor. First, Bayesian inference was rerun, with an absolute prior (= 1.00), on the constrained topology consistent with previous phylogenetic hypotheses. The predictive value of the constrained harmonic mean likelihoods (H_{1-6}) were then compared to the original, unconstrained likelihoods (H_0) using a Bayes factor comparison, B_{10} = Harmonic Mean Ln Likelihood H_1 − Harmonic Mean Ln Likelihood H_0 (Kass and Raftery, 1995). Six hypotheses were treated this way: (1) H_1 = *Brachoria* (*sensu* Keeton, 1959) constrained to be monophyletic versus H_0 = unconstrained molecular topology; (2) H_2 = *Sigmoria s.l.* constrained to be monophyletic versus H_0 = unconstrained molecular topology; (3) H_3 = SW86 in Fig. 3A versus H_0 = unconstrained molecular topology; (4) H_4 = SW86 in Fig. 3B versus H_0 = unconstrained molecular topology; (5) H_5 = SW86 in Fig. 3A versus H_0 = unconstrained morphological topology; (6) H_6 = SW86 in Fig. 3B versus H_0 = unconstrained morphological topology.

2.7. Character mapping and ancestral state reconstruction

Morphological character histories and ancestral character states were inferred under stochastic models of evolution using likelihood-based posterior mapping (Huelsenbeck et al., 2003; Nielsen, 2002) in the program SIMMAP 1.0b2.02 (Bollback, 2005). Posterior mapping allows a probabilistic reconstruction that is compatible with the observed character states and an evolutionary model of character change. Unlike the parsimony approach, which forces character history onto a single topology without taking into account uncertainty (Huelsenbeck et al., 2003), character mapping under likelihood predicts mappings under an explicit model of change while taking into account probability of change proportional to branch lengths and uncertainty in the topology, observed character states, and other estimated parameters.

Because the cingulum has played an important role in defining the genus—i.e., because it is the diagnostic feature for nominal *Brachoria*—the ancestral states of this character on the apheloriine tree are of interest. The cingulum is the only character for which we reconstructed ancestral states and traced onto the phylogeny. This binary character (0, absent; 1, present) was iteratively mapped, using 25 realizations drawn from its posterior character history distribution, onto the consensus tree from the combined analysis (intersection data set) to determine where on the tree and how often a cingulum is likely to have evolved. Ancestral character states for the cingulum were reconstructed at seven ancestral nodes using the Mk+Γ model integrating uncertainty over observed character states with the MCMC posterior distribution of trees and with estimated parameters. Estimates (including model parameters, trees, and branch lengths) were taken from the combined Bayesian analysis (intersection data set). Finally, every morphological character was stochastically mapped, using ten realizations drawn from its posterior history distribution, onto the set of post-burnin trees sampled from the MrBayes MCMC posterior distribution (combined intersection analysis) to estimate the number of transformations per character, dwell time per state, and homoplasy index (calculated: HI = 1 − minimum transformations/estimated number of transformations).

3. Results

3.1. Sequence alignment and phylogenetic inference

The alignment generated using the HKY85 substitution model in Prank was preferred over the JC69 model based on visual inspection; in particular, we concentrated on difficult-to-align regions like loop regions, e.g.: sites 576–674. We preferred sequence length variation to be accounted for by single insertions (with the gaps indicating the absence of the insertion lined up in the other taxa) instead of multiple, independent deletions staggered between sequences (Löytynoja and Goldman, 2005). Alignment of raw sequences (mean length = 1352 bp) resulted in approximately 1538 bp which was subsequently divided into three partitions—16S (1–1209), tRNA-Valine (1210–1280), and 12S (1281–1538). Of the 1538 sequence characters, 828 were constant, 204 variable

characters were parsimony uninformative, and 506 were parsimony informative. Mean base composition was A = 0.43289, C = 0.22787, G = 0.07825, T = 0.26099 and nucleotide frequency was homogeneous across taxa ($\chi^2 = 47.5181$, df = 156, $P > 0.995$). With constant sites removed, the base-composition bias was 0.27598 (with 0.0 being no bias at all and 1.0 being severely biased to all one base).

The following substitution models were inferred for the partitions under the hLRT in MrModeltest: 16S (GTR+I+G), tRNA-Valine (HKY+G), and 12S (GTR+G). MCMC was run for 2M generations. Stabilization and convergence of likelihood values occurred after 1,390,000 generations. Of the trees, 19,401 were retained post burn-in and summed to create a majority rule tree (Fig. 4A).

A polyphyletic *Brachoria* is recovered, with *Apheloria*; *Deltotaria philia* (Chamberlin, 1949b); *Dixioria* Chamberlin, 1947; and *Rudiloria* Causey, 1955 embedded inside. Two separated *Brachoria* clades are evident, each supported by posterior probabilities of 1.0. Of 17 genera with at least two exemplar specimens, eight are monophyletic with posterior probability (Pp) values between 0.65 and

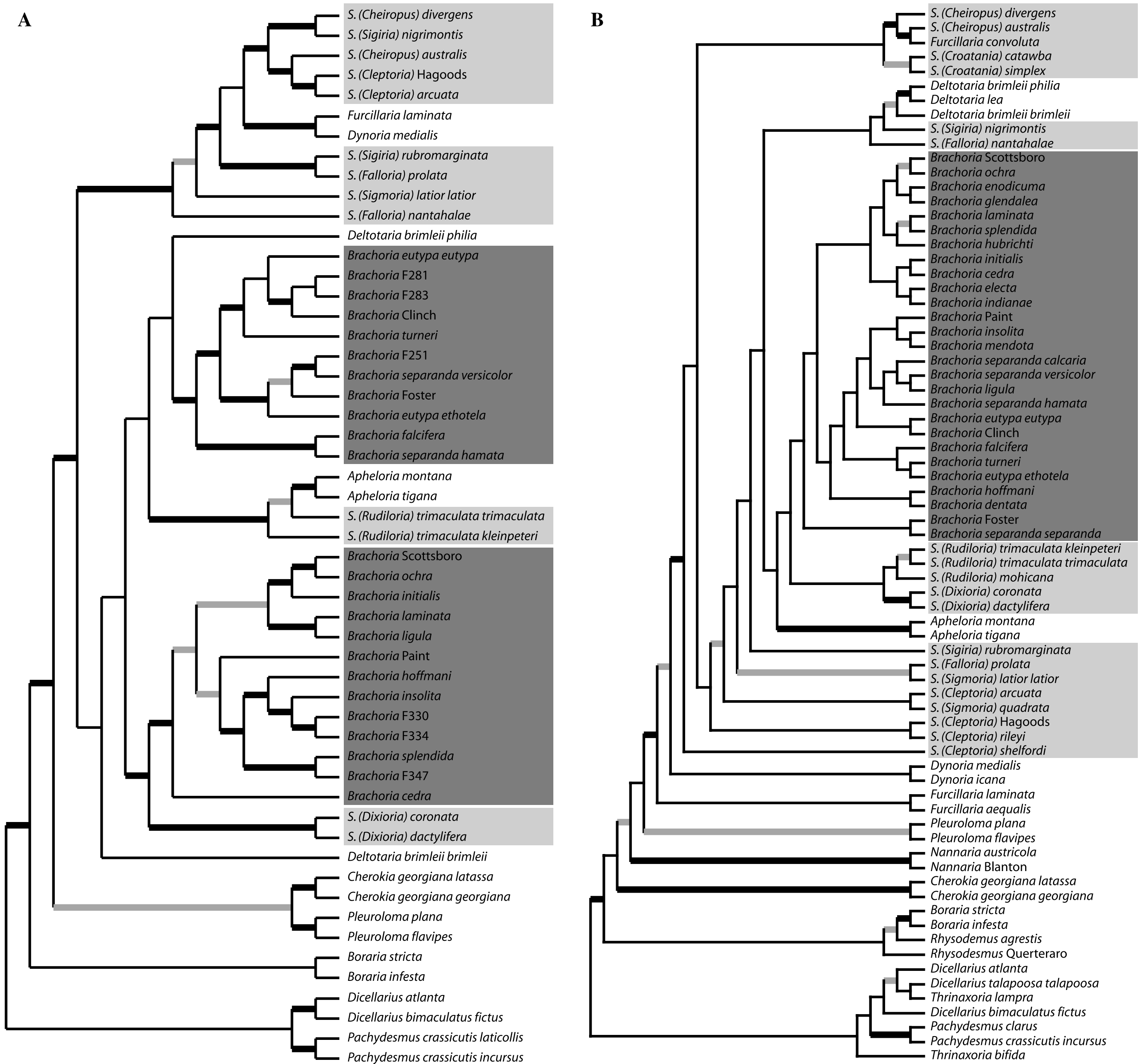

Fig. 4. Phylogenies reconstructed using Bayesian inference from molecular and morphological data sets. (A). Molecular phylogeny, harmonic mean likelihood = −13953.67 and (B) Morphological phylogeny, harmonic mean likelihood = −2,047.83 (thickened, black branches denote Pp values between 0.90 and 1.00; gray branches denote values between 0.70 and 0.89). Light gray boxes, nominal *Sigmoria s.l.* species, dark gray boxes, nominal *Brachoria* species.

1.00. The genus *Sigmoria s.l.* is polyphyletic and occurs throughout the phylogeny multiple times. Apheloriini, Pachydesmini, and (*Cherokia* Chamberlin, 1949a + *Pleuroloma* Rafinesque, 1820) clades are supported with Pp values of 1.0, 1.0, and 0.88 respectively, and the tribe Rhysodesmini is paraphyletic with respect to Apheloriini. (The molecular phylogeny is hereafter referred to as "Pp-D," D for DNA, in reference to posterior probability values.)

3.2. Morphological character coding, scoring, and phylogenetic analysis

Of 68 binary and multi-state characters scored for 73 taxa, 40 were derived from male genitalia, 5 from female genitalia, and 23 from the integument. Of these 68 characters, 67 were parsimony-informative. The Mk+Γ model of character change was chosen over the simpler Mk using a Bayes factor comparison. The evidence against the Mk model without gamma-distributed rates was "very strong" (cf. Bayes factor interpretation table Kass and Raftery, 1995:777) with a Bayes factor of 134.16. For the Mk+Γ analysis, stabilization, and convergence of likelihood values occurred after 31M generations. Of the trees, 43,288 were retained post burn-in and summed to create a majority rule tree (Fig. 4B). A very weakly supported monophyletic *Brachoria* is recovered (Pp = 0.19). Of 22 genera with at least two exemplar specimens, 13 are monophyletic with Pp values between 0.52 and 0.95. The genus *Sigmoria s.l.* is polyphyletic and occurs throughout the phylogeny several times. An Apheloriini clade is supported with a Pp value of 0.97 and the tribes Rhysodesmini and Pachydesmini are paraphyletic with respect to Apheloriini (The morphological phylogeny is hereafter referred to as "Pp-M" in reference to posterior probability values.).

3.3. Total evidence

Combined Bayesian analyses of the intersection data set (using taxa with complete morphological and molecular characters) ran for 2.2M generations with stabilization and convergence of likelihood values occurring after 1.1M generations. Of the trees, 21,681 were retained post burn-in and summed to create a majority rule consensus (Fig. 5). A polyphyletic *Brachoria* is recovered with *Apheloria*, *Dixioria*, and *Rudiloria* embedded inside. The two discrete *Brachoria* clades (*Brachoria* and *Appalachioria* n. gen.: see Appendix C) are both supported by Pp values of 1.00. Of 17 genera with at least two exemplar specimens, ten are monophyletic with Pp values between 0.65 and 1.00. The genus *Sigmoria s.l.* is polyphyletic and occurs throughout the phylogeny multiple times. Apheloriini, Pachydesmini, and (*Cherokia* + *Pleuroloma*) clades are supported with Pp values of 1.00, 1.00, and 0.79, respectively. The tribe Rhysodesmini is paraphyletic with respect to Apheloriini. (The combined intersection data set/phylogeny is hereafter referred to as "combined-I" or "Pp-I" in reference to posterior probability values.) The combined-I tree is chosen as the preferred topology for stochastic character-mapping and classification purposes because its reconstruction was based on all of the evidence available to us at this time.

For the combined union data set (total evidence), MCMC analysis was run for 20M generations but convergence of likelihood values did not occur (standard deviation of split frequencies, SDSF = 0.023). As a result, another analysis was run starting with a 'usertree'—i.e., the last tree sampled in the posterior distribution from the previous analysis—and two perturbations ('nperts = 2'). The analysis was then run for an additional 14.5M generations sampling a tree every 300 generations. In the second analysis, convergence still did not occur (SDSF = 0.02), but parameter values appeared to have stabilized and converged upon examination of overlay plots from the separate runs in the program Tracer. Furthermore, topologies from the two simultaneous runs did not appear different except for the placement of a few taxa (i.e., those with a large amount of missing data). Of the trees, 21,869 were retained post burn-in and summed to create a majority rule tree (Fig. 6). A polyphyletic *Brachoria* is recovered with *Apheloria*, *Dixioria*, and *Rudiloria* embedded inside. Two discrete *Brachoria* clades are both weakly supported with Pp values of 0.27 (for the clade sister to *Dixioria*) and 0.43 (for the *Appalachioria* clade sister to *Apheloria* + *Rudiloria*). Of 22 genera with at least two exemplar specimens, ten are monophyletic with Pp values between 0.36 and 1.00. The genus *Sigmoria s.l.* is polyphyletic and occurs throughout the phylogeny multiple times. Apheloriini, Pachydesmini, and Nannariini clades are supported with Pp values of 1.00, 1.00, and 0.89, respectively. The tribe Rhysodesmini is paraphyletic with respect to (Apheloriini+Nannariini). (The combined union data set/phylogeny is hereafter referred to as "combined-U" or "Pp-U" in reference to posterior probability values.)

3.4. Bayes factor comparisons of alternative phylogenetic hypotheses

Bayes factor comparisons are summarized in Table 1. The likelihoods of the unconstrained topologies (H_0, from Fig. 4A and B) all had much larger values than the alternative constrained topologies ($H_{1–2}$, *Brachoria s.l.* and *Sigmoria s.l.* constrained to be monophyletic and $H_{3–6}$, from Fig. 3A and B) and were therefore always favored, with Bayes factors between −74.54 and −862.36. Because the objective of the comparison was to assess the relative predictive values of the topological likelihoods, the size of the negative number was of interest. In order of increasing Bayes factor (= most to least discordant, or a decreasing absolute value) the comparisons are as follows: H_3 = SW86 in Fig. 3A against molecular topology (Fig. 4A), H_4 = SW86 in Fig. 3B against molecular topology (Fig. 4A), H_2 = *Sigmoria s.l.* constrained to be monophyletic against molecular topology (Fig. 4A), H_6 = SW86 in Fig. 3B against morphological topology (Fig. 4B), H_5 = SW86 in Fig. 3A against morphological topology (Fig. 4B),

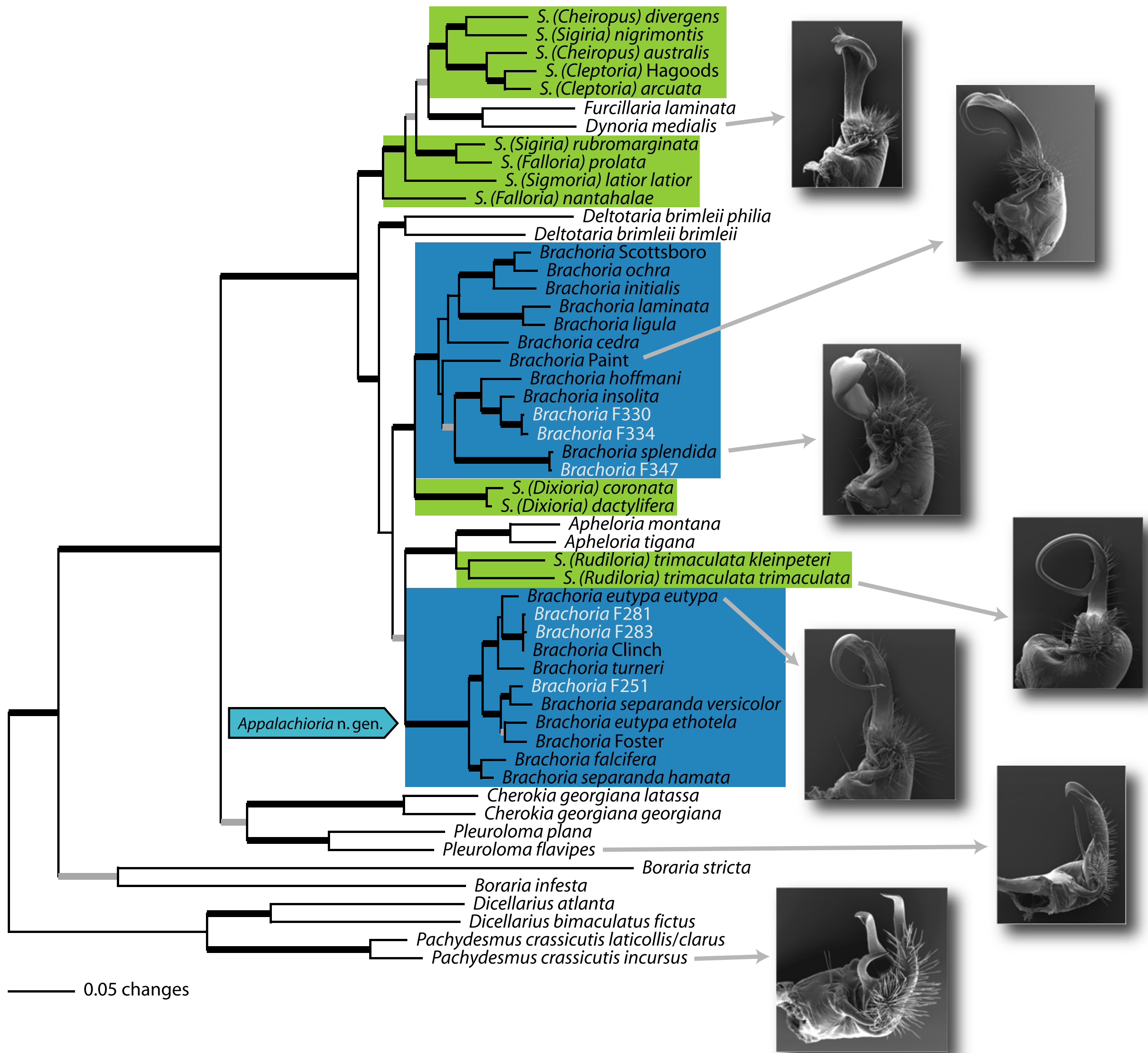

Fig. 5. Preferred phylogeny reconstructed using Bayesian inference from the combined intersection data set (using taxa with complete morphological and molecular characters and six unidentified female specimens), harmonic mean likelihood = −15,575.61 (thickened, black branches denote Pp values between 0.90 and 1.00; gray branches denote values between 0.70 and 0.89). SEMs of some example male gonopods to the right (left gonopod, medial view). Green boxes, nominal *Sigmoria s.l.* species, blue boxes, nominal *Brachoria* species. Exemplars in lightly-colored type denote unidentified female specimens as *Brachoria s.l.*

H_1 = *Brachoria* (*sensu* Keeton, 1959) constrained to be monophyletic against molecular topology (Fig. 4A).

3.5. Character mapping and ancestral state reconstruction

The Bayesian ancestral state reconstruction found a low probability ($P = 0.0966$) of a cingulum in the ancestral node of the separate *Brachoria* clades (Fig. 7, node 4). Probabilities that the cingulum occurred instead in ancestral nodes of *Appalachioria* + *Apheloria* + *Rudiloria* and *Dixioria* + *Brachoria* (their most recent common ancestors, MRCAs) were higher, at 0.3534 and 0.5178 respectively. Probability that a cingulum occurred on the ancestral node of *Dixioria* + *Brachoria* (node 2: $P = 0.5178$) was higher than *Appalachioria* + *Apheloria* + *Rudiloria* (node 6: $P = 0.3534$). A shorter branch length at node 1 (Fig. 7) corresponds with a higher probability of ancestral presence of a cingulum at the MRCA (node 2: $P = 0.5178$) of *Dixioria* + *Brachoria*, vice-versa in nodes seven and six. That is to say, *Brachoria* (at node one) is phylogenetically closer to its MRCA with its sister group (*Dixioria*) than *Appalachioria* is to its MRCA with its sister group (*Rudiloria* + *Apheloria*). Iteratively drawing 25 samples from the probability distribution of histories for character 29 (presence of a cingulum) and superimposing the resultant maps onto a single tree predict high probability of change on the branches leading to each separate *Brachoria s.l.* clade. During the 25 iterations, cingulum presence (Fig. 7, in black) also appeared on the

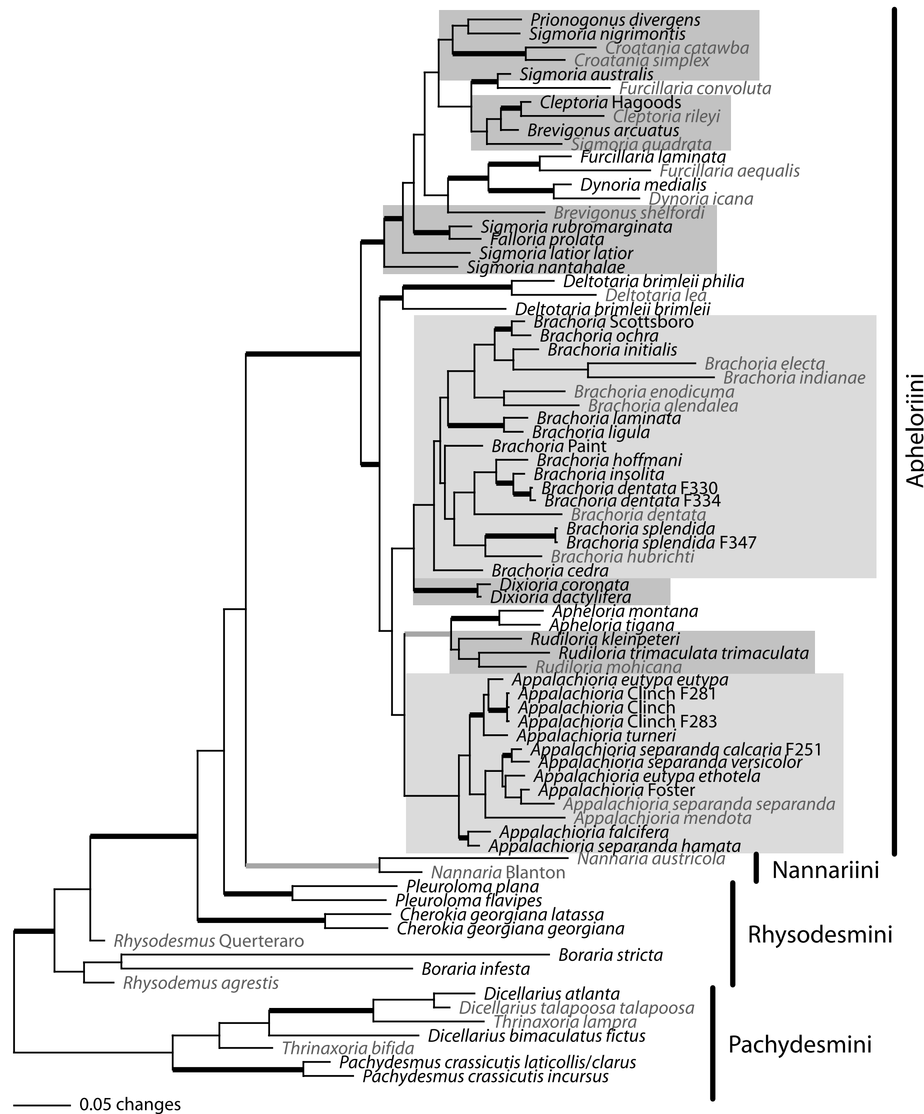

Fig. 6. Phylogeny reconstructed using Bayesian inference from the combined union data set (using all available data), harmonic mean likelihood = −16,284.12 (thickened, black branches denote Pp values between 0.90 and 1.00; gray branches denote values between 0.70 and 0.89). Nomenclature updated to reflect new classification. Dark gray boxes, nominal *Sigmoria s.l.* species, light gray boxes, nominal *Brachoria* species. Names in gray type denote exemplars with only morphological data and missing molecular. Those with an "F" and a number denote previously unidentified female specimens, which have been identified based on a combination of phylogenetic position and branch length (and the specimens' locality).

Table 1
Summary of Bayes factor comparisons of alternative phylogenetic hypotheses

Hypothesis	LnL: constrained	LnL: unconstrained	LnL: difference	Bayes factor [2ln(B_{10})]
H_1: *Brachoria* monophyly; H_0: Molecular tree (Fig. 4A)	−13990.94	−13953.67	−37.27	−74.54
H_2: *Sigmoria* monophyly; H_0: Molecular tree	−14105.4	−13953.67	−151.73	−303.46
H_3: SW86, Preliminary (Fig. 3A); H_0: Molecular tree	−14384.85	−13953.67	−431.18	−862.36
H_4: SW86, Revised (Fig. 3B); H_0: Molecular tree	−14325.74	−13953.67	−372.07	−744.14
H_5: SW86, Preliminary; H_0: Morphological tree (Fig. 4B)	−2097.2	−2047.83	−49.37	−98.74
H_6: SW86, Revised; H_0: Morphological tree	−2098.97	−2047.83	−51.14	−102.28

A relatively smaller Bayes factor value indicates lower predictive value of the alternative hypothesis (H_x) over the unconstrained topology (H_0). All Bayes factors imply "very strong" evidence (i.e., with an absolute value >10) in favor of the unconstrained null hypothesis based on the table described by Kass and Raftery (1995).

branch leading to the genus *Dixioria*, but change was predominately concentrated on those leading to the two separate *Brachoria* clades. Each character is annotated with the estimated number of transformations, dwell time per state, and homoplasy index (Appendix B). For presence of a cingulum, incorporating topological and parameter uncer-

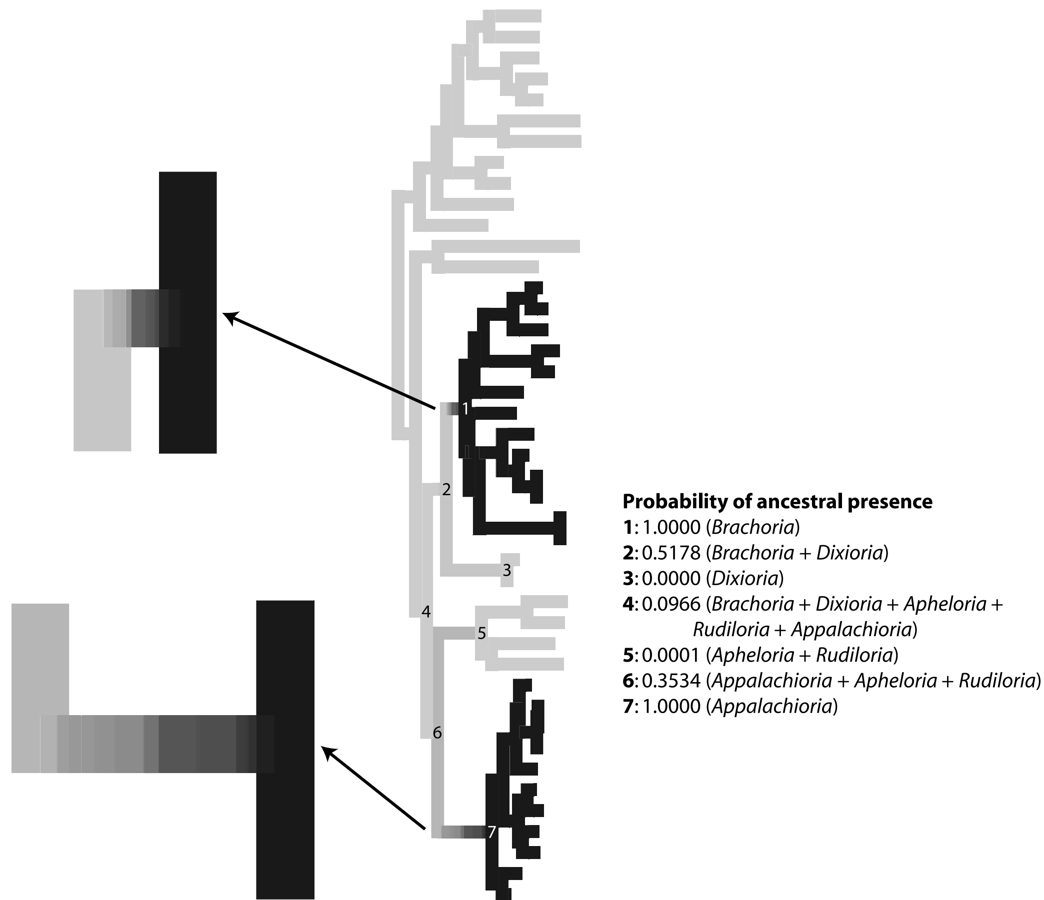

Fig. 7. Iterative posterior map and Bayesian ancestral-state reconstruction of the gonopodal cingulum using stochastic character mapping. Superimposition of 25 iterations drawn from the posterior distribution of characters histories for character 29, the diagnostic character of the genus *Brachoria* (gray, absence; black, presence). Full Bayesian ancestral state reconstruction (integrating uncertainty over posterior distribution of trees, estimated parameters and observed character states) of the gonopodal cingulum on seven nodes of the combined-I phylogeny.

tainty, the number of transformations = 2.1581; dwell time for each state, absent = 0.8317 and present = 0.1683; and homoplasy index = 0.5366 (Appendix A, Character 29).

4. Discussion

Combined phylogenetic inference (Figs. 5 and 6) and Bayes factor comparisons (Table 1) demonstrate that the current classification scheme as proposed by Shelley (2000a,b, 2002, 1986) and generic delineations within the tribe Apheloriini lack support and require revision. The genus *Brachoria*, a focal taxon, is polyphyletic and comprises two independently derived clades (Pp-D, I = 1.00, for both clades, Figs. 4A, and 5). One of these clades, (*Appalachioria* n. gen.: see Appendix C) is a sister-group to (*Apheloria* + *Rudiloria*) (Pp-I = 0.84, Fig. 5), and the other to *Dixioria* (Pp-D, I = 1.00, Figs. 4A, and 5). The genus *Sigmoria s.l.* is polyphyletic and comprises at least three independent lineages (one of which, the southern clade, is paraphyletic relative to the genera *Furcillaria* and *Dynoria*, Figs. 4A, 5, 6). However, several nominal taxa proved to be monophyletic, confirming prior taxonomic hypotheses: e.g., Apheloriini, Pachydesmini, Nannariini, and several genus–groups names.

4.1. Phylogenetic classification of Apheloriini

Prior phylogenetic hypotheses (SW86, Fig. 3A and B) are incompatible with the one presented here, except for a few relationships (i.e., Apheloriini monophyly, etc.). Consequently, Bayes factors were highly negative for all topological comparisons and suggested "very strong" evidence—i.e., with an absolute value >10—in favor of the unconstrained null hypothesis based on the table described by Kass and Raftery (1995). Of interest in calculating Bayes factors was not necessarily to absolutely accept or reject a topological hypothesis, but to assess the relative sizes of the posterior odds or the predictive likelihoods (Kass and Raftery, 1995; Nylander et al., 2004) of the constrained models (H_X). This is an evaluation of the Bayes factors; that is to say, how much more negative ("worse") are they from several alternative classification hypotheses (i.e., H_1:H_0 vs. H_2:H_0 vs. H_3:H_0, …), or how great of a margin is the predictive likelihood of a constrained tree smaller than another constrained tree? In comparison with the molecular topology (Fig. 4A), the topology constrained to a monophyletic *Sigmoria* (H_2) has a lower Bayes factor than the one constrained to a monophyletic *Brachoria* (H_1, Table 1). This is

evident from the fact that *Sigmoria s.l.* is "more" polyphyletic and occurs in three places on the tree while *Brachoria* (*sensu* Keeton, 1959) is in two. The preliminary phylogenetic hypothesis (H_3) of SW86 (Fig. 3A) contained both monophyletic *Brachoria* and *Sigmoria* and was derived from putative apomorphies of the gonopods; whereas the revised hypothesis (H_4) (Fig. 3B) was also built on these same morphological characters, but it heavily weighted relatedness with regard to geographical proximity (see the mosaic evolution concept, Whitehead *in* SW86). Both hypotheses have a very low (negative) Bayes factor, i.e., with a high absolute value, but the preliminary hypothesis has a slightly lower one ($H_3 = -862.36$ cf., $H_4 = -744.14$, Table 1). Therefore, geographical distributions did not provide definitive resolution to the evolutionary history.

The morphological data (Fig. 4B) fit the preliminary (Fig. 3A) and revised hypothesis (Fig. 3B) of SW86 better than the molecular data (H_5 and H_6, vs. H_3 and H_4, Table 1). The predictive likelihoods of the constrained topologies were closer to the unconstrained values (Bayes factors, $H_5 = -98.74$ and $H_6 = -102.28$). Is this due to the same confounding sources of morphological bias affecting both phylogenetic reconstructions similarly (i.e., the present one and the ones from SW86)? Because primary-homology hypotheses of past authors were thoroughly reassessed here, supplemental characters from several different authors used, and new characters added, it appears that the same confounding sources of bias affected both ours and previous analyses, resulting in similar spurious results. This further supports the idea that available morphological characters are of poor quality (evident from a high stochastically calculated homoplasy index) and may have precluded accurate phylogenetic reconstructions.

The preferred phylogenetic tree (combined-I, Fig. 5) is the basis of the new classification (Appendix C). The taxonomic modifications presented here take a conservative approach, deferring a detailed classification (one which will include more of the ~65 species in the southern clade) to future phylogenetic analyses. Nominal *Brachoria* species comprise two well-supported clades (Pp-D, I = 1.00, for each, Figs. 4A and 5). Consequently, a new genus for the nominal *Brachoria* clade is named, *Appalachioria*. Neither a monophyletic *Sigmoria s.l.* nor the supra-specific formulation in SW86 is consistent with the molecular Bayesian inferred topology (i.e., $H_2 - H_4$ all have Bayes factors < -300). Reflecting this, two genera previously classified in *Sigmoria s.l.*, *Dixioria*, and *Rudiloria*, are more closely related to nominal *Brachoria* species than they are to other *Sigmoria s.l.* species. Otherwise, excluding these two genera, *Sigmoria s.l.* with *Dynoria* and *Furcillaria* forms a monophyletic group, the "southern clade" (top clade in Fig. 5). However, only two supra-specific taxa (*Croatania* and *Dynoria*) of eight in the southern clade are monophyletic (Pp-U = 0.97 and 1.00, respectively in Fig. 6). Although not reflected in our taxonomic sampling, the southern clade contains the majority of the species in Apheloriini (~65 spp.) and almost certainly has a more complex evolutionary history than this study could accommodate. This analysis presents the need for a more thorough phylogenetic analysis of this southern clade.

The present phylogeny is more consistent with the genus–group delineations of Hoffman (1999) than those of SW86—that is, with a narrowly defined *Sigmoria* and recognition of the genera *Brevigonus* Shelley, 1980a; *Lyrranea* Hoffman, 1963; *Prionogonus* Shelley, 1982; and *Stelgipus* Loomis, 1944. However, our preferred phylogeny does not agree with several of Hoffman's hypotheses of genus monophyly (i.e., *Brachoria*, *Brevigonus*, *Cleptoria*, etc.) and its lack of phylogenetic resolution above the genus-level. As a result, we retain the generic nomenclature of Hoffman (adding a new genus, *Appalachioria*) and combine it with our supra-generic phylogeny to update the current classification (Appendix C).

4.2. Tribal phylogenetic relationships

Though tribal relationships were not of primary interest in this study, they are equally problematic. Contrary to prior hypotheses (SW86 in Fig. 3A and B), a sister-group relationship between Apheloriini and Pachydesmini is inconsistent with the phylogenetic hypotheses presented here (see Fig. 5). Although monophyly of the tribe Pachydesmini is universally supported (Pp-M = 0.99, Pp-D, I, U = 1.00), it is always placed outside of [Apheloriini + (*Cherokia* + *Pleuroloma*)] or [((Apheloriini + Nannariini) + *Pleuroloma*) + *Cherokia*], even with alternative rootings. A clade comprising the rhysodesmine genera *Cherokia* and *Pleuroloma* (Pp-D = 0.88, Pp-I = 0.79) was always sister to Apheloriini (Pp-D, I = 1.00), except in the combined-U phylogeny (Fig. 6); this relationship was hypothesized previously based primarily on female cyphopodal characters (Hoffman, 1950, 1960; Shelley, 1980a). On the other hand, the relationship between (*Cherokia* + *Pleuroloma*) and *Boraria* Chamberlin, 1943b suggested by Shelley (1980a) is unsupported by the analyses presented here. The sister-group relationship between *Cherokia* and *Pleuroloma* in the molecular and combined-I trees, however, may be artificial. In the combined-U analysis (Fig. 6), these genera do not form a monophyletic group as in the molecular and combined-I trees. Because the combined-U analysis, which was more thoroughly-sampled, split *Cherokia* and *Pleuroloma* into separate paraphyletic clades, a monophyletic group comprising these rhysodesmine genera may be an artifact of incomplete taxon sampling and long-branch attraction.

In all analyses, the tribe Rhysodesmini (represented in this analysis by *Cherokia*, *Pleuroloma*, *Boraria*, and *Rhysodesmus*) is paraphyletic with respect to Apheloriini. Because only a fraction of rhysodesmine diversity, which includes about 80 species in 11 genera (Hoffman, 1999), has been included in the present analysis, the statement that "Rhysodesmini is paraphyletic to the Apheloriini + Nannariini" does not nearly do justice for a summary of the evolutionary history of the tribe. Rhysodesmini is a "dumping-ground" for a multitude of enigmatic eastern xystodesmid genera with long, acicular prefemoral processes (e.g.,

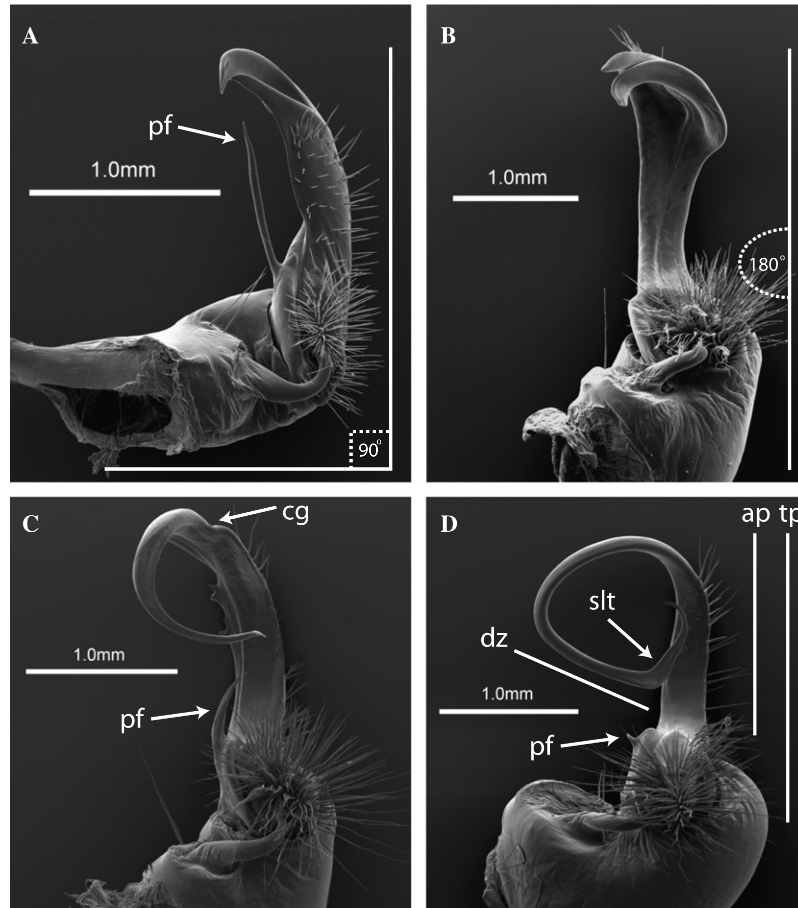

Fig. 8. Diagnostic gonopodal features of apheloriine millipedes (medial view of the left gonopod): (A) *Pleuroloma flavipes*, telopodite–coxa articulated at a 90° angle; (B) *Dynoria medialis*, telopodite–coxa articulated at a 180° angle (uniquely derived for Apheloriini), prefemoral process (pf) absent (prefemoral process present, short and stout is uniquely derived for Apheloriini, but further modified to be absent in *Dynoria* and other taxa); (C) *Appalachioria eutypa eutypa*, prefemoral process present (pf), short and stout (uniquely derived for Apheloriini), cingulum (cg) present; (D) *Rudiloria trimaculata trimaculata*, cingulum absent, distal zone (dz), sickle-like tip (slt), acropodite (ap), telopodite (tp).

the "micro-xystodesmids": *Caralinda* Hoffman, 1978a; *Gonoessa* Shelley, 1984a; *Lourdesia* Shelley, 1991, and *Parvulodesmus* Shelley, 1983). Future phylogenetic analyses are required to explore the intricacies of this tribe, which is likely to remain paraphyletic. These analyses should include a broader sampling of genus-exemplars to assess evolutionary relationships of xystodesmid genera and tribes, especially with respect to relationships between the eastern and western Nearctic faunas.

4.3. Supraspecific phylogenetic relationships

Though the phylogeny presented here departs radically from prior hypotheses of supra-specific relationships, several morphological apomorphies are consistent with its branching pattern. Each of the two *Brachoria* clades is very well-supported (Pp-D, I = 1.00, for both clades). Additionally, the two clades have clear sister-groups: i.e., *Dixioria* and (*Apheloria* + *Rudiloria*). The clade containing [*Brachoria*, now *Appalachioria* + (*Apheloria* + *Rudiloria*)] (Pp-I = 0.84) is consistent with several gonopodal apomorphies (Appendix B; see Fig. 8D for terminology). *Brachoria falcifera* Keeton, 1959 (now *Appalachioria*), a species at the base of the clade (Figs. 4A, 5, and 6), has an extremely slender acropodite with a sickle-like tip and a distal zone surface (containing the prostatic groove) one-quarter twisted and directed posterolaterally, similar to the species *Sigmoria* (*Rudiloria*) *trimaculata trimaculata* (Wood, 1864), now *Rudiloria*. Fundamentally the gonopodal acropodite of *B. falcifera* is very similar to the *R. trimaculata* acropodite (Fig. 8D), but with a cingulum. The other *Brachoria* clade is sister to *Dixioria* and strongly supported by Pp values (Pp-D, I = 1.00) and two shared, derived nucleotide site substitutions (site number in parentheses, alignment available for download at www.treebase.org matrix Accession No. M2738): A(993) and C(1210). The sister-group containing *Apheloria* + *Rudiloria* is strongly supported by posterior clade probabilities (Pp-D = 1.00, Pp-I = 1.00, and Pp-U = 0.79) and the unique combination of derived characters (Appendix B): presence of a medial flange on the basal zone of the acropodite, telopodite with a well-defined anterior twist strongly twisted cephalically, and a long distal zone greater than or equal to half the length of the acropodite. This relationship has been previously mentioned by

Shear (1972), who synonymized *Rudiloria* into *Apheloria*, and Hoffman (1978b), who suggested a close affiliation but a generic distinction. The genus *Deltotaria* Causey, 1942 is polyphyletic in the molecular tree, but is a well-supported clade in the morphological and combined trees (Pp-M = 0.71, Pp-I = 0.90, Pp-U = 0.95) and is supported by a unique combination of derived characters: presence of a gonopodal coxal apophysis and a flat anterior dorsolateral paranotal disc. The phylogenetic placement of this genus is not consistent with the preliminary, nor revised phylogenetic hypotheses of SW86. The phylogenetic analysis also recovered a polyphyletic *Sigmoria s.l.* The two *Sigmoria s.l.* subgenera, *Dixioria* and *Rudiloria*, are not related to the other subgenera; instead they are more closely related to *Brachoria s.l.* species. The genus *Sigmoria s.l.* is polyphyletic and comprises three separately derived lineages: *Dixioria*, *Rudiloria*, and the "southern clade". The southern clade is well-supported (Pp-D = 1.00, Pp-I = 0.99, Pp-U = 0.92) and made up of exemplars from the *Sigmoria s.l.* subgenera *Cheiropus* Loomis, 1944; *Cleptoria* Loomis, 1944; *Sigiria* Chamberlin, 1939; *Falloria* Hoffman, 1948; and from exemplars in the genera *Furcillaria* Shelley, 1981 and *Dynoria* Chamberlin, 1939. All of the nominal genus–group names in the tribe (except for *Croatania*, which is represented in the combined-U data set) are represented in the analysis, but only a fraction of about 65 nominal species are included. In addition, nearly all of the nominal genus–groups are non-monophyletic. Therefore, the diversity of the clade may be more complicated than this phylogeny presents. The southern clade certainly contains more genera (including *Dynoria* and *Furcillaria*) than the single genus *Sigmoria* can accommodate. The tribe Apheloriini, in which these genera reside, is a well-supported clade (Pp-M = 0.97, Pp-D, I, U = 1.00) confirming prior hypotheses of tribal monophyly (Hoffman, 1979, 1999; Shelley and Whitehead, 1986). Synapomorphies that support this clade are: telopodite-coxa articulated at a 180° angle (Fig. 8A); prefemoral process present, short and stout (Fig. 8C and D), or absent (Fig. 8B), never long and acicular (Fig. 8A); and nucleotide site substitutions (site number in parentheses, alignment available for download at www.treebase.org matrix Accession No. M2738): T(92), C(1077).

4.4. Classification problems

Modern phylogenetic methodology is seldom applied to classification problems in the Diplopoda. However, phylogenetic relationships should underlie classifications (Franz, 2005; Hennig, 1966; Platnick, 1977; Ronquist, 2004; Wiley et al., 1991), and accurate and repeatable methods for phylogeny reconstruction should be used (Prendini and Wheeler, 2005). Unfortunately, these techniques have largely been unexploited for inferring the evolutionary history of many millipede taxa. Although quicker and non-repeatable approaches to taxonomy should be adopted within a context of biodiversity and conservation (Erwin and Johnson, 2000), or when results are needed rapidly, repeatable and accurate methods should be used for beta-taxonomy (e.g., revisions and classifications).

As mentioned in the introduction, the traditional method to build classifications in millipedes is a "bottom-up" approach where all available museum material (including *every* species of a focal taxon) is compared to make hypotheses about relationships using morphological diagnosibility (i.e., the establishment of taxa based on gaps or discontinuities in morphological features—apomorphies). In our opinion, this perspective can be cumbersome and sometimes impede taxonomy if all species must be examined to build a classification. For example, if a taxonomist wishes to build a classification system for the family Xystodesmidae using this bottom-up technique, s/he must examine 300+ species for important diagnostic characters (a conservative species estimate for this family and small for most arthropod families). The number of specimens involved becomes overwhelmingly large—and the task unmanageable—when multiple specimens per species (female paratypes/non-type material) are included. The species-rich and widespread genera *Apheloria* and *Brachoria* have never been globally considered within the larger context of Apheloriini because they contained a large number of species, particularly undescribed ones. As a result, they were provisionally located in equivocal positions near the base of the inferred phylogeny (SW86, Figs. 3A and B).

Incorporating testable, repeatable, and accurate methods for the phylogenies underlying millipede classification is of principal importance to the advancement of the field. When phylogenetic methodology is implemented, multiple character systems are used, and species are the terminal units, classification schemes based on such approaches are explicit, testable hypotheses. As a consequence, they have a greater potential to be more accurate, precise, and stable. As evidenced from the past instability in Apheloriini classification, hypotheses that lack explicit phylogenetic methodology and character support are doomed to vacillate from the opinions of one worker to another. Therefore, the burden of overturning a classification requires more than an unrepeatable visual estimation of morphological gaps and dubious conjecture regarding geographical distribution. Minimally, transparent and repeatable phylogenetic methods such as those employed here should be fully utilized so future workers can reproduce the analysis. Overturning, or ignoring our classification system on the basis of unsupported opinion or deference to authority should be unacceptable to the systematics and diplopod community as a whole (see Prendini and Wheeler, 2005 for a salient discussion of this issue). If there is a preexisting exemplar phylogeny, as now is the case for the tribe Apheloriini, subsequent taxonomic workers can *easily* incorporate additional taxa, include more characters, and test these hypotheses to change the classification we have proposed.

The preponderant use of male genitalia (i.e., gonopodal characters) in traditional millipede taxonomy may have negatively impacted prior classifications in the Apheloriini through biased character choice. The use of a single data set

built almost entirely on gonopodal characters may have misled the phylogeny behind the classification systems. Indeed, taxonomy built largely on these characters and on the premise of rapid and divergent genitalic evolution has recently been called into question (Bond et al., 2003; Bond and Sierwald, 2003).

The problem of bias in morphological characters has been addressed in this study by the simultaneous analysis of combined evidence, i.e., female and male genitalia, somatic (Appendix B), and DNA sequence characters. Phylogenies reconstructed independently from morphology and sequence characters, however, results in two different topologies (e.g., monophyly versus polyphyly of *Brachoria*, Figs. 4A and B). Of note, the morphological tree is partially congruent with the phylogenies of SW86. Similar biases may have led both phylogenies (i.e., the morphology-inferred phylogeny from the present analysis and the hypotheses of SW86) to converge on a similar but incorrect result (see above regarding Bayes factors between unconstrained versus constrained morphological topologies).

The morphology-derived tree is made less reliable due to a large amount of homoplasy in the morphological characters. As already mentioned, the phylogenies reconstructed separately from morphological and molecular characters were incongruent. These differences in topology and Pp values are attributed to a large amount of confounding homoplasy in the morphological-character partition. Homoplasy indices ($HI = 1 - \text{minimum transformations/estimated number of transformations}$), which were stochastically calculated from the combined-I tree for morphological characters, were on average rather high, but were slightly lower in the genitalic (male gonopods, $HI = 0.6227$; female cyphopods, $HI = 0.6596$) than in the somatic characters ($HI = 0.6915$). Furthermore, the phylogeny inferred using only morphology had a larger quantity of unsupported clades ($Pp < 0.71$) than did the molecular phylogeny. Homoplasy is therefore apparent in the data set, although no specific case of homoplasy, such as adaptive convergence (Wiens et al., 2003) or character nonindependence (Wiens et al., 2005) can be seen.

The diagnostic character for *Brachoria*, the cingulum, has a homoplasy index of 0.5366 and it transitioned about two times (2.1581) on the apheloriine exemplar phylogeny (Appendix B, Character 29), suggesting an independent derivation in two different clades (Fig. 7). The character map of the cingulum, iteratively sampled from the posterior character-history distribution, shows that state change from absence (gray) to presence (black, Fig. 7) is largely concentrated on branches leading to the two separate *Brachoria* clades; moreover, the probability of ancestral presence of a cingulum is equal to one for both the nominal clades (Fig. 7, node 1 and 7) and sharply decreases to $P = 0.0966$ towards their common ancestor farther down the tree (Fig. 7, node 4). Based on this low probability, it is unlikely a cingulum occurred in the ancestral node of the separate *Brachoria* clades. As a result, the cingulum as a primary character is in itself inappropriate for taxonomy and must be qualified with additional characters for diagnosis of these clades.

5. Conclusions and future directions

Our study of Apheloriini phylogeny shows considerable disagreement with the preexisting classification scheme. Combining mitochondrial ribosomal DNA and morphology, we tested previous taxonomic hypotheses using repeatable methods. Specimens included exemplars representing all of the genus-group names in the tribe Apheloriini, an expanded sample of species in the genus *Brachoria* (22 nominal species groups out of 32 and four undescribed species) and three closely related tribes: Nannariini, Pachydesmini, and Rhysodesmini. The total-evidence tree of Fig. 5 (combined-I), our preferred hypothesis, recovers both nominal *Brachoria* and *Sigmoria s.l.* as polyphyletic and is inconsistent with the most widely accepted prior hypotheses (SW86, in Fig. 3A and B). Genus–group classification provisionally reverts back to Hoffman (1999), which split-up genera more than SW86, for the tribe Apheloriini (Appendix C) with addition of a new genus *Appalachioria*. Although a heterogeneous view of genus–group nomenclature is more congruent with the preferred phylogeny in this paper, it is not “heterogeneous enough”. Prior classifications and the present study likely underestimate true underlying diversity, especially in the speciose “southern clade.”

Despite the rather definitive conclusions we draw from the combined data set presented in Figs. 5 and 6, the higher-level classification of the Apheloriini will undoubtedly undergo future refinement. One limitation is that our molecular data were sampled from two linked markers drawn from the mitochondrial genome; a nuclear marker that corroborates these results would be ideal. However, the available nuclear genes that we have sampled so far (Marek and Bond, unpublished data) do not provide the level of resolution needed for the taxonomic breadth of this study. Such markers that work in diplopod taxa are currently under development. Also, we would expand the study to include more taxa. Areas in need of increased taxonomic sampling include the tribe Rhysodesmini, additional species from the southern clade (e.g., *Sigmoria*, *Dynoria*, *Furcillaria*), and the micro-xystodesmids. It is our hope that this work will serve to provide a new framework for documenting and describing the incredible evolutionary diversity in this interesting arthropod group.

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Appendix A

List of taxa sampled

Taxon	Spc#—M	Spc#—F[3]	Latitude	Longtitude	State, county	NCBI Accession No.
Apheloriini						
Apheloria montana (Bollman, 1887) (mrp[1], dna[2])	SPC000134	SPC000133	35.73446	−82.08378	North Carolina, McDowell	DQ490660
A. tigana Chamberlin, 1939 (mrp, dna)	SPC000311	A4274	35.84460	−78.75750	North Carolina, Wake	DQ490687
Brachoria cedra Keeton, 1959 (mrp, dna)	SPC000276	—	36.66032	−83.18390	Virginia, Lee	DQ490680
B. dentata Keeton, 1959 (mrp)	PMLN0022	PMLN0022	36.80180	−82.92180	Virginia, Lee	—
B. electa Causey, 1955 (mrp)	PMLN0019	PMLN0019	37.82690	−84.72540	Kentucky, Mercer	—
B. enodicuma Keeton, 1965 (mrp)	PMLN0083	PMLN0083	34.63190	−85.93330	Alabama, Jackson	—
B. eutypa ethotela Chamberlin, 1942 (mrp, dna)	SPC000293	SPC000291	36.88190	−81.52340	Virginia, Smyth	DQ490685
B. eutypa eutypa Chamberlin, 1939 (mrp, dna)	SPC000226	SPC000229	36.07860	−81.77860	North Carolina, Avery	DQ490668
B. falcifera Keeton, 1959 (mrp, dna)	SPC000259	—	37.12882	−81.87666	Virginia, Tazewell	DQ490677
B. glendalea (Chamberlin, 1918a,) (mrp)	PMLN0059	PMLN0059	36.06810	−86.88060	Tennessee, Davidson	—
B. hoffmani Keeton, 1959 (mrp, dna)	SPC000261	SPC000265	37.29292	−82.30893	Virginia, Dickenson	DQ490678
B. hubrichti Keeton, 1959 (mrp)	PMLN0015	PMLN0015	35.09220	−85.64670	Tennessee, Marion	—
B. indianae (Bollman, 1888) (mrp)	PMLN0109	PMLN0109	38.48170	−85.50980	Indiana, Clark	—
B. initialis Chamberlin, 1939 (mrp, dna)	SPC000083	SPC000084	31.73469	−88.19488	Alabama, Choctaw	DQ490658
B. insolita Keeton, 1959 (mrp, dna)	SPC000275	—	36.89532	−82.60513	Virginia, Wise	DQ490679
B. laminata Keeton, 1959 (mrp, dna)	SPC000258	SPC000264	37.16602	−81.70370	Virginia, Tazewell	DQ490676
B. ligula Keeton, 1959 (mrp, dna)	SPC000324	SPC000322	37.43356	−81.57610	West Virginia, McDowell	DQ490688
B. mendota Keeton, 1959 (mrp)	PMLN0028	—	36.95340	−82.05480	Virginia, Russell	—
B. ochra (Chamberlin, 1918a,) (mrp, dna)	SPC000077	SPC000091	34.30959	−87.39433	Alabama, Lawrence	DQ490655
Brachoria F251 (F[4]) (dna)	—	SPC000251	37.13190	−80.52802	Virginia, Montgomery	DQ490673
B. separanda hamata Keeton, 1959 (mrp, dna)	SPC000325	—	37.08271	−81.30132	Virginia, Tazewell	DQ490689
B. separanda separanda Chamberlin, 1947 (mrp)	PMLN0078	PMLN0078	38.48620	−79.67090	Virginia, Highland	—
Brachoria Foster (mrp, dna)	SPC000296	SPC000294	36.89021	−80.83755	Virginia, Wythe	DQ490686
B. separanda versicolor Hoffman, 1963 (mrp, dna)	SPC000257	PMLN0120	37.06032	−81.29519	Virginia, Bland	DQ490675
Brachoria Scottsboro (mrp, dna)	SPC000071	SPC000070	34.60658	−86.11101	Alabama, Jackson	DQ490653
Brachoria Paint (mrp, dna)	SPC000220	SPC000217	36.97738	−82.84390	Tennessee, Greene	DQ490666
Brachoria Clinch (mrp, dna)	SPC000282	—	36.72317	−82.29852	Virginia, Washington	DQ490682
Brachoria F283 (F) (dna)	—	SPC000283	36.72317	−82.29852	Virginia, Washington	DQ490683
Brachoria F281 (F) (dna)	—	SPC000281	36.72317	−82.29852	Virginia, Washington	DQ490681
B. splendida (Causey, 1942) (mrp, dna)	SPC000341	SPC000344	36.73537	−83.73924	Kentucky, Bell	DQ490693
B. turneri Keeton, 1959 (mrp, dna)	SPC000288	SPC000289	36.81711	−81.92088	Virginia, Washington	DQ490684
Brachoria F347 (F) (dna)	—	SPC000347	36.73537	−83.73924	Kentucky, Bell	DQ490694
Brachoria F330 (F) (dna)	—	SPC000330	36.80174	−82.92245	Virginia, Lee	DQ490690
Brachoria F334 (F) (dna)	—	SPC000334	36.80174	−82.92245	Virginia, Lee	DQ490691
Sigmoria (Cheiropus) australis Shelley, 1986 (mrp, dna)	SPC000080	SPC000082	30.57910	−84.94170	Florida, Liberty	DQ490657
S. (C.) divergens Chamberlin, 1939 (mrp, dna)	SPC000039	SPC000041	35.11707	−82.63942	South Carolina, Greenville	DQ490650
S. (Cleptoria) arcuata (Shelley, 1981) (mrp, dna)	SPC000423	SPC000420	34.40480	−82.57786	South Carolina, Anderson	DQ490699
S. (C.) rileyi (Bollman, 1887) (mrp)	A2610	A2610	33.95340	−83.53790	Georgia, Oconee	—
S. (C.) shelfordi Loomis, 1944 (mrp)	A1548	A1548	34.09820	−82.35790	South Carolina, Abbeville	—
S. (*C.*) Hagoods (mrp, dna)	SPC000389	SPC000390	33.21206	−81.31976	South Carolina, Barnwell	DQ490697
S. (Croatania) catawba (Shelley, 1977) (mrp)	A1505	A1505	34.85290	−81.67430	South Carolina, Union	—
S. (C.) simplex (Shelley, 1977) (mrp)	A1489	A1489	34.68250	−81.24170	South Carolina, Chester	—
S. (Dixioria) coronata (Hoffman, 1949) (mrp, dna)	SPC000166	SPC000163	36.65547	−81.58620	Virginia, Smyth	DQ490665

Appendix A (*continued*)

Taxon	Spc#—M	Spc#—F[3]	Latitude	Longtitude	State, county	NCBI Accession No.
S. (D.) dactylifera (Hoffman, 1956) (mrp, dna)	SPC000223	—	36.40880	−81.58610	North Carolina, Ashe	DQ490667
S. (Falloria) nantahalae Hoffman, 1958 (mrp, dna)	SPC000244	SPC000249	35.34880	−83.97680	North Carolina, Graham	DQ490670
S. (F.) prolata Shelley, 1986 (mrp, dna)	SPC000145	SPC000147	35.72054	−83.39545	Tennessee, Sevier	DQ490663
S. (Rudiloria) mohicana (Causey, 1955) (mrp)	A5362	A5362	40.61060	−82.31290	Ohio, Ashland	—
S. (R.) trimaculata kleinpeteri (Hoffman, 1949) (mrp, dna)	SPC000164	—	36.71343	−81.46003	Virginia, Grayson	DQ490664
S. (R.) trimaculata trimaculata (Wood, 1864) (mrp, dna)	SPC000253	SPC000252	37.42802	−80.49935	Virginia, Giles	DQ490674
S. (Sigiria) nigrimontis (Chamberlin, 1947) (mrp, dna)	SPC000246	A665	35.76480	−82.26510	North Carolina, Yancey	DQ490671
S. (S.) rubromarginata (Bollman, 1888) (mrp, dna)	SPC000055	—	34.75646	−84.70615	Georgia, Murray	DQ490652
S. (Sigmoria) latior latior (Brölemann, 1900) (mrp, dna)	SPC000227	A8698	36.07860	−81.77860	North Carolina, Avery	DQ490669
S. (Sigmoria) quadrata Shelley, 1981 (mrp)	A8184	A8184	34.09590	−81.13350	South Carolina, Richland	—
Deltotaria brimleii brimleii Causey, 1942 (mrp, dna)	SPC000142	—	35.63648	−83.49181	Tennessee, Sevier	DQ490662
D. brimleii philia (Chamberlin, 1949a,b) (mrp, dna)	SPC000047	—	34.80362	−83.12994	South Carolina, Oconee	DQ490651
D. lea Hoffman, 1961 (mrp)	A0713	—	35.18250	−81.27390	North Carolina, Gaston	—
Furcillaria aequalis Shelley, 1981 (mrp)	A1462	A1462	34.53170	−81.84910	South Carolina, Laurens	—
F. convoluta Shelley, 1981 (mrp)	A1562	A1562	34.53170	−81.84910	South Carolina, Laurens	—
F. laminata Shelley, 1981 (mrp, dna)	SPC000421	SPC000424	34.40480	−82.57786	South Carolina, Anderson	DQ490698
Dynoria icana Chamberlin, 1939 (mrp)	A4626	A4626	35.04840	−83.44990	North Carolina, Macon	—
D. medialis Chamberlin, 1949 (mrp, dna)	SPC000431	SPC000427	33.25058	−83.92334	Georgia, Butts	DQ490700
Nannariini						
Nannaria austricola Hoffman, 1950 (mrp)	SPC000352	SPC000353	35.06337	−83.43687	North Carolina, Macon	—
Nannaria Blanton (mrp)	SPC000177	SPC000181	36.85942	−83.38239	Kentucky, Harlan	—
Rhysodesmini						
Pleuroloma flavipes Rafinesque, 1820 (mrp, dna)	SPC000338	SPC000340	36.92891	−83.19141	Kentucky, Bell	DQ490692
P. plana Shelley, 1980 (mrp, dna)	SPC000119	SPC000115	30.57910	−84.94170	Florida, Liberty	DQ490659
Boraria infesta (Chamberlin, 1918a,) (mrp, dna)	SPC000248	SPC000232	35.76480	−82.26510	North Carolina, Yancey	DQ490672
B. stricta (Brölemann, 1896) (mrp, dna)	SPC000135	SPC000241	35.83420	−82.40938	North Carolina, Yancey	DQ490661
Cherokia georgiana georgiana (Bollman, 1889) (mrp, dna)	SPC000354	SPC000356	35.06337	−83.43687	North Carolina, Macon	DQ490695
C. georgiana latassa Hoffman, 1960 (mrp, dna)	SPC000073	SPC000072	34.60658	−86.11101	Alabama, Jackson	DQ490654
Rhysodesmus agrestis Shelley, 1999 (mrp)	A8427	A8427	35.90210	−83.95530	Tennessee, Knox	—
Rhysodesmus Querteraro (mrp)	A3966	A3966	19.33889	−96.56667	Mexico, Querteraro	—
Pachydesmini						
Dicellarius atlanta (Chamberlin, 1946) (mrp, dna)	SPC000428	A1855	33.25058	−83.92334	Georgia, Butts	DQ490648
D. bimaculatus fictus (Chamberlin, 1943a,b) (mrp, dna)	SPC000079	—	30.57910	−84.94170	Florida, Liberty	DQ490656
D. talapoosa talapoosa (Chamberlin, 1939) (mrp)	A3117	A3117	33.47530	−85.81940	Alabama, Cleburne	—
Pachydesmus clarus (Chamberlin, 1918a,) (mrp)	A4371	A4371	31.73600	−92.40000	Louisiana, Grant	—
P. crassicutis incursus Chamberlin, 1939 (mrp, dna)	SPC000380	SPC000397	33.13690	−81.43390	South Carolina, Barnwell	DQ490696
P. crassicutis laticollis (Attems, 1899) (dna)	—	SPC000010	35.90401	−84.99195	Tennessee, Cumberland	DQ490649
Thrinaxoria bifida (Wood, 1864) (mrp)	A1847	A2607	34.76160	−85.00350	Georgia, Whitfield	—
T. lampra (Chamberlin, 1918a,) (mrp)	A8921	A8921	33.39230	−94.09240	Texas, Bowie	—

Alphabetical by species (tribe listed at top). [1]mrp, specimen used in the morphological partition; [2]dna, specimen used in the molecular partition; [3]female-specimen localities not shown, available by request from the corresponding author; and [4]unidentified female specimens (F). Taxa with a non-italicized locality-name in place of a specific epithet are undescribed. Spc#, specimen number. Specimens with a "SPC" prefix are presently housed in the millipede collection at East Carolina University and will ultimately be deposited in the Field Museum of Natural History, Chicago, Illinois, USA; those with an "A" prefix are stored at the North Carolina Museum of Natural Sciences, Raleigh, North Carolina, USA; those with a "PMLN" are from the Virginia Museum of Natural History, Martinsville, Virginia, USA.

Appendix B

Qualitative, morphological characters used in the analysis (binary and multistate). 1–40, male gonopodal characters; 41–45, female cyphopodal characters; 46–68, male somatic characters. Stochastic character statistics calculated from the combined-I data set. DT, dwell time spent in state *i*; TR, number of character transitions; HI, homoplasy index. *Characters/character states used in combined-U analysis and not in the combined-I analysis.

(1) ♂ Gonopodal coxal sternum, presence: present, coxae connected with sternum (0), DT = 0.3351; absent, coxae connected with membrane only (1), DT = 0.6649. TR = 2.2322; HI = 0.5520. Notes: SW86:214 suggest that the absence of a sternum between the gonopodal coxae of segment seven is an apomorphy uniting Apheloriini, Pachydesmini, and Nannariini. The presence of a sternum is a character of the tribe Rhysodesmini (Hoffman, 1960; Shelley, 1980a).

(2) ♂ Gonopodal coxal apophysis, presence: absent (0), DT = 0.9198; present (1), DT = 0.0802. TR = 2.4750; HI = 0.5960. Notes: A coxal apophysis is present in the genera *Deltotaria*, *Pachydesmus*, and *Xystodesmus* Cook, 1895 (Hoffman, 1956, 1958; Shelley, 1984b; Shelley and Whitehead, 1986).

(3) ♂ Gonopodal telopodite–coxa angle: 90° articulation between telopodite and coxa (0), DT = 0.4950; 180° articulation between telopodite and coxa (1), DT = 0.5050. TR = 1.0898; HI = 0.0824. Notes: Shelley, 1980a, p. 135 states that a 90° articulation between the telopodite and coxa is a tribal feature of Rhysodesmini.

(4) ♂ Gonopodal prefemoral process, presence: present (0), DT = 0.9431; absent (1), DT = 0.0569. TR = 3.2341; HI = 0.6908.

(5) ♂ Gonopodal prefemoral process, shape: present, long, acicular (0), DT = 0.4949; present, short, stout (1), DT = 0.4487; absent (2), DT = 0.0564. TR = 4.2128; HI = 0.5253. Notes: except for the pachydesmine species *Dicellarius okefenokensis* (Chamberlin, 1918b), the prefemoral process is longer and needle-shaped in Rhysodesmini, Nannariini, and Pachydesmini compared to a short and stout or absent prefemoral process in Apheloriini (SW86:216).

(6) ♂ Gonopodal basal zone inner surface, orientation relative to a perpendicular axis arising ventrally from the gonopodal coxa: twisted anteromedially (0), DT = 0.4989; medially (1), DT = 0.5011. TR = 8.6996; HI = 0.8851.

(7) ♂ Gonopodal basal zone tubercle one, presence: absent (0), DT = 0.9785; present (1), DT = 0.0215. TR = 2.1034; HI = 0.5246.

(8) ♂ Gonopodal basal zone tubercle two, presence: absent (0), DT = 0.9955; present (1), DT = 0.0045. TR = 2.1311; HI = 0.5308.

(9) ♂ Gonopodal basal zone tubercle two, size: absent (0), DT = 0.9961; enlarged (1), DT = 0.0013; large spur (2), DT = 0.0026. TR = 2.0733; HI = 0.0354.

(10) ♂ Gonopodal basal zone medial flange, presence: absent (0), DT = 0.9603; present (1), DT = 0.0397. TR = 3.7566; HI = 0.7338.

(11) ♂ Gonopodal anterior bend + apical curve, presence: absent (0), DT = 0.5319; present (1), DT = 0.4681. TR = 2.1838; HI = 0.5421. Notes: *Brevigonus shelfordi* has an anterior bend, but no apical curve or distal zone. In this species, the acropodite terminates at the distal extremity of the peak (SW86).

(12) ♂ Gonopodal basal and distal zones, planation: distal zone absent (0), DT = 0.5033; not coplanar (1), DT = 0.4840; coplanar, distal zone at a right angle from the peak (2), DT = 0.0128. TR = 4.1877; HI = 0.5224. Notes: short distal zones, coplanar with the basal zones and directed perpendicularly from the peak, are present in five species of *Cleptoria* (SW86:13).

(13) ♂ Gonopodal anterior twist, definition: anterior twist absent (0), DT = 0.5086; broad and poorly defined, slightly twisted cephalically (1), DT = 0.2720; well-defined and strongly twisted cephalically (2), DT = 0.2194. TR = 10.0882; HI = 0.8017. Notes: anterior twist = medial bend and a cephalic twist; occurs at the same place as the anterior bend. Distinct from the anterior bend (geniculation) and torsion (rotational twisting).

(14) ♂ Gonopodal "medial" flange, presence: absent (0), DT = 0.7794; present (1), DT = 0.2206. TR = 9.8839; HI = 0.8988. See SW86:14 for discussion of this character.

(15) ♂ Gonopodal acropodite medial margin tooth, presence: absent (0), DT = 0.9056; present (1), DT = 0.0944. TR = 7.4118; HI = 0.8651. Notes: see SW86:14 for discussion of this character.

(16) ♂ Gonopodal peak tooth, presence: absent (0), DT = 0.9678; present (1), DT = 0.0322. TR = 4.2371; HI = 0.7640.

(17) ♂ Gonopodal acropodite medial margin accessory tooth, presence: absent (0), DT = 0.9979; present (1), DT = 0.0021. TR = 1.0836; HI = 0.0772. Notes: see SW86:14 for discussion of this character.

(18) ♂ Gonopodal distal zone-peak, angle: straight, 0° (0), DT = 0.5327; bent, non-circular angle to 90° (1), DT = 0.4623; circular (2), DT = 0.0051. TR = 3.1617; HI = 0.3674. Notes: see SW86:12 for discussion of this character.

(19) ♂ Gonopodal distal zone, area distal to apical curve, orientation: distal zone absent, straight gonopod (0), DT = 0.5028; curved medially (1), DT = 0.4763; curved laterally (2), DT = 0.0209. TR = 6.3097; HI = 0.6830. Notes: see SW86:12 for discussion of this character.

(20) ♂ Gonopodal distal zone, length: distal zone absent (0), DT = 0.5301; short, less than 0.4× length of

acropodite (1), DT = 0.3921; long, greater than or equal to 0.5× length of acropodite (2), DT = 0.0778. TR = 13.4620; HI = 0.8514. Notes: see SW86:12 for discussion of this character.

(21) ♂ Gonopodal lateral flange, presence: absent (0), DT = 0.2961; present (1), DT = 0.7039. TR = 11.5275; HI = 0.9133. Notes: see SW86:14 for discussion of this character.

(22) ♂ Gonopodal lateral flange, orientation: absent (0), DT = 0.2961; anterolaterally (1), DT = 0.5830; anterodorsally (2), DT = 0.0748; dorsolaterally (3), DT = 0.0290; posterolaterally (4), DT = 0.0170. TR = 17.3123; HI = 0.7690.

(23) ♂ Gonopodal lateral flange, shape: absent (0), DT = 0.3072; laminate (1), DT = 0.5019; lobe-like (2), DT = 0.1908. TR = 17.5844; HI = 0.8863.

(24) ♂ Gonopodal acropodite distal zone surface, orientation relative to a perpendicular axis arising ventrally from the gonopodal coxa: prolaterally, linear acropodite (0), DT = 0.4947; anterolaterally, twisted 1/4 turn clockwise (1), DT = 0.3307; posterolaterally, twisted 1/4 turn counterclockwise (2), DT = 0.0993; ventrally, twisted 3/4 turn clockwise (3), DT = 0.0752. TR = 15.4396; HI = 0.8057.

(25) ♂ Gonopodal acropodite, expansion: tapering to a point distally (0), DT = 0.7985; constant width (1), DT = 0.1056; expanded distally (2), DT = 0.0959. TR = 11.0047; HI = 0.8183.

(26) ♂ Gonopodal additional apical process, presence: absent (0), DT = 0.4084; present (1), DT = 0.5916. TR = 11.1170; HI = 0.9100.

(27) ♂ Gonopodal solenomere (those with an additional apical process), position: absent (0), DT = 0.4764; medial (1), DT = 0.0395; anterolateral (2), DT = 0.0743; posterolateral (3), DT = 0.4099. TR = 14.8550; HI = 0.7980.

(28) ♂ Gonopodal fold, presence: absent (0), DT = 0.9591; present (1), DT = 0.0409. TR = 1.0906; HI = 0.0831.

(29) ♂ Gonopodal cingulum, presence: absent (0), DT = 0.8317; present (1), DT = 0.1683. TR = 2.1581; HI = 0.5366.

(30) ♂ Gonopodal cingulum, location: absent (0), DT = 0.8322; proximal (1), DT = 0.0065; distal (2), DT = 0.1613. TR = 3.1289; HI = 0.3608.

(31) ♂ Gonopodal acropodite anterior bend, presence: absent (0), DT = 0.4082; present (1), DT = 0.5918. TR = 2.0837; HI = 0.5201.

(32) ♂ Gonopodal acropodite apical curve, presence: absent (0), DT = 0.5318; present (1), DT = 0.4682. TR = 2.1852; HI = 0.5424. Notes: see SW86:14 for discussion of this character.

(33) ♂ Gonopodal acropodite peak, percent of acropodite distal from peak: 0, straight acropodite (0), DT = 0.5321; 17–33 (1), DT = 0.1739; 40 (2), DT = 0.1879; 40-70 (3), DT = 0.1061. TR = 17.4532; HI = 0.8281.

(34) ♂ Gonopodal apical acropodite, percent divided: 0, not divided (0), DT = 0.6889; 16.6 (1), DT = 0.0637; 30 (2), DT = 0.2042; 100 (3), DT = 0.0432. TR = 8.6797; HI = 0.6544.

(35) ♂ Gonopodal solenomere tip, shape: sharp (0), DT = 0.8635; blunt (1), DT = 0.1365. TR = 7.5865; HI = 0.8682. Notes: see SW86:13 for discussion of this character.

(36) ♂ Gonopodal third branch, presence: absent (0), DT = 0.9500; present (1), DT = 0.0500. TR = 2.1517; HI = 0.5353.

(37) ♂ Gonopodal acropodite, curvature: linear (0), DT = 0.5035; irregular circle (1), DT = 0.4742; smoothly continuous circle (2), DT = 0.0223. TR = 3.1435; HI = 0.3638. Notes: see SW86:12 for discussion of this character.

(38) ♂ Gonopodal acropodite arc (viewed ventrally), curvature: linear, no arc (0), DT = 0.4384; ventrally (1), DT = 0.1087; laterally (2), DT = 0.3554; cephalically (3), DT = 0.0975; caudally* (4). TR = 12.7009; HI = 0.7638. Notes: see Keeton, 1959, p. 53 for discussion of this character.

(39) ♂ Gonopodal acropodite torsion, presence: absent (0), DT = 0.5034; present (1), DT = 0.4966. TR = 2.1493; HI = 0.5347. Notes: see SW86:212 for discussion of this character.

(40) ♂ Gonopodal acropodite, bulk: thin (0), DT = 0.8830; bulky (1), DT = 0.1170. TR = 8.7923; HI = 0.8863. Notes: gonopodal bulk was the primary distinguishing character for the genus "*Tucoria*" (Keeton, 1959).

(41) ♀ Cyphopodal receptacle, presence: present (0), DT = 0.9564; absent (1), DT = 0.0436. TR = 1.3003; HI = 0.2309.

(42) ♀ Cyphopodal receptacle, size at its widest part: absent (0), DT = 0.0470; shorter than prefemur length (1), DT = 0.4003; subequal to prefemur length (2), DT = 0.2015; wider than prefemur length (3), DT = 0.3512. TR = 16.0791; HI = 0.8134.

(43) ♀ Cyphopodal valves, symmetry: symmetric (0), DT = 0.7989; asymmetric (1), DT = 0.2011. TR = 11.2005; HI = 0.9107.

(44) ♀ Cyphopodal valves, orientation: ventrally (0), DT = 0.4656; anteroventrally (1), DT = 0.3116; posteroventrally (2), DT = 0.0379; twisted posterior (3), DT = 0.1848. TR = 13.2318; HI = 0.7733.

(45) ♀ Cyphopodal receptacle cuticle, surface: absent (0), DT = 0.0447; smooth (1), DT = 0.0570; sculptured (2), DT = 0.8983. TR = 4.6471; HI = 0.5696.

(46) ♂ Gnathochilarium lateral emargination, presence: present (0), DT = 0.9849; absent (1), DT = 0.0151. TR = 2.1888; HI = 0.5431.

(47) ♂ Antennomere one distal cuticle, conformation*: cylindrical, not wrapped around cones (0); wrapped around cones (1).

(48) ♂ Collum ridges, presence: present on anterolateral margin (0), DT = 0.9359; absent (1), DT = 0.0641. TR = 4.2511; HI = 0.7648. Notes: see Keeton, 1959, p. 55 for discussion of this character in *Brachoria*.

(49) ♂ Caudolateral corners, paranota I–X, shape: acute, projecting caudally (0), DT = 0.7042; rounded cephalically (1), DT = 0.2958. TR = 8.6654; HI = 0.8846. Notes: caudal projection = projecting beyond paranotal caudal edge. See SW86:17 and Keeton, 1959, p. 55 for discussions of this character.
(50) ♂ Caudolateral corners, paranota I–XIX, shape: acute, projecting caudally on all segments (0), DT = 0.6081; rounded cephalically on segments I–X only (1), DT = 0.1805; rounded cephalically on segments I–X, rounded throughout remaining segments (XI–XIX) (2), DT = 0.2115. TR = 15.8558; HI = 0.8739.
(51) ♂ Metatergal pores, metatergites IX+X, presence: present (0), DT = 0.9842; absent (1), DT = 0.0158. TR = 2.1329; HI = 0.5312.
(52) ♂ Metatergal linear bump-pores, presence: absent (0), DT = 0.7129; present (1), DT = 0.2871. TR = 2.3813; HI = 0.5801.
(53) ♂ Lateral wrinkles, tergites IX+X, presence: tightly wrinkled (0), DT = 0.3376; loosely wrinkled (1), DT = 0.6624. TR = 11.3345; HI = 0.9118.
(54) ♂ Longitudinal paranotal wrinkles, presence: present (0), DT = 0.1192; absent (1), DT = 0.8808. TR = 1.5718; HI = 0.3638.
(55) ♂ Repugnatorial glands, paranota IX+X, orientation: laterally (0), DT = 0.5201; dorsal (1), DT = 0.4799. TR = 9.5001; HI = 0.8947.
(56) ♂ Metatergal dorsal slits, presence: present (0), DT = 0.9879; absent (1), DT = 0.0121. TR = 2.2080; HI = 0.5471.
(57) ♂ Metatergal dorsal microsculpture, mesh shape: isodiametric (0), DT = 0.8716; anisodiametric (1), DT = 0.1284. TR = 1.6438; HI = 0.3917.
(58) ♂ Paranota-dorsum, segments IX+X, angle: 130° (0), DT = 0.3594; 180° (1), DT = 0.6406. TR = 2.2277; HI = 0.5511. Notes: see Shelley, 1980a, p. 135 and Causey (1951) for discussions of this character.
(59) ♂ Paranotal segments IX+X, width: thick (0), DT = 0.1374; thin (1), DT = 0.8626. TR = 2.7365; HI = 0.6346.
(60) ♂ Anterior dorsolateral paranotal disc, segments IX+X, concavity: puffed out (0), DT = 0.1201; flat (1), DT = 0.1122; scooped out (2), DT = 0.7677. TR = 12.1859; HI = 0.8359.
(61) ♂ Gonapophyses, shape: cylinder-shaped (0), DT = 0.4278; goblet-shaped (1), DT = 0.5722. TR = 13.3560; HI = 0.9251.
(62) ♂ Sternal knobs, 4th leg pair, presence∗: absent (0); present (1). Notes: See SW86:214 for discussion of this character.
(63) ♂ Pleural process, segments IX+X, presence: absent (0), DT = 0.6207; present (1), DT = 0.3793. TR = 7.8149; HI = 0.8720.
(64) ♂ Sternal triangular spines, segments IX+X, presence: present (0), DT = 0.3321; absent (1), DT = 0.6679. TR = 7.1743; HI = 0.8606. Notes: see SW86:214 and Shelley, 1980a,b, p. 135 for discussion of this character.
(65) ♂ Sternal median bulge, segments IX+X, presence: absent (0), DT = 0.8492; present (1), DT = 0.1508. TR = 1.1186; HI = 0.1060.
(66) ♂ Setae, sterna IX+X, presence: present (0), DT = 0.5126; absent (1), DT = 0.4874. TR = 6.6070; HI = 0.8486.
(67) ♂ Ventral excavation, sterna IX+X, presence: present (0), DT = 0.4402; absent (1), DT = 0.5598. TR = 6.7085; HI = 0.8509.
(68) ♂ Pregonopodal tarsal claws, shape: curved (0), DT = 0.1503; bisinuately curved (1), DT = 0.8497; twisted, spatulate∗ (2). TR = 4.0010; HI = 0.7501. Notes: see SW86:214 for discussion of this character.

Appendix C

Taxonomy of Apheloriini. Clade diagnoses based on a parsimony map of all unequivocal state changes for the combined-I data set in MacClade 4.0 (Maddison and Maddison, 2000). [1]Unique and uniform for the clade; [2]Unique for the clade but modified in some taxa; [3]Unique for the clade but shared with other taxa, diagnostic when combined. Asterisks denote taxa not in the analysis—inclusion hypothesized retrospectively from diagnostic characters.

C.1. Tribe Apheloriini Hoffman

Apheloriini Hoffman, 1979, p.158; Shelley *in* Shelley and Whitehead, 1986: p. 205; Hoffman, 1999: 304.

Genera: *Apheloria* Chamberlin, 1921; *Appalachioria* n. gen.; *Brachoria* Chamberlin, 1939; *Brevigonus* Shelley, 1980b; *Cheiropus* Loomis, 1944; *Cleptoria* Chamberlin, 1939; *Croatania* Shelley, 1977; *Deltotaria* Causey, 1942; *Dixioria* Chamberlin, 1947; *Dynoria* Chamberlin, 1939; *Falloria* Hoffman, 1948; *Furcillaria* Shelley, 1981; *Lyrranea* Hoffman, 1963∗; *Prionogonus* Shelley, 1982; *Rudiloria* Causey, 1955; *Sigmoria* Chamberlin, 1939; *Stelgipus* Loomis, 1944∗.

Apheloriini is placed with other taxa in the subfamily Xystodesminae based on the combination of the following characters (Hoffman, 1978c): Female cyphopodal characters: sternum II triangular medially with slender lateral extensions that articulate against a condyle on the pleurotergum. Coxae II length subequal to width, not broadened laterally. Cyphopod nestled in shallow membranous sac, not enlarged, observable ventrally when retracted.

Diagnosis: Male gonopodal characters: [1]Telopodite–coxa articulated at a 180 degree angle (Fig. 8B–D). [2]Prefemoral process present, short and stout (Fig. 8C and D); or absent (Fig. 8B); never long and acicular (Fig. 8A). Nucleotide site substitutions (site number in parentheses, alignment available for download at www.treebase.org matrix Accession No. M2738): [1]T(92). [1]C(1077). [3]A(730). [3]T(1090).

Additional characters that may be helpful in distinguishing Apheloriini from other xystodesmine taxa: male gonopodal characters: coxal sternum absent, coxae connected with membrane. Distal zone (*sensu stricto*) usually present; absent in 421 and 431. Basal and distal zones (*sensu lato*) usually not coplanar; coplanar, distal zone at a right angle with peak in *Brachoria laminata* and *B. splendida*; absent in *Furcillaria laminata*. Anterior twist usually broad and poorly defined, slightly twisted cephalically; or occasionally well-defined and strongly twisted cephalically; absent in *Dynoria medialis*. Distal zone-peak juncture usually bent, non-circular angle to 90°; circular in *Apheloria montana*; straight, 180° in *Furcillaria laminata* and *Dynoria medialis*. Area distal to apical curve (distal zone, *sensu lato*) usually curved medially; recurved laterally in *Prionogonus divergens*, *Sigmoria australis*, *S. latior latior*, *Brachoria ligula*; straight, distal zone (*sensu lato*) absent in *Furcillaria laminata*. Distal zone (*sensu stricto*) usually short, less than 0.4 length of acropodite; or occasionally long, greater than or equal to 0.5 length of acropodite; distal zone (*sensu stricto*) absent in *Furcillaria laminata* and *Dynoria medialis*. Distal zone (*sensu stricto*) surface usually directed anterolaterally, twisted 1/4 turn clockwise; or occasionally directed posterolaterally, twisted 1/4 turn counterclockwise; or rarely directed ventrally, twisted 3/4 turn clockwise. Anterior bend present. Apical curve usually present; absent in *Furcillaria laminata* and *Dynoria medialis*. Percent of acropodite length distal from peak usually between 17% and 70%; straight acropodite, distal zone (*sensu stricto*) absent in *Furcillaria laminata* and *Dynoria medialis*. Telopodite form usually an irregular circle; smoothly continuous circle in *Apheloria montana* and *A. tigana*; linear in *Furcillaria laminata*. Acropodite arc (viewed ventrally) usually directed laterally; or occasionally directed ventrally; or rarely cephalically. Telopodite torsion usually present; absent in *Dynoria medialis*. Female cyphopodal characters: valves usually directed anteroventrally; or occasionally directed posteroventrally; or occasionally twisted posteriorly; directed ventrally in *Dynoria medialis*.

Large (4–6 cm) and broad, "flat-backed" millipedes. Females are typically larger and more convex. Bright aposematic coloration in yellow, orange, red, and violet. Pleasant cherry, almond aroma (benzaldehyde, byproduct of cyanide-producing reaction used for defense).

Distribution: found mainly in the Appalachian Highlands of eastern North America with a center of species diversity in the Southern Appalachian Mountains (Fig. 2).

Ecology: Apheloriini are found predominately in moist, hardwood, deciduous forests beneath leaf litter on the forest floor. Many also occur in *Rhododendron* coves, especially those from the Southern Blue Ridge province. A few species are also known from cedar glades. Apheloriine millipedes feed on dead moistened maple, tulip poplar, oak, and dogwood leaves. Primarily diurnal, though a few species can be found at night in large quantities.

C.1.1. Genus Appalachioria ***n. gen.***

Type species: *Brachoria falcifera* Keeton, 1959

Species: *A. eutypa eutypa* (Chamberlin, 1939), n. comb.; *A. eutypa ethotela* (Chamberlin, 1942), n. comb.; *A. falcifera* (Keeton, 1959), n. comb.; *A. mendota* (Keeton, 1959), n. comb.; *A. separanda separanda* (Chamberlin, 1947), n. comb.; *A. separanda calcaria* (Keeton, 1959), n. comb.; *A. separanda versicolor* (Hoffman, 1963), n. comb.; *A. separanda hamata* (Keeton, 1959), n. comb.; *A. turneri* (Keeton, 1959), n. comb.

Appalachioria is placed with other taxa in the tribe Apheloriini based on the following characters: male gonopodal characters: [1]Telopodite–coxa articulated at a 180° angle (Fig. 8B–D). [2]Prefemoral process present, short and stout (Fig. 8C and D); or absent (Fig. 8B); never long and acicular (Fig. 8A). Nucleotide site substitutions (site number in parentheses, alignment available for download at www.treebase.org ,matrix Accession No. M2738): [1]T(92). [1]C(1077). [3]A(730). [3]T(1090).

Diagnosis: Male gonopodal characters: [3]cingulum present. [3]Cingulum distal (located a distance greater than 1/2 the length of the acropodite distally from the coxa). Nucleotide site substitutions (site number in parentheses, alignment available for download at www.treebase.org matrix Accession No. M2738): [1]G(350). [1]A(696). [1]C(789). [3]G(204). [3]A(207). [3]A(309). [3]T(643). [3]A(647). [3]T(698). [3]T(726). [3]C(785). [3]C(790). [3]C(795). [3]A(1152). [3]A(1164). [3]A(1185). [3]A(1265). [3]C(1277). [3]G(1311). [3]T(1312). [3]A(1318).

Additional characters that may be helpful in distinguishing *Appalachioria* from other apheloriine taxa: male exoskeletal characters: collum ridges absent. Caudolateral corners of paranota rounded cephalically on anterior segments (I–X) and rounded throughout remaining segments (XI–XIX). Male gonopodal characters: acropodite distal zone surface usually directed posterolaterally, twisted 1/4 turn counterclockwise; directed anterolaterally, twisted 1/4 turn clockwise in *Appalachioria* Foster; directed ventrally, twisted 3/4 turn clockwise in *Appalachioria turneri*.

Large (4–5 cm) and broad, "flat-backed" millipedes. Narrower than many apheloriines. Rounded paranota. Bright aposematic coloration in yellow, orange, red, and violet that commonly mimics other sympatric apheloriine genera (frequently mimics the yellow and black alternating bands of *Apheloria virginiensis corrugata*).

Distribution: found mainly in the valley and ridge province of the Appalachian Highlands (Fig. 2).

Ecology: *Appalachioria* are predominately found in moist, hardwood, deciduous forests beneath leaf litter on the forest floor. Some also can be found in *Rhododendron* coves. Primarily diurnal.

Etymology: gender, f. *Appalachioria* is a latin neologism compounded from the Appalachian Mountains, after the mountains in which these millipedes occur and the latin ending "-oria", which is common for other genera in the tribe, Apheloriini.

C.1.2. Genus Brachoria Chamberlin, 1939

Brachoria Chamberlin, 1939. Type species: *B. initialis* Chamberlin, 1939; by original designation.

Species: *B. calceata* (Causey, 1955)∗; *B. cedra* Keeton, 1959; *B. conta* Keeton, 1965∗; *B. dentata* Keeton, 1959; *B. divicuma* Keeton, 1965∗; *B. electa* Causey, 1955; *B. enodicuma* Keeton, 1965; *B. evides* (Bollman, 1887)∗; *B. glendalea* (Chamberlin, 1918a); *B. gracilipes* (Chamberlin, 1947)∗; *B. hansonia* Causey, 1950∗; *B. hoffmani* Keeton, 1959; *B. hubrichti* Keeton; 1959; *B. indianae* (Bollman, 1888); *B. initialis* Chamberlin, 1939; *B. insolita* Keeton, 1959; *B. kentuckiana* (Causey, 1942)∗; *B. laminata* Keeton, 1959; *B. ligula* Keeton, 1959; *B. ochra* (Chamberlin, 1918a); *B. plecta* Keeton, 1959∗; *B. splendida* (Causey, 1942); *B. viridicolens* Hoffman, 1948∗.

Brachoria is placed with other taxa in the tribe Apheloriini based on the following characters: male gonopodal characters: [1]telopodite–coxa articulated at a 180° angle (Fig. 8B–D). [2]Prefemoral process present, short and stout (Fig. 8C and D); or absent (Fig. 8B); never long and acicular (Fig. 8A). Nucleotide site substitutions (site number in parentheses, alignment available for download at www.treebase.org matrix Accession No. M2738): [1]T(92). [1]C(1077). [3]A(730). [3]T(1090).

Diagnosis: Male gonopodal characters: [3]cingulum present. [3]Cingulum proximal (located a distance less than 1/2 the length of the acropodite distally from the coxa), or distal (located a distance greater than 1/2 the length of the acropodite distally from the coxa). Nucleotide site substitutions (site number in parentheses, alignment available for download at www.treebase.org matrix Accession No. M2738):[3]T(1114).

Additional characters that may be helpful in distinguishing *Brachoria* from other apheloriine taxa: male exoskeletal characters: collum ridges usually present. Caudolateral corners of paranota usually rounded cephalically on anterior segments (I–X) and acute, projecting caudally (beyond paranotal caudal edge) throughout remaining segments (XI–XIX); acute, projecting caudally on all segments (I-XIX) in *Brachoria* Scottsboro, *B. ochra*, *B. initialis*. Male gonopodal characters: acropodite distal zone surface usually directed anterolaterally, twisted 1/4 turn clockwise; or occasionally directed posterolaterally, twisted 1/4 turn counterclockwise.

Large (4–6 cm) and broad, "flat-backed" millipedes. Bright aposematic coloration in yellow, orange, red, and violet that commonly mimics other sympatric apheloriine genera (like *Appalachioria*, *Brachoria* individuals frequently mimic the yellow and black alternating bands of *Apheloria virginiensis corrugata*).

Distribution: found mainly in the Cumberland Plateau section of the Appalachian Highlands (Fig. 2).

Ecology: *Brachoria* are predominately found in moist, hardwood, deciduous forests (*B. cedra* occurs in cedar glades) beneath leaf litter on the forest floor. Primarily diurnal.

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